Abstracts of the 2018 American Dairy Science Association® Annual Meeting

June 24–27, 2018
Knoxville, Tennessee

Journal of Dairy Science®
Volume 101, Supplement 2

JOURNAL OF DAIRY SCIENCE® *SINCE 1917*

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American Dairy Science Association®

Sunday, June 24, 2018

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Workshop: National Animal Nutrition Program (NANP) Models

1 Introduction and model construction: Part I (lecture). T. J. Hackmann*[1], M. D. Hanigan[2], and V. L. Daley[3], [1]*University of Florida, Gainesville, FL,* [2]*Virginia Tech, Blacksburg, VT,* [3]*National Animal Nutrition Program, University of Kentucky, Lexington, KY.*

This lecture will provide an overview of mathematical models, their types, and their construction. The general objective of mathematical modeling is to take a hypothesis, convert it a system of equations, and determine how well the equations describe reality. The specific objectives depend on the application, but could include predicting nutrient digestibility or intake. In this way, modeling is no different from any other scientific exercise—the first step is for the investigator to identify the hypothesis and objective. There are different types of models, and the investigator should choose a type suited to the specific objectives. In defining its type, a model can be categorized as static or dynamic, empirical or mechanistic, and deterministic or stochastic. Historically, nutrient requirement models have been static, empirical, and deterministic; they provided snapshots in time, did not describe mechanisms underlying responses, and did not consider inherent biological variance. These models were easy to derive, and have served the community well for more than a century. The Molly cow model is dynamic, mechanistic, and deterministic predicting responses through time based on underlying elements of digestion and metabolism without consideration of biological variation. After the investigator identifies the hypothesis, objective, and model type, the next step of constructing a model is to draw a block diagram. This diagram organizes the model conceptually. Rectangles in the diagram represent state variables, and arrows connecting the rectangles show the relationship of the variables. In a model of carbohydrate digestion in the rumen, for example, rectangles would represent pools of fiber, starch, and sugars, and an arrow connecting fiber and sugars would represent hydrolysis of fiber. This approach of representing pools or compartments within a system is referred to as compartmental modeling. In the remaining steps of constructing a model, the investigator translates the block diagram into system of equations, defines values of equation parameters, and solves the model so it can generate predictions. If evaluation of the model shows predictions are inadequate, earlier steps are repeated to refine the model.

Key Words: mathematical model, dynamic, rumen

2 Introduction and model construction: Part II (exercises). M. D. Hanigan*[1], V. L. Daley[2], and T. J. Hackmann[3], [1]*Virginia Tech, Blacksburg, VA,* [2]*National Animal Nutrition Program, University of Kentucky, Lexington, KY,* [3]*University of Florida, Gainesville, FL.*

The principles of mathematical modeling in agricultural sciences are well described by France and Thornley (1984). They categorized models as static or dynamic, empirical or mechanistic, and deterministic or stochastic, although, in practice, these categories are a continuum. This talk and exercise will focus on the mechanics of building and solving a compartmental model of intestinal N metabolism. A simple regression equation is often used to represent static processes; for example, dCP = CP_In × 0.65. This approach only considers fractional digestion of CP in the gut, and ignores any effects of other factors such as passage rate or microbial activity. In this simple model, a fast rate of passage would have the same digestibility as a slow rate. If one wants to represent residence time effects on CP digestion, then consideration of the pool size is needed. Mechanisms controlling CP digestion in the rumen can be incorporated into the model to yield better estimates. A dynamic model with a rumen pool of CP and a representation of rates of passage and degradation driven by microbial activity can be constructed and fitted to data to derive information on those mechanisms An intestinal model can be linked to the rumen model to further predict intestinal digestions and amino acid absorption. If rates of passage and degradation are known, it can also be used to predict outcomes when system inputs are manipulated. A representation of this system will be built by participants using R and fit to example data. The model can easily be extended, as there is no mathematical limit to the complexity that can be incorporated. Pool size, and thus the fluxes driven by pool size, can be solved numerically using a computer and numerical integration algorithms. As demonstrated with the example problem, compartmental modeling is very useful for modeling nutrient metabolism and animal performance as nutrient flow through a series of compartments and into product or excreta can be represented.

Key Words: mathematical model, type, review

3 Model evaluation: Part I (lecture). E. Kebreab*, *University of California, Davis, Davis, CA.*

Model evaluation indicates the level of accuracy and precision of model performance by assessing the credibility and reliability of a model in comparison to measured observations. Quantitative statistical model evaluation methods can be classified into 3 types including (1) standard regression statistics, which determines strength of linear relationship, (2) error index, which quantifies deviation in observed units, and (3) relative model evaluation that are dimensionless. Within the first category, analysis of residuals involves regressing residuals against predicted or other model variables. In this method, the model is unbiased if residuals are not correlated with predictions and the slope is not significantly different from zero. Predicted values can also be centered making the slope and intercept estimates in the regression orthogonal and thus, independent. This allows for mean biases to be assessed using the intercepts of the regression equations, and the slopes to determine the presence of linear biases. Mean square error of prediction (MSEP) and its square root (RMSEP) are commonly used methods of error index type of evaluation. In general, RMSEP values less than half of observed SD may be considered having a good performance. The MSEP can be decomposed into (1) error due to overall bias of prediction, (2) error due to deviation of the regression slope from unity, and (3) error due to the disturbance. Examples of the third category include the concordance correlation coefficient (CCC). The CCC can be represented as a product of 2 components: a correlation coefficient estimate that measures precision (range 0 to 1, where 1 = perfect fit) and a bias correction factor that indicates how far the regression line deviates from the line of unity (range from 0 to 1 and 1 indicates that no deviation from the line of unity has occurred). During model evaluation, a combination of the methods described above should be used to gain insight on model performance.

Key Words: evaluation, model

4 Model evaluation: Part II (exercises). E. Kebreab*, *University of California, Davis, Davis, CA.*

The objective of the model evaluation exercise is to familiarize users on various tools used to evaluate models. The exercise will use the R statistical software due to its relatively straightforward use, which is also freely available on the internet. A data set containing observed

and predicted data will be made available to the participants. Based on principles covered, the participants will be asked to calculate the mean square error of prediction (MSEP) and its square root (RMSEP), which are one of the most commonly used methods of model evaluation. Furthermore, the exercise includes calculated the MSEP decomposition into (1) error due to overall bias of prediction, (2) error due to deviation of the regression slope from unity, and (3) error due to the disturbance. The participants will be asked to calculate another model evaluation category, which is the concordance correlation coefficient (CCC). The participants are expected to express CCC as a product of 2 components: a correlation coefficient estimate that measures precision (range 0 to 1, where 1 = perfect fit) and a bias correction factor that indicates how far the regression line deviates from the line of unity (range from 0 to 1 and 1 indicates that no deviation from the line of unity has occurred). Finally, participants will be asked to compare results from the 2 different categories of model evaluation.

Key Words: model performance, modeling, prediction accuracy

5 Meta-analysis: Part I (lecture). R. R. White*, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Meta-analysis of literature is used in animal nutrition research to gain a more comprehensive understanding of the response being studied. Using weighted, mixed-effects, regression, most meta-analytical data sets can be evaluated. In these analyses, data are first gathered using clearly defined search criteria. Collected data should include the response variables of interested, standard errors reported, and explanatory variables under consideration. Once data are compiled, they should be checked for outliers and possible errors when transferring data. To remove individual study statistical analysis effects, data should be partitioned into mixed- and fixed-effect analyses and standardized. When data are clean and errors are standardized, backward, stepwise regression with fixed-effects for variables of interest and random-effects for things like study and location can be conducted. Variables should be removed from the model according to a predetermined cutoff, usually a *P*-value of 0.05. When all variables in the model are significant, removed variables can be iteratively re-tested to ensure that factors were not removed due to model instability. Correlation between factors can then be assessed using variance inflation factors (VIF). Parameters with a VIF above 10 should only be kept when parameters are correlated by calculation. When variables are correlated, the variable with highest VIF can be removed. Backward, stepwise regression, elimination at a significance cutoff, and elimination based on correlation should be iterated until model has only significant parameters and is not highly correlated. This procedure provides a framework for most animal nutrition meta-analyses, but may require some adjustments based on available data.

6 Meta-analysis: Part II (exercises). D. M. Liebe* and R. R. White, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

This meta-analysis workshop will work through an example data set using a common analysis procedure to illustrate the usefulness of meta-analysis as a tool in ruminant nutrition research. The workshop will focus on use of R and R Studio for conducting meta-analysis. The example data set includes literature reporting how microbial N outflows from the rumen are influenced by dietary nutrient intakes, marker type, sampling location, rumen pH and rumen volatile fatty acid or ammonia concentrations. The workshop will walk through a multi-step process used to evaluate data for common errors, correct standard errors, and derive models using a backward, stepwise regression procedure. Packages reviewed will include those required to read in data (xlsx, XLConnect, googlesheets), those required to handle data (dplyr, reshape2), those required to visualize data (ggplot2), and those required to fit linear mixed effect models (nlme, lme4, lmerTest). The workshop will walk participants through how data should be structured in text files or spreadsheets; how packages can be used to read data in from these external formats; how data can be handled and queried once read into R or R Studio; how data can be visualized using the ggplot2 package; and how models can be derived using linear mixed-effects models weighted for the inverse of study standard errors. At the end of the workshop, participants should be able to (1) organize data for use in a meta-analysis; (2) read data into R from a variety of formats; (3) visualize data distributions for assessment of common data entry errors; (4) calculate weights for use in meta-analysis; (5) derive a model using a multi-step backward elimination approach; and (6) evaluate the fit of a model using common fit statistics.

Key Words: meta-analysis, nutrition, regression

7 Opportunities for federal funding of modeling research. S. I. Smith* and M. A. Mirando, *USDA-National Institute of Food and Agriculture, Institute of Food Production and Sustainability, Washington, DC.*

The Food, Conservation, and Energy Act of 2008 (Public Law 110–246; i.e., the 2008 Farm Bill) established the National Institute of Food and Agriculture (NIFA) within the US Department of Agriculture (USDA). NIFA directs federal funding to advance agricultural research, education, and extension to solve agricultural challenges. In FY2017, NIFA invested a total of approximately $1.5 billion: $854 million in Research and Education Activities, $478 million in Extension Activities, $159.6 million in Mandatory and Endowment Funding, and $36.0 million in Integrated Activities, as designated in the 2017 Congressional Appropriations Act (Public Law No: 115–31). This investment is broadly split between Capacity Funding and Competitive Grant Funding. NIFA administers more than 30 Competitive Programs with broad eligibility. NIFA's flagship competitive program is the Agriculture and Food Research Initiative (AFRI). Mathematical modeling is recognized as a powerful tool that can be effectively applied to organize and interconnect what is known, to highlight knowledge gaps and areas needing research, and to prompt investigators to ask better questions. As a result, there are currently 80 active AFRI livestock projects with a modeling component, 8 of which directly involve the dairy enterprise. These dairy projects address a broad array of topics including ruminal metabolism, genomic prediction, whole genome selection, host-pathogen interaction, metabolic diseases, antimicrobial resistance and nutrition. NIFA has published 3 AFRI Request for Applications (RFAs) in FY 2018; (1) the Foundational and Applied Science Program RFA, (2) the Education and Workforce Development RFA and (3) the Sustainable Agricultural Systems RFA. It should be noted that modeling is specifically invited in 9 of the Foundational program area priorities, as well as the program area priority for Sustainable Agricultural Systems. A list of NIFA RFAs can be found at https://nifa.usda.gov/rfa-list

Key Words: modeling, nutrition, funding

Workshop: Spore Sources and Transmission from Farm to Fork—Detection and Control Strategies

8 Introduction to dairy-relevant sporeformers and detection methodologies. M. Wiedmann*, *College of Food Science, Cornell University, Ithaca, NY.*

Spore-forming bacteria are a diverse group of bacteria that can grow at various temperatures, survive extreme conditions, and persist in farm and processing environments for years. These organisms are capable of growing in and affect the quality of fluid milk, cheese, and dairy powders. The dairy industry must adopt a systems approach to reducing the impact of spore-forming bacteria. Key to this goal is the development and implementation of appropriate testing methods for spore-forming bacteria, from spoilage organisms to pathogens. This session will introduce participants to the most common spore-forming bacteria encountered in the dairy industry, and how methodologies have emerged to detect, enumerate, and track these organisms.

9 On-farm sources and control strategies. N. Martin*, *Cornell University, Ithaca, NY.*

Spore-forming bacteria are found ubiquitously in natural environments, including on dairy farms. Manure, soil, water and other materials in cow environments can harbor millions of spores, exposing the cow and, ultimately, raw milk to these organisms. Research indicates that various on-farm management practices are associated with the presence and levels of spore-forming bacteria in bulk tank raw milk. This session will explore the types of management practices in various locations across the United States that are associated with psychrotolerant, mesophilic and thermophilic spores in bulk tank raw milk. Additionally, attendees will learn about approaches to reducing spore levels in raw milk through simple and inexpensive intervention strategies.

10 Introduction to dairy relevant sporeformers and detection methodologies. T. Erickson*, *Ecolab, St. Paul, MN.*

Spore-forming bacteria are a diverse group of bacteria that can grow at various temperatures, survive extreme conditions and persist in farm and processing environments for years. These organisms are capable of growing in and affect the quality of fluid milk, cheese, and dairy powders. The dairy industry must adopt a systems approach to reducing the impact of spore-forming bacteria. Key to this goal is the development and implementation of appropriate testing methods for spore-forming bacteria, from spoilage organisms to pathogens. This session will introduce participants to the most common spore-forming bacteria encountered in the dairy industry, and how methodologies have emerged to detect, enumerate, and track these organisms.

ADSA 2018 Mini Symposium: Priorities for Fiber Research (DC33 Follow-Up)

11 Priorities for future research to improve fiber utilization by animals. D. R. Mertens*, *Mertens Innovation & Research LLC, Belleville, WI.*

The objective is to summarize the results of the 33rd ADSA Discover Conference, *Integrated Solutions to Fiber Challenges*, held September 2017; convey future research priorities that were identified; and promote discussion and networking to implement needed research for fiber utilization. Conference sessions included plants and climate; fiber analysis for animals; impact of plants on animals; animal by fiber interactions: getting the most out of fiber; and modeling fiber utilization by animals. It provided a unique opportunity for professional interactions that fostered discussion and identified areas of research that would improve fiber utilization. The final session of the conference summarized recommendations and challenges facing future research. Participants concluded that advances in chemical analyses have been significant, and the use of crude fiber for feed tags and regulation should be abandoned. Six broad categories of nutritional fiber research were identified: (1) chemical analysis, (2) biological (digestion) analysis, (3) physical analysis, (4) fiber fermentation, (5) fiber modeling, and (6) next transformational change in utilization. Survey participants ranked these categories (highest priority first): 2, 4, 3, 1, 5, and 6. These categories were divided into 35 specific items and ranked 0 to 100. Three topics averaged >75: (1) continue research on fiber utilization of forage and by-products, (2) additional graduate training using regional/national research teams, and (3) investigating fiber physical characteristics. Five topics, which averaged 70–74, were combined: improve biological methods of measuring microbial fiber digestion and their interactions, and create a consortium of animal producers, allied seed/feed industries, and governments to prioritize and fund fiber research and improve analytical and mathematical skills of future researchers. Remaining topics will be discussed. Attendees at the conference identified important priorities and discussed how to meet these priorities for the dairy industry. This mini-symposium is intended to broaden the discussion about the future of fiber utilization research with scientists at the annual ADSA meeting.

Key Words: analyses, digestion, modeling

ADSA Graduate Student Symposium: Manuscript Writing for Graduate Students

12 Tips and tricks for turning your ideas into peer-reviewed publications. M. A. G. von Keyserlingk* and D. M. Weary, *University of British Columbia, Vancouver, BC, Canada.*

A major goal for researchers is to tell our colleagues (and others) about our work. This is formally done through the publishing of our findings in the peer-reviewed literature, but for many of us this is a daunting task. How do we take our data and tell the story that will result in publication? In this talk, we provide some simple steps to help ease the process. The work starts with the formulation of an interesting and important research question. This question needs to be situated within the current literature, building upon existing ideas, and helping to fill a recognized gap. A clear research question may also help in identifying predictions that inform experimental design and what is measured, allowing you to focus on a few well-reasoned ideas, and avoid including measures simply because they are easy to collect. Before starting data collection, try to write a first draft of the Introduction and Methods sections of your paper, using the format of your target journal. This will force you to clearly describe your research question and to link your proposed methods with the main ideas. The Methods section should also describe your proposed statistical analysis and the power analysis you used to calculate sample size, and follow one of the reporting guidelines specified by the journal (such as ARRIVE). As you begin data collection, you can revise the Methods to reflect any changes you have made. Before you begin statistical analysis, carefully scrutinize the raw data, using plots to check for outliers. Take special effort to develop the figures and tables describing your main findings—these will be the stars of your paper. Before you start, try to develop an eye for what types of graphical reporting you find most helpful, and use these ideas when presenting your results. The discussion should carefully integrate your results into the literature, identify new ideas and gaps for future research, and end with a clear and specific conclusion. A high-quality paper requires many drafts, so be prepared to take the time needed to polish your efforts, including seeking out critical comments from readers whose work you admire.

Key Words: authorship, publishing, scientific writing

13 Collaborating with co-authors: Writing, presenting, and publishing. D. M. Barbano*, *Cornell University, Ithaca, NY.*

Organization, planning, and inclusive communication among co-authors are the keys to success. Collaboration in writing, presenting, and publishing will flow a bit differently for review papers versus research papers, but the basic principles are the same. In both cases, the co-authors need to agree on the target journal and who will be the corresponding author. For review papers, each co-author is normally responsible for a section(s) and the lead author will do the integration. For original research papers, the sequence is a bit different. The manuscript development and planning has to start with clear and measurable objectives, an experimental design, and a plan for statistical analysis of the data. This should be done before you do the research. In writing, start with a title page, an introduction section with only the last sentence(s) written (i.e., the objectives of the research), and descriptive first-level and second-level section titles for the remainder of the paper. Step 1: Write the materials and methods in complete detail (best if this is done while doing the research). Step 2: Analyze data and make final form data tables and figures with all statistical analysis included. Have all co-authors agree on the main messages from each table and figure. You are not ready to start writing the results and discussion section until step 2 is complete. This is the step where people waste too much time writing before the data (and co-authors) are ready for them to write. Step 3: Write your story about your data (don't worry about the literature yet). Have all co-authors review and provide input before going to step 4. Step 4: Next, bring in appropriate discussion of literature citations to compare with your story. Some previous work may agree and some may differ. Provide a balanced perspective. Step 5: Write a short conclusion about your results, not the literature. Stick to facts that are statistically significant. Have all co-authors review and revise. Step 6: Write the introduction including only background references that are necessary to understand the topic and to logically lead the reader to your objective statement that was written earlier. Step 7: Write the abstract with the objective(s), a brief experimental approach, and then add the conclusions that match step 5.

Key Words: writing, publishing, presenting

14 Manuscript preparation, navigating journal submission, and the peer-review process. L. E. Armentano*, *University of Wisconsin, Madison, WI.*

A good paper starts with a good experiment, which in turn starts with a clearly stated hypothesis. The introduction must end with this hypothesis, and the final paragraph of your paper must say what you conclude about that hypothesis. Write a draft introduction before you start your experiment. You will update this before submission, but your hypothesis should not change. As you do your research, start writing sentences for your methods. Make sure they describe exactly what you are doing. This means you have read the original methodology, even if citing it through another paper. If you are also keeping track of the references you will cite, that makes 3 big parts of your paper that you have drafted before finishing your study. I prefer combining results and discussion text in one section and put most of my results in either a table or figure. Some reviewers want discussion of every result, but I submit papers that contain "minor" results in some table rows that I do not discuss. If a reviewer asks, add text to the discussion about these results. The less you say in a discussion, the more you focus on the core objective. I write for my reviewers, so I would rather have to add distracting discussion text at their request after they have read my focused discussion. Certainly, anything else interesting that comes up is worth mentioning but don't elaborate or speculate on ideas beyond the scope of your experiment. Do know how your results compare with the existing literature to make sure something is not way off—you do have to discuss anything that seems unusual. A good paper comprises unambiguous, direct, short, minimally complicated sentences that each convey a single idea. Integrate related ideas using paragraphs of simple sentences. Have others read your paper, especially people outside your immediate research group. Writing is an iterative process, so expect to modify your precious writing in response to reviewers. Respect the input of all your reviewers, and if you failed to make them understand something, rewrite your paper so that anyone reading the final version is not similarly misled. Know the journal guidelines.

Key Words: journal submission, manuscript, peer review

15 Will your research impact dairy farmers? C. Geiger*, *Hoard's Dairyman, Fort Atkinson, WI.*

Scientists do a marvelous job conducting research and sharing those findings in scientific journals. However, will that work ultimately change how dairy producers care for cows and produce nutritious dairy products for consumers? Writing for a lay audience, including dairy farmers and their consultants, is a far different proposition than authoring material for scientific journals. To be successful, authors need to convert detailed research into an easy-to-read article while still maintaining the integrity of the technical work. When writing, remember your reader is a busy person who puts in more work hours than the average American. When you write, outline your article, marshal your facts, and tell your story with personal candor. Express yourself simply and concisely. Keep your sentences short and uncomplicated. Short paragraphs add to readership comprehension. Present just one idea per paragraph. Highly technical acronyms are readership busters. To improve comprehension, consider commonly used vernacular. If the sentence cannot be comprehended the first time, it needs further editing. Anticipate practical reader questions, and answer them in the article; if you cannot, say so and why. Get to the meat of your message immediately. Tell the reader something in the first paragraph. Unless historical background is essential, omit it. Too many readers will flip the page if the first few paragraphs don't have anything to offer. Word counts should not go over 1,100 to 1,200 words. Put action in your title. Avoid label-type titles such as, "Breeding cows." Compelling titles, 48 characters or less, and strong subtitles add to readership. That review-type subtitle will stimulate curiosity in your material and can help the reader know what you think is important about the material. Keep titles to one line and move all details to footnotes. Also, inserting subheads in the article can call out important points between paragraphs. A theme setter-type photograph can add tremendously to the presentation of the article. That photo should be related to the material presented and clarify points made in the article. Graphics also aid readership. Good charts, graphs, and tables can help the author break up the text and make the page more pleasing to the eye.

Key Words: research, dairy farmers

M1 Development and validation of a rapid method for measurement of casein in raw milk using front-face fluorescence spectroscopy and chemometrics. Y. B. Ma* and J. K. Amamcharla, *Food Science Institute, Animal Sciences and Industry, Kansas State University, Manhattan, KS.*

The casein content in raw milk is important for the industry as it influences the cheese yield. The casein content is determined by the difference between true protein and non-casein protein in raw milk. The objective of this study was to develop a rapid quantification method for casein in raw milk using front-face fluorescence spectroscopy (FFFS). To prepare milk samples for calibration, raw skim milk was obtained from Kansas State University's dairy farm and ultrafiltered to increase the protein concentration. The casein content of retentate and permeate were measured by a reference method. The retentate and permeate were combined at different ratios to make 10 calibration samples with casein content ranging from 0.37 to 3.7%. Sample preparation for the FFFS involved thoroughly mixing 7 mL calibration sample with 0.6 mL acetic acid (10% wt/wt) to precipitate the casein. Sample mixture was vortexed and transferred immediately to a quartz cuvette. Tryptophan emission spectrum of the mixture was immediately measured by a spectrofluorometer with a 1% attenuator (excitation wavelength at 280 nm; emission wavelength range from 300 to 440 nm) at 25°C. The process was repeated twice to obtain a sample size of 20 for the calibration model. Prediction models were developed using principal component regression and partial least square regression (PLSR) and validated with the leave-one-out cross-validation (LOOCV). The principal component regression and PLSR models showed LOOCV correlation coefficients of 0.970 and 0.988, root mean square error (RMSE) of 0.39% and 0.24%, and ratio of prediction to deviation of 4.5 and 4.7, respectively. The developed models were independently validated by 5 raw milk samples collected on different days. Principal component regression and PLSR predictions had a mean difference of 0.12% and 0.11% casein compared with the reference method and RMSE of 0.19% and 0.19%, respectively. The mean bias of 2 prediction models is not significantly different from 0 ($P > 0.05$). The FFFS method showed potential quantification of casein in raw milk, but validation on a large sample set is further required.

Key Words: partial least square regression (PLSR), principal component regression, front-face fluorescence spectroscopy (FFFS)

M2 Hunter versus CIE color measurement systems for analysis of milk-based beverages. N. Cheng*[1], D. Barbano[2], and M. A. Drake[1], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Both Hunter (L, a, b) and International Commission on Illumination (CIE; L*, a*, b*) color measurement systems are used for instrumental measurement of food color but which system is best for fluid milk is not known. The objective of our work was to determine the differences in sensitivity of Hunter and CIE methods at 2 different viewer angles for measurement for whiteness, red/green, and blue/yellow color of milk based beverages. Sixty combinations of milk-based beverages were formulated (2 replicates) with a range of fat level from 0.2 to 2%, true protein level from 3 to 5%, and casein as a percent of true protein from 5 to 80% to provide a wide range of milk-based beverage color. In addition, commercial skim, 1% and 2% fat HTST pasteurized fluid milks were analyzed. All beverage formulations were HTST pasteurized and cooled to 4°C before analysis. Measurement viewer angle (2 versus 10°) had very little impact on objective color measures of milk-based beverages with a wide range of composition for either the Hunter or CIE color measurement system. Temperature (4, 20, and 50°C) of color measurement had a large impact ($P < 0.05$) on the results of color measurement in both the Hunter and CIE measurement systems. The effect of milk beverage temperature on color measurement results was the largest for skim milk and the least for 2% fat milk ($P < 0.05$). This highlights the need for proper control of beverage serving temperature for sensory panel analysis of milk-based beverages with very low fat content and for control of milk temperature when doing objective color analysis for quality control in manufacture of milk-based beverages. The Hunter system of color measurement was more sensitive to differences in whiteness among milk based beverages than the CIE system ($P < 0.05$), while the CIE system was much more sensitive to differences in yellowness among milk based beverages ($P < 0.05$). There was little difference between the Hunter and CIE system in sensitivity to green/red color of milk based beverages. In defining milk based beverage product specifications for objective color measures for dairy product manufacturers, the viewer angle, color measurement system (CIE versus Hunter) and sample measurement temperature should be specified along with type of illuminant.

Key Words: color, milk, whiteness

M3 Optimizing the emulsification properties of heated whey protein isolate (WPI)-pectin complexes for emulsions containing 20% oil at pH 5.0. A. Kotchabhakdi* and B. Vardhanabhuti, *University of Missouri, Columbia, MO.*

There has been increasing interest in developing food ingredients for clean-label applications. We have previously shown that heated whey protein and pectin complexes (HCPX) formed at pH above pI have improved emulsification properties and stability when emulsions contained 5% oil. However, it is not fully understood whether these HCPX could stabilize emulsions containing higher oil content as in sauces and salad dressings. The objective of this study was to optimize the emulsification properties of HCPX in emulsions containing 20% oil at pH 5.0. The HCPX were formed by heating mixed 3 wt% whey protein isolate (WPI) and pectin (0, 0.3, 0.45 wt%) at pH 5.5, 5.8, and 6.2 at 85°C for 15 min. Emulsions were made, followed by pH adjustment to 5.0. Final emulsions contained 20 wt% oil, 2 wt% protein and 0 to 0.3 wt% pectin. Emulsification properties were assessed by measuring droplet size, ζ-potential, rheological properties and creaming stability. Emulsions stabilized by heated WPI without pectin had the average droplets sizes >36 μm and ζ-potentials ranging from −27.2 to −19.8 mV. They were not stable and separated into 2 layers within a few hours. The HCPX-stabilized emulsions showed significant improvement in emulsification properties and stability. Mean droplet sizes significantly decreased ($P < 0.05$) and ranged from 1.6 to 21 μm while droplets became more negatively charged with ζ-potential ranging from −37 to −40.9 mV. Both heating pH and pectin concentration during HCPX formation played important roles on the emulsification properties of the HCPX. The most stable emulsions (>30 d) were those stabilized by HCPX formed with 0.45% pectin at heating pH of 5.5 and 5.8. Formation pH also influenced the rheological behavior of the emulsions with those stabilized by HCPX formed at pH 6.2 being more viscous.
These results indicate that emulsification properties of heated WPI and pectin complexes formed at pH > pI can be optimized to stabilize

emulsions containing higher oil content. They can be utilized as clean-label ingredients in applications such as sauces and dressings.

M4 Evaluation of the drying kinetics of micellar casein concentrate and reduced-mineral micellar casein concentrate at different solids concentrations. H. N. Vora* and L. E. Metzger, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Micellar casein concentrate (MCC) was prepared by microfiltration and diafiltration of skim milk to produce a retentate with approximately 22% total solids (95% casein as a percentage of true protein). Modified (reduced mineral) micellar casein concentrate (MMCC) was prepared by diluting the MCC retentate to 7% solids and injecting CO_2 to pH 5.9 and ultrafiltered to produce a retentate with approx. 22% total solids. Three sets of trials were performed on separate skim milk lots for both MCC and MMCC. The drying kinetics of the MCC and MMCC from each trial were then studied using single droplet drying (SDD). The SDD approach involves a single droplet suspended on the tip of a glass filament, where changes in droplet diameter, mass, and temperature can be measured during drying. The aim of this study was to develop and compare a predictive model generated using SDD for MCC and MMCC which can be used as a tool to optimize the drying conditions and reduce costly plant trials when developing new ingredients with unique functional properties. In this study, 2 ± 0.05 µL droplets of MCC and MMCC were dried using SDD at 2 different levels of total solids: 10% and 20% at 90°C with hot air at a velocity of 0.8 m/s. Droplet diameter and mass change data were collected and processed using Adobe After Effects 7.0 to enable the extraction of images. Although the pattern of change in average diameter data obtained from SDD was same for both MCC and MMCC, there was a significant difference observed during the average diameter change between MCC and MMCC ($P < 0.05$) at both the solids level MCC showed a rapid change in average diameter compared with MMCC. The curves of average mass change obtained from SDD were plotted against time. It was observed that as the total solids level increases the drying time increases, which is mainly due to the formation of crust on the particle and subsequent slower moisture migration to the surface of the particle with higher total solids level in both MCC and MMCC.

Key Words: single droplet drying, micellar casein concentrate, reduced-mineral micellar casein concentrate

M5 Whey proteins enhance color and stability of anthocyanin pigments. G. Miyagusuku-Cruzado*, R. Jimenez-Flores, and M. M. Giusti, *The Ohio State University, Columbus, OH.*

Current trends show that the food industry is moving away from synthetic colorants and looking for natural alternatives. Anthocyanins (ACN) are plant flavonoids with vivid colors that range from red to blue, but their application as food colorants is restricted by their limited stability, particularly at pH close to neutral. Nevertheless, some studies have shown that ACN have higher stability in dairy systems than in buffers at the same pH. We hypothesized that milk components such as proteins can interact with ACN, and that certain ACN may have a higher affinity for proteins due to specific structural conformations. Our objective was to evaluate whether whey proteins can interact with ACN leading to enhanced color and stability. Model solutions were prepared by diluting ACN from different sources with pH 3 citric acid – Na_2HPO_4 buffer until a $\lambda_{vis\text{-}max}$ absorbance of 0.7 was reached, followed by addition of whey protein isolate (WPI) at different concentrations (0, 0.01, 0.05, 0.1, 0.5 and 1.0 mg/mL model solution). Absorption spectra was measured after 15 min and color parameters (CIELab) were calculated using ColorBySpectra software. Model solutions were heated to 90°C for up to 500 min to test heat stability, with samples taken every 50 min. ACN content was measured using the pH differential method. Addition of WPI resulted in a significant absorption increase ($P < 0.05$) at the $\lambda_{vis\text{-}max}$ up to 17%. Color of the model solutions was enhanced ($\Delta E > 5$), becoming noticeably darker with WPI concentrations as low as 0.05 mg/mL for *Berberis boliviana*, 0.5 mg/mL for purple corn and grape skin, and 1.00 mg/mL for black carrot and red cabbage. The absorption increase at the $\lambda_{vis\text{-}max}$ was dependent on WPI concentration, some fitting a linear model while others an exponential one, suggesting that some ACN may have higher affinity for WPI than others. Also, WPI addition significantly increased thermal resistance of ACN ($P < 0.05$). Further studies will focus on different anthocyanin chemical structures and the possible utilization of acid whey to stabilize ACN pigments. This could facilitate the transition from synthetic colorants to natural and healthier alternatives.

Key Words: whey protein isolate (WPI), acid whey, natural colorant

M6 Production and storage stability of liquid micellar casein concentrate. A. R. A. Hammam* and L. E. Metzger, *South Dakota State University, Brookings, SD.*

Micellar casein is a high protein ingredient that can be used as a valuable source of intact casein in process cheese formulations. The objective of this study was to produce a highly concentrated micellar casein (HC-MC) and evaluate its storage stability. Skim milk was pasteurized at 72°C for 16 s and kept at ≤ 4°C until the following day when it was heated in a plate heat exchanger to 50°C and microfiltered with a ceramic GP MF system (0.1µm) in a feed and bleed mode to produce a 3 × MF retentate (1 kg of retentate:2 kg of permeate). Subsequently, the retentate was diluted 2× with soft-water (2 kg of water:1 kg of retentate) and again microfiltered at 50°C to a 3× concentration as described previously. The retentate was then cooled to 4°C, and stored overnight. The following day, the retentate was heated to 65°C and microfiltered in a recirculation-mode until the total solid reached approximately 22%. Subsequently, the temperature was increased to 74°C and microfiltration was continued until the permeate flow rate reached less than 5 L/h. The HC-MC retentate was transferred at 74°C to sterilized vials and stored at 4°C. This trial was repeated 3 times using 3 separate batches of raw milk. During microfiltration, the mean cumulative SP removal in the first, second, and third stages was 46, 77, and 83%, respectively. The mean HC-MC at time zero contained 25.42% total solids (TS), 20.20% true protein (TP), 0.09% NPN, 0.55% NCN, 19.80% CN, 2.0% ash, 97.70% CN%TP, and 0.45% SP. The NCN content increased significantly ($P < 0.05$) from 0.55 to 0.76% during 2 mo of storage. The NPN also increased over time from 0.095% at time zero to 0.12% after 2 mo of storage. The mean aerobic bacterial count in HC-MC at time zero was 2.6 ± 0.16 log cfu/mL and increased to 3.5 ± 0.89 and 4.3 ± 0.97 log cfu/mL after 1 and 2 mo of storage, respectively. Coliform, yeasts, and mold were not detected at any time point. This study determined that HC-MC could be manufactured using ceramic MF membranes with over 25% TS and greater than 95% CN%TP. The impact of the small increase in NCN and NPN during 2 mo of storage on process cheese characteristics will be evaluated in subsequent studies.

Key Words: microfiltration, micellar casein, shelf life

M7 Use of micro- and nano-bubbles for improving the functional properties of Greek-style yogurt. K. S. Babu*, D. Z. Liu, and J. K. Amamcharla, *Kansas State University, Manhattan, KS.*

Increased awareness of health benefits has driven the popularity of Greek-style yogurt (GSY) in recent years. However, increased protein content in the GSY leads to increased graininess, higher viscosity, and chalky mouthfeel. The objective of this study was to investigate the efficiency of micro- and nano-bubbles for improving the physical, rheological, and functional properties of GSY. In this study, a custom-built system was used to incorporate micro- and nano-bubbles (MNB) in GSY. The base for GSY was formulated to a protein content of 10% (wt/wt) using nonfat dry milk, micellar casein concentrates, and water. Control GSY (C-GSY; GSY pumped through the positive displacement pump without attaching the MNB generator) and MNB-treated GSY (MNB-GSY) were compared and evaluated for physical, rheological characteristics such as apparent viscosity, % loss-of-structure (measure of the rate of thixotropic breakdown), syneresis, water-holding capacity (WHC) before and after storage at 5°C for 1, 2, 3, and 4 weeks. Two replicates of C-GSY and MNB-GSY were manufactured and the data were analyzed as repeated measures (SAS Institute Inc.). The density of freshly prepared MNB-GSY and C-GSY was 0.97 and 1.04 g/cm^3, respectively. When compared with C-GSY, the syneresis, WHC, and grain counts were significantly different ($P < 0.05$) after the MNB treatment and subsequent storage time. After the wk 2, 3, and 4, the MNB-GSY samples showed ~58.4%, ~43.1%, and ~50.1% lesser apparent viscosity compared with the corresponding weeks C-GSY samples. The syneresis of the MNB-GSY was significantly lower ($P < 0.05$), ~19% than C-GSY after storage for 4 wk. After storage for 4 wk, the % loss-of-structure for the C-GSY and MNB-GSY was 32% and 20.1%, respectively. Before storage, the grain counts of C-GSY and MNB-GSY were ~143 and ~37 grain counts/g of yogurt, respectively. After storage for 4 wk, the grain counts of C-GSY and MNB-GSY were ~178 and ~4 grain counts/g of yogurt, respectively. Overall, the incorporation of MNB into GSY showed significant improvements in the rheological and functional properties of GSY.

Key Words: micellar casein concentrate, rheology, micro- and nano-bubbles

M8 Ratiometric fluorescence spectroscopy—A novel technique for rapid detection of bacterial endospores. N. Awasti* and S. Anand, *Midwest Dairy Food Research Center, Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

The current spore detection methods rely on cultural techniques, having limitations of time, efficiency, and sensitivity. Spore coat contains calcium dipicolinic acid (CaDPA) as a major constituent, which can serve as a biomarker for bacterial endospores. We report a rapid and sensitive technique for detection of bacterial endospores by using ratiometric fluorescence-based sensors. This method is based on the detection of CaDPA that enhances luminescence of lanthanide ion, when complexed with a semiconducting polymer. A CaDPA standard curve was generated at excitation-emission wavelength of λ_{284}-λ_{528} by using Synergy 2 fluorescence spectrophotometer. Intensity was recorded after chelating semiconducting fluorescent polyfluorene (PFO) dots with terbium ions, sensitized by different volumes of CaDPA (0.1 μ*M*). All trials were conducted in the replicates of 3 and mean ± SE were calculated. The standard curve so generated showed a linear relationship ($R^2 = 0.98$) in experimental concentration range of 2.5 to 25 n*M* of CaDPA, with corresponding intensity (a.u.) of 545 to 2130. Endospores of an aerobic spore former, *Bacillus licheniformis* ATCC 14580, were produced at 37°C for 15 d, on Brain Heart Infusion agar. The efficiency of sporulation was evaluated by spore staining and plating techniques. Total CaDPA content in spores was estimated after suspending reducing concentrations of spores (logs 9.0 through 1.0 cfu/mL, at 1-log intervals) in HPLC-grade water. For higher spore spiking levels such as 9.2 ± 0.03, 8.4 ± 0.05, 7.1 ± 0.13 and 6.3 ± 0.02 logs, the mean CaDPA content values, observed from the standard curve, were 9.4, 7.2, 6.2 and 5.3 n*M*, whereas, for lower levels of 4.2 ± 0.05, 3.1 ± 0.04, 2.0 ± 0.11, and 1.36 ± 0.09 logs, we observed 3.8, 3.3, 2.2 and 1.3 n*M* mean CaDPA content. Our results indicated a linear relationship of the CaDPA content of endospores with that of the endospore counts, and the standard curve of CaDPA concentration. This study provides a proof of concept for a potential application of this technique to rapidly detect bacterial endospores in dairy and food industry. Further studies are in progress in our laboratory to standardize this technique for dairy product matrices such as cheese, whey proteins, and powders.

Key Words: spore, fluorescence, *Bacillus*

M9 Response of Holstein dairy cattle to a sodium propionate supplement fed postpartum. M. Wukadinovich* and H. A. Rossow, *University of California, Davis, Davis, CA.*

Subclinical (SCK) and clinical (CK) ketosis is a metabolic disease common in dairy cattle and can decrease milk production, reproductive efficiency, and increase risk of being culled from the herd. Traditionally, cows have been supplemented with glucogenic precursors either by drenching or inclusion in the TMR to decrease ketone formation and increase blood glucose levels. The objective of this experiment was to examine the incidence of SCK and CK, levels of ketones and glucose in blood, and milk yield in Holstein dairy cattle fed a molasses-based sodium propionate supplement (Innovative Liquids LLC) for the first 14 DIM. On a commercial dairy in California, a total of 226 cows and 102 heifers were systematically enrolled in C with a subset of 74 cows and 39 heifers bled, and 200 cows and 106 heifers were enrolled in GP with a subset of 81cows and 36 heifers bled in a switchback design. Blood glucose and β-hydroxybutyric acid (BHB) concentrations were measured on 3, 7, and 14 DIM using NovaMax meters (Nova Diabetes Care Inc., Billerica, MA). Ketosis was defined as BHB levels of 1.0–1.4 mmol/L for SCK or BHB levels of > 1.4 mmol/L for CK. Glucose concentrations were defined as low (≤40 mg/dL) or adequate (>40 mg/dL). Data were analyzed using the Mixed Procedure of SAS (v. 9.4, SAS Institute, 2015). Results are presented as least squares means ± standard error. Average blood BHB and glucose concentrations did not differ between treatments for primiparous or multiparous cows (C 0.53 ± 0. 02, GP 0.55 ± 0.02 mmol/L BHB, $P = 0.5$; C 44 ± 0.77, GP 43 ± 0.78 mg/dL glucose, $P = 0.6$). Concentration of blood glucose was inversely related to BHB ($P < 0.01$). Treatment GP did not affect milk yield compared with C for primiparous or multiparous cows during the first 21 DIM (C 29.5 kg milk /d ± 0.94, GP 30.0 ± 0.96 kg milk/d; $P = 0.5$). The incidence of SCK and CK was low during this study; 9 C cows and 7 GP cows were subclinical, and 6 C cows and 9 GP cows were clinical. Therefore, in this study there was little improvement in the incidence of CK and SCK or increase in milk yield with supplementation.

Key Words: β-hydroxybutyric acid (BHB), subclinical ketosis, clinical ketosis

M10 Effects of timing of local anesthesia on cortisol and adrenocorticotropic hormone levels in calves after dehorning. A. J. Mathias*[1], C. C. Williams[1], C. Scully[2], and S. J. Blair[1], [1]*Louisiana State University AgCenter, Baton Rouge, LA,* [2]*Louisiana State University School of Veterinary Medicine, Baton Rouge, LA.*

Dehorning is a painful animal management procedure that is commonly performed in dairy calves. The use of local anesthesia lessens the physiological and behavioral effects of dehorning in calves. Twenty-four intact Holstein heifer calves (6 to 8 wk of age) were assigned to 1 of 4 treatments (n = 6 calves/treatment) to evaluate effects of timing of local anesthesia on physiological indicators of stress associated with pain of dehorning. Treatments included anesthesia without dehorning (CON); dehorning without anesthesia (NO_ANET); anesthesia followed by immediate dehorning (ANET_0); and anesthesia with a 10-min delay before dehorning (ANET_10). Approximately 2 h before dehorning, jugular catheters (14 gauge, 3.5 inch; MILA International, Inc., Erlanger, KY) were inserted. Blood samples were collected 10 min before and immediately before (0-min sample) the initiation of the experiment. For the groups that received local anesthesia, a cornual nerve block was performed with 5 mL of 2% lidocaine hydrochloride on both horns. In NO_ANET, calves received 5 mL of 0.9% saline in place of lidocaine. Blood samples were collected at 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 after dehorning. Plasma concentrations were measured by radioimmunoassay for cortisol at every time point and adrenocorticotropic hormone (ACTH) through 30 min. At 25 min after dehorning, cortisol concentrations were lower ($P < 0.05$) in CON than ANET_0, whereas ANET_10 and NO_ANET were not significantly different from either CON or ANET_0. At 1 and 2 min post-dehorning, ACTH concentrations were lower ($P < 0.05$) in ANET_10 and CON than ANET_0 and NO_ANET. At 3, 4, 5, and 10 min post-dehorning, ACTH concentrations were lower in CON than ANET_0 and NO_ANET, and ANET_10 did not differ significantly from any treatment groups. Because the observed differences in plasma ACTH concentrations dissipated within 5 min and plasma cortisol concentrations returned to pretreatment levels within 1 h of dehorning for all calves, it is inconclusive as to whether or not there is a benefit to waiting 10 min after the administration of lidocaine to dehorn calves.

Key Words: dehorning, calves, cortisol

M11 Feeding a low-starch fresh cow diet may increase NDF digestibility. C. E. Knoblock*[1], W. Shi[1], I. Yoon[2], and M. Oba[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Diamond V, Cedar Rapids, IA.*

The objective of this study was to determine the total-tract nutrient digestibility of Holstein dairy cows (n = 38) supplemented with or without a *Saccharomyces cerevisiae* fermentation product (SCFP, NutriTek, Diamond V, Cedar Rapids, IA) during the periparturient period. All animals were fed a common basal close-up diet containing 13% starch, and fed high- or low-starch diets (HS vs. LS; 27 and 21%, respectively) immediately after calving. Animals were assigned to 1 of 4 treatments (CON+HS, CON+LS, SCFP+HS, SCFP+LS) in a randomized block design. Apparent total-tract nutrient digestibility was determined at wk 1 and 3 after calving using indigestible neutral detergent fiber (NDF), determined after 288 h in situ digestion, as an internal marker. There was no treatment effect of SCFP supplementation on the total-tract nutrient digestibility. Although dry matter or organic matter digestibility was not affected by dietary starch content, cows fed the LS diets increased NDF digestibility compared with those fed the HS diets on wk 1 (40.7 vs. 35.3%; $P = 0.01$), and multiparous cows on the LS treatments tended to increase NDF digestibility on wk 3 (40.6 vs. 35.8%; $P = 0.08$). For wk 1, there were no correlations ($P > 0.05$) between NDF digestibility and dry matter intake or any rumen fermentation variables measured in the current study, including mean pH, minimum pH, the duration under pH 5.8, and volatile fatty acid profile. However, NDF digestibility for wk 3 was positively correlated with dry matter intake (r = 0.40; $P = 0.01$) and molar proportion of acetate in rumen fluid (r = 0.49; $P = 0.04$). Feeding the low starch diets increased total-tract starch digestibility of multiparous cows on wk 1 (98.7 vs. 97.8%; $P = 0.01$) and wk 3 (98.7 vs. 97.7%; $P < 0.01$), but decreased starch digestibility of primiparous cows on wk 1 (98.6 vs. 99.1%; $P = 0.04$) and wk 3 (98.7 vs. 99.3%; $P = 0.04$). These data suggest that feeding fresh cows a low-starch diet may

increase total-tract NDF digestibility, but that its effects on total-tract starch digestibility are not consistent.

Key Words: *Saccharomyces cerevisiae* fermentation product, NDF digestibility, rumen pH

M12 Determining immune-modulating components of *Saccharomyces cerevisiae* with RAW 264.7 murine macrophages. S. E. Sivinski*, R. A. Rusk, J. L. McGill, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

Feed components can modulate the immune system, but in vivo data are expensive and rare; thus, an effective screening tool for evaluating such nutrients is needed to enable informed selection of candidate immunomodulators for in vivo investigation. This study used RAW 264.7 murine macrophages as an innate immunity in vitro screening tool to identify immune-modulating properties of *Saccharomyces cerevisiae* and some of its components. Treatments were 0.01, 0.1, or 1 mg/mL of whole *S. cerevisiae* cells (WC), mannan, Zymosan (includes cell wall protein-carbohydrate complexes, mannans, and β-glucans), or D-mannose at either pH 3 or 7. A pH of 3 was used to mimic the acidic conditions of the stomach to assess potential alterations of component activity. The cells were transfected with a vector that drove expression of an alkaline phosphatase reporter gene upon activation of NFκB. Cells (n = 6 wells/treatment) were incubated with treatments for 18 h. Cell supernatants were then incubated for 2 h with alkaline phosphatase (AP) substrate media (QUANTI-Blue) to assay enzyme activity. After normalizing values across plates using unstimulated cells and LPS (1 ng/mL) stimulated cells as negative and positive controls, intra- and inter-assay CVs were 3.9% and 15.8%, respectively. The effects of treatment, pH, and dose(treatment) were evaluated by mixed models. Treatment and dose(treatment) affected AP activity (both $P < 0.001$) whereas pH did not have an effect ($P = 0.31$). WC at 1 mg/mL and mannan at 0.1 mg/mL tended to increase AP activity (both $P = 0.07$), whereas WC at 0.1 mg/mL, mannan and D-mannose at 1 mg/mL, and Zymosan at all 3 concentrations increased AP activity (all $P < 0.001$), compared with the unstimulated cell control. Zymosan stimulated a greater immune response than all other components (all $P < 0.001$), and WC stimulated a greater response than D-mannose ($P = 0.03$). Overall, this innate immunity in vitro screening method was useful for quickly determining immune-modulating properties and concentrations of *S. cerevisiae* components.

Key Words: bioactive nutrient, nutritional immunology, in vitro screening method

M13 Evaluating forage and feed cost per hundredweight of milk sold on 4 dairy farms double cropping winter annuals with corn silage in northern and western Pennsylvania. E. J. Ranck* and L. L. Holden, *Pennsylvania State University, State College, PA.*

Double cropping winter annuals with corn silage can reduce the nutrients that enter watersheds while providing farmers with extra forage to improve profitability. This study evaluated the effect of double cropping on milk yield and feed and forage costs in areas where double cropping was not common. Four case study farms were chosen from northern and western Pennsylvania, based on willingness to provide data and experience using double cropping. Each farm provided yearly summaries of crop, animal, and financial data for 2016 and 2017. Farmers self-reported crop yields by total crop harvested. Milk yield and milk income were calculated using year-end milk statements. Feed costs were calculated using feed purchased and market prices for crops produced in 2016. For 2017, animal rations and market prices were used to calculate cost of feed. Farms ranged from 54 to 663 (±260.1) cows and 26 to 552 (±221.9) ha. Percentage of hectares double cropped ranged from 8.6 to 23.8 (±7.00) and total hectares double cropped ranged from 6.3 to 58.7 (±21.42). Farm data were analyzed using a one-way ANOVA to determine difference by farm with least significant difference for mean separation. Milk yield and milk component yield averaged 33.9 (±3.32) and 2.29 (±0.213) kg/d, respectfully, and differed ($P < 0.05$) by farm. Forage cost per hundredweight of milk averaged $5.64 (±1.248) and differed ($P < 0.05$) by farm, whereas feed cost per hundredweight of milk averaged $12.05 (±1.958), but did not differ by farm. Lower forage costs were associated with the farm with the highest percentage of land double cropped but did not result in improved milk yield or components per cow, indicating that other management factors and not the use of double cropping had greater effects on milk yield.

Key Words: double cropping, winter annuals, feed cost

M14 Effects of different organic pastures on dairy heifer growth. J. A. Hadfield*[1], M. Rose[2], R. Stott[1], B. L. Waldron[2], A. Young[1], S. C. Isom[1], K. A. Rood[1], and K. J. Thornton[1], [1]*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT,* [2]*Forage and Range Research, USDA–Agricultural Research Service, Logan, UT.*

Dairy heifers developed in certified organic programs, especially those on primarily pasture-based management schemes, have a lower rate of gain than heifers raised in conventional production systems. Slower growing heifers have a delayed onset of puberty, which can result in economic inefficiency for producers. Furthermore, it is suggested that organically raised heifers are at an increased risk for internal parasitism because some commercial anthelmintics are limited by rule. This study investigates the effects that different forages in a rotational grazing system have on growth and performance of organically raised dairy heifers. We hypothesized that different pastures will affect growth and reproductive efficiency of developing heifers. To test this hypothesis, a trial was conducted over a period of 2 yr (year 1: n = 48, year 2: n = 81). A conventional dry lot control and 8 different pasture treatments were utilized. Treatments included tall fescue (TF), meadow brome grass (MB), orchard grass (OG), perennial rye (PR) and each of these grasses interseeded with the legume birdsfoot trefoil (BFT). Yearling Jersey heifers (n = 129) were randomly assigned to 1 of 8 different pasture treatments (n = 15) or a dry lot control (n = 9). Every 35 d, during a 105-d period, heifers were weighed, measured for hip height, and blood and fecal samples collected to determine serum insulin-like growth factor-1 (IGF-1) and blood urea nitrogen (BUN) concentrations, along with fecal egg per gram counts. At the end of the 105-d period, heifers were bred and checked 30 d post-breeding to determine conception rate. Repeated-measures analyses were performed using PROC MIXED in SAS (version 9.4) to determine how treatment affected each measurement over the total 105-d period. Pasture altered weight gain ($P = 0.01$) and serum BUN concentrations ($P < 0.01$) over the 105-d period. Specifically, animals that consumed BFT had increased ($P < 0.01$) weight gain and serum BUN concentrations compared with animals that consumed a grass monoculture pasture. However, no differences ($P > 0.05$) between treatments relative to hip height, serum IGF-1 concentration, fecal egg counts, or conception rate were observed over the 105-d period. These data provides insight into how different forages in organic pastures may affect growth and productivity of dairy heifers.

Key Words: organic, grazing

M15 Automatic feed push-up frequency effects on dairy cattle behavior and milk production. M. D. Adamczyk[1], L. N. Grinter*[1], A. R. Lee[2], J. M. Bewley[3], and J. H. C. Costa[1], [1]*Dairy Science Program, Dept. of Animal and Food Sciences, University of Kentucky, Lexington, KY,* [2]*Department of Animal Science, University of Tennessee Knoxville, Knoxville, TN,* [3]*CowFocused Housing, Bardstown, KY.*

Feed push-up improves the feed availability for dairy cows between feed deliveries and may increase intake and milk production. The study objective was to compare the effects of different feed push-up frequency of an automatic feed push-up system (Juno, Lely, Maasluis, Netherlands) on dairy cow behavior and milk production. Forty-eight cows (mean ± SD; 173 ± 101 DIM; 73.6 ± 23.3 lbs/d; 1.3 ± 0.5 parity) were divided into 2 groups, balanced by parity and DIM. A crossover design compared 2 treatments from April to June 2017: 2 feed push-ups/d (2FPU; 1000 and 1950 h) and 12 feed push-ups/d (12FPU; 0000, 0200, 0345, 0500, 0820, 1000, 1200, 1400, 1610, 1950, 2100, and 2200 h), for 3 wk/treatment, separated by 1 wk washout. Rumination time and eating time were measured by a behavior monitoring eartag; SmartBow (SmartBow GmbH, Jutogasse, Austria) and SensOor (Agis Harmelen, the Netherlands) respectively. Rest time was recorded by a behavior monitoring leg tag (Afi Act II, Afimilk, Afikim, Israel). An in-line milk assessment tool (AfiLab, Afimilk, Afikim, Israel) measured milk yield, milk fat percentage, and protein percentage. A mixed linear model (SAS 9.4) was used to determine the relationship of feed push-up frequency with behavior and milk production variables. Date was specified as a repeated measure, and cow as subject, using a compound-symmetry structure. The model contained the fixed effects of DIM, parity (1 or 2), treatment, sequence of treatment, THI, and all 2-way interactions (fixed effects remained regardless of significance; manual backward elimination removed non-significant 2-way interactions). Daily resting time was greater for 2FPU than 12FPU (640 ± 12 vs 615 ± 12 min/d; $P < 0.001$) and rumination tended to increase for 2FPU (366 ± 9 vs 359 ± 9 min/d; $P = 0.06$). Daily eating time and energy corrected milk were not different between treatments ($P > 0.10$). Feed push-up frequency influenced cattle behavior; however did not have a significant effect on feeding or production. Further research with feed push up frequency should consider intermediary frequencies and other variables as intake rate and feeding bouts.

Key Words: feed availability, push-up frequency, precision dairy technology

M16 Effects of pasture quality on feed efficiency and production of organic dairy cows in the southeastern United States. H. R. Bailey*[1], D. M. Butler[1], G. M. Bates[1], G. M. Pighetti[1], P. D. Krawczel[1], S. R. Smith[2], K. Mercier[2], D. W. McIntosh[1], and A. G. Rius[1], [1]*Department of Animal Science, University of Tennessee, Knoxville, TN,* [2]*College of Agriculture, Food and Environment, University of Kentucky, Lexington, KY.*

Empirical data on forage mixtures maximizing quality and productivity is lacking for the Southeast US. Our objective was to (1) compare production of 4 forage mixtures in the spring grazing season, and (2) evaluate forage quality on predicted milk production and feed efficiency using the Integrated Farm System Model. Mixtures contained a combination of cool and warm season legumes and/or grasses and/or brassicas with differences in legume species: (A) red clover, (B) crimson clover and cowpea, (C) alfalfa and red clover, and (D) cowpea. Mixtures were planted in 0.5-acre plots at 5 organic farms located in Kentucky and Tennessee. It was hypothesized that increased mixture diversity would increase pasture performance. Grab samples were collected from April to June 2017 to determine crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) content. Data from NIRS analysis were analyzed in SAS 9.4 using the GLIMMIX procedure. Samples ranged from 16 to 21% CP throughout the season, with mixture C having the greatest average concentration of CP at 20.4% ($P = 0.03$). Forage ADF ranged from 25 to 37% and NDF ranged from 45 to 51% with a lower NDF values in mixture C later in the grazing season ($P = 0.09$). Mean forage quality data were entered in the model and simulations mimicked 3 farm systems: (1) Holsteins on pasture for 12 h/d (H12), (2) small Holsteins on pasture for 24 h/d (SH24), and (3) Jerseys on pasture for 24 h/d (J24) during the grazing season. Simulations over 4 yr were conducted to determine predicted grazing efficiency (pasture DMI kg/ milk production kg) and feed efficiency (total DMI kg/milk yield kg) on forage mixtures to achieve target milk yields (Table 1). Mixture C may help increase forage quality and productivity on organic dairies. Results predicted that use of alfalfa and red clover may help enhance forage quality and increase efficiency of grazing cows.

Table 1 (Abstr. M16). Grazing efficiency (DMI kg/ milk production kg) and feed efficiency (total DMI kg/milk yield kg) per cow

	Grazing Efficiency			Feed Efficiency		
Mixture	H12	SH24	J24	H12	SH24	J24
A	5.8	24.6	15.7	29.1	52.0	29.4
B	5.9	26.5	15.5	29.1	54.2	29.2
C	6.3	28.9	16.5	29.5	55.1	29.6
D	5.9	24.3	15.5	29.1	51.9	29.2

Key Words: organic, pasture, dairy cow

M17 Effect of extended colostrum feeding on plasma glucagon-like peptide 1 concentration in newborn calves. Y. Inabu*[1], J. Pyo[2], S. Pletts[2], M. Steele[2], and T. Sugino[1], [1]*The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan,* [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Glucagon-like peptide 1 (GLP-1) plays a role in the regulation of appetite and glucose homeostasis via the stimulation of insulin secretion. The objective of this study was to evaluate the effect of extended colostrum feeding on plasma concentrations of GLP-1. Holstein bull calves (n = 18) were fed pooled colostrum at 7.5% of BW at 2 h after birth, then fed mature milk (M), mixture at a ratio of 50:50 for pooled colostrum and milk (CM), or pooled colostrum (C; n = 6 for each treatment) at 5% of BW at 12 h after birth, and every 12 h thereafter until 72 h after birth. Blood samples were obtained before (1 and 2 h after birth) and after (until 75 h after birth) the first colostrum feeding, and plasma concentrations of GLP-1, insulin and glucose were measured. Data were analyzed by ANOVA of JMP 13 with treatment, time and treatment by time interaction as fixed effects. Treatment by time interaction was observed for plasma insulin and glucose concentrations ($P < 0.01$), which was mainly the result of lower concentrations from 14 to 27 h after birth (from 1 to 2 d after birth) for CM or C than for M. Conversely, on 3 d after birth, difference between treatments was not observed for insulin and glucose. Plasma GLP-1 concentration tended to be higher ($P = 0.05$) for C (2.26 ± 0.24 ng/mL; LSM ± SEM) compared with M (1.37 ± 0.24 ng/mL). A treatment by time interaction was observed for GLP-1 ($P < 0.01$), but unlike glucose and insulin, this was mainly the result of higher concentrations from 54 to 70 h after birth (3 d after birth) for C than for CM or M. Plasma concentration of glucose was not correlated with that of GLP-1 but positively correlated with that of insulin on d 1 (r = 0.48, $P < 0.01$) and d 2 (r = 0.49, $P < 0.01$) after birth. On 3 d after birth, plasma concentration of insulin was not correlated with that of glucose but positively correlated with that of GLP-1 (r = 0.34, $P < 0.01$). In conclusion, these results indicate that extended colostrum feeding may increase plasma GLP-1 concentrations, especially on 3 d after birth, but further study is necessary to determine the effect on plasma insulin and glucose concentrations.

Key Words: glucagon-like peptide 1 (GLP-1), colostrum, calf

M18 Fetuin-A modulates lipid mobilization in bovine adipose tissue by enhancing lipogenic activity of adipocytes. C. Strieder-Barboza* and G. A. Contreras, *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Fetuin-A (FetA) is an adipokine and free fatty acids (FFA) transporter linked to adipose tissue (AT) function in transition cows. Plasma and AT FetA decrease after parturition coinciding with reduced lipogenesis and increased lipolysis. In monogastrics, FetA enhances lipogenesis, but its role on lipid mobilization of ruminants is unclear. Our objective was to determine the effects of FetA on lipogenesis and lipolysis in bovine adipocytes. Preadipocytes from tailhead subcutaneous AT of dairy cows (n = 6) were induced to differentiate in a coculture system and used in the experiments. Lipolytic responses of adipocytes were evaluated after a 2-h β-adrenergic stimulation with 1 μ*M* isoproterenol (ISO) alone or combined with 0.1 mg/mL of FetA (FETA+ISO). Medium alone (CON) or mixed with 0.1 mg/mL of FetA (FETA) served as controls. Lipogenic responses were assessed in adipocytes treated with CON or FETA for 48 h by quantification of FFA uptake (kinetic assay for 1h) and triacylglycerol (TG) accumulation (Adipored) and gene (qPCR) and protein expression (Western blot) of lipogenic markers. Adrenergic stimulation with ISO increased lipolysis compared with CON, as reflected in the release of glycerol (12 ± 0.04 vs 0.04 ± 0.02 n*M*/cell, $P = 0.003$) and FFA (15 ± 13 vs 6.2 ± 2.4 n*M*/cell, $P = 0.04$). Lipolysis induced by ISO was attenuated by FetA (FETA+ISO) as reflected by a lower glycerol (0.06 ± 0.04 n*M*/cell, $P = 0.02$) and FFA (5.7 ± 2.7 n*M*/cell, $P = 0.01$) release. The treatment with FetA enhanced lipogenic responses compared with CON as demonstrated by a 1.5 times increment in FFA uptake ($P = 0.02$) and TG accumulation ($P = 0.05$); and the upregulation of 1-acylglycerol-3-phosphate acyltransferase (AGAPT2) gene expression ($P = 0.04$) and protein content ($P = 0.08$). In conclusion, FetA attenuates lipolytic response and enhances lipogenesis in bovine adipocytes. The upregulation of the rate-limiting lipogenic enzyme AGAPT2 by FetA suggests a potential pathway by which this adipokine promotes TG synthesis in adipocytes.

Key Words: adipokine, adipocyte, lipid mobilization

M19 Ruminal, diet, and environmental factors that affect dairy calf performance. C. A. Ceh*, R. R. White, and K. M. Daniels, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Although impacts of dietary and environmental factors on calf performance have been investigated, no studies have investigated how rumen development, independent of diet and environment, influences dairy calf performance. The objective was to summarize the literature on calf performance and derive equations that relate rumen (e.g., rumen pH, reticulo-rumen weight, papillae area) and non-rumen factors (e.g., feed composition, form of feed, housing) to animal performance [e.g., intake of milk replacer (MR), starter, and forage; average daily gain (ADG); and feed efficiency]. In total, 146 treatment means from 36 trials were obtained under the following selection criteria: study reported dairy calves only; calf between 0 to 24 wk of age; calves had to be fed MR for some part of the study; study reported one or more rumen variables; and ADG >0.2 kg/d. Forward selection, multiple regression was used to derive equations to estimate variables that influenced the response variable in each model; models were weighted by the inverse of the standard error of the mean. Models were evaluated based on root estimated variance and concordance correlation coefficients (CCC). A positive association was seen in ADG between final body weight, weaned calves, and total amount of starter intake, while negatively associated with calf age, Holstein breed calves, and initial body weight (CCC = 0.961). Feed to gain ratio was positively associated with the weight of the ruminal contents (CCC = 0.904). Daily forage intake was negatively associated with the percent of the diet that was starter or MR (CCC = 0.999). Daily starter intake was positively associated with higher acid detergent fiber in the starter, a pelleted starter, and diets including starter and forage (CCC = 0.986). Daily MR intake was negatively associated with the percentage of the diet that was starter and ruminal pH (CCC = 0.940). Although dietary and environmental factors are closely associated with calf performance, ruminal factors appear to have additional, additive influences on calf performance.

Key Words: rumen development, meta-analysis, calf

M20 Epigenetic regulation of nuclear factor erythroid 2 like 2 (NFE2L2) signaling pathway through methionine supply during the periparturient period in liver of dairy cows. F. Batistel*[1], S. Moeez[1], L. Han[1], C. Parys[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

Nuclear factor erythroid 2 like 2 (NFE2L2) is a transcription factor that regulates the expression of antioxidant proteins. We investigated the effect of increasing Met supply during the periparturient period on NFE2L2, its targets and the potential for epigenetics in its regulation. Multiparous cows were assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH) to ensure a ratio of Lys to Met in the metabolizable protein close to 2.8:1. Mepron (0.09–0.10% of the dry matter intake) was fed from −28 to 30 d relative to parturition. Liver was sampled from 8 cows/treatment at −10, +10, and +30 d relative to parturition. NFE2L2 and DNA methyltransferases (DNMT) were analyzed by protein blotting, while NFE2L2 targets and NFE2L2 DNA methylation were analyzed by RT-PCR. Glutathione, global DNA methylation, and histone H3 lysine 4 tri-methylation (H3K4me3) were analyzed by colorimetric commercial kits. Data were analyzed using a Mixed model considering block as random effect and treatment, time and its interaction as fixed effect. Compared with control, MET-fed cows had greater ($P = 0.04$) protein expression of phosphorylated NFE2L2 and tended to have greater ($P = 0.07$) total NFE2L2. These results agree with the lower ($P = 0.03$) DNA methylation of the NFE2L2 promoter, which indicates a higher rate of NFE2L2 transcription. Among the 7 NFE2L2 target genes analyzed, *TXNRD1*, *HMOX1, PIR*, and *NQO1* were upregulated ($P \leq 0.05$) by MET supply. Expression of *TNX*, *FECH*, and *FTH1* was not affected ($P \geq 0.10$) by MET. Hepatic glutathione was greater ($P = 0.01$) and global DNA methylation lower ($P = 0.04$) in MET-supplemented cows. The protein expression of the de novo DNMT3A and the maintenance DNMT1 were not affected ($P \geq 0.10$) by MET-supply; however, DNMT3A protein expression increased ($P = 0.05$) over time. A treatment × time interaction ($P = 0.05$) was observed for the protein expression of the de novo DNMT3B because of an increase over time. Increasing MET-supply in the diet enhanced ($P = 0.04$) the concentration of H3K4me3 especially in the postpartum period. Together, these data demonstrate that MET supply during the periparturient period might prevent liver damage by inflammation and oxidative stress through its effect on the NFE2L2 pathway, which is at least in part regulated epigenetically.

Key Words: DNA methylation, histone, oxidative stress

M21 Milking intervals of cows with contrasting production. F. Masía*[1,3], N. Lyons[2], M. Piccardi[1,3], M. Balzarini[1,3], R. Hovey[4], and S. Garcia[5], [1]*Cátedra de Estadística y Biometría de la Facultad de Ciencias Agropecuarias de Universidad Nacional de Córdoba, Córdoba, Argentina,* [2]*Intensive Livestock Industries, NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle NSW, Australia,* [3]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina,* [4]*Department of Animal Science, University of California, Davis, CA,* [5]*School of Life and Environmental Sciences and Sydney Institute of Agriculture, The University of Sydney, Camden, NSW, Australia.*

In automatic milking systems (AMS), there is variability in milking intervals (MI) within and between cows. Extended MI (particularly greater than 16 h in pasture-based systems) have a negative effect on milk yield (MY). Having cows that tolerate extended MI without negative effects on MY could improve overall system performance. The aim of this study was to describe MI of cows with contrasting production milked in pasture-based AMS. A database containing records of milking events for 917 multiparous cows for one year (July 2015 – June 2016) from 2 AMS farms in Australia was used. Each record contained farm, cow, lactation, days in milk, MI and MY. Daily yields were then calculated as the sum of milking events. Lactation curves were adjusted with an incomplete gamma function (Wood, 1967) with a random intercept. Daily yields were expected to be auto-correlated. The adjustments were made with PROC NLMIXED from SAS. Predicted curves of average daily production according to lactation (2 vs 3 or more) and calving season (warm vs cool) were obtained. The best linear unbiased prediction (BLUP) allowed categorization of cows and lactations as having either high or low milk production (positive and negative BLUP, respectively). Then, each MI were categorized as belonging to short (<16 h) or long (≥16 h) MI. Differences between categories were compared using the binomial test of proportions. There were significant differences in the distribution of MI within each production level ($P < 0.0001$). High production cows had 34% of milking events with intervals greater than 16 h and were 4.13 times more likely than low production cows to have low MI (<16 h). Preliminary results also indicate that high production cows do not have such a negative effect of longer MI on MY as the low production cows do. This study indicates that there are cows that have long MI and still maintain high levels of production. Identifying and selecting for these cows should enable improve robot performance.

Key Words: non-lineal models, BLUP, robotic milking

M22 Evaluating the effects of fibrolytic enzymes derived from *Trichoderma reesei* fungal extraction on rumen fermentation, omasal nutrient flow and production performance in dairy cows during early lactation. B. Refat*[1], D. Christensen[1], J. McKinnon[1], A. Beattie[2], T. McAllister[3], W. Yang[3], O. AlZahal[4], and P. Yu[1], [1]*Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada,* [2]*Crop Development Center, Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada,* [3]*Lethbridge Research and Development Centre, Lethbridge, AB, Canada,* [4]*AB Vista, Marlborough, United Kingdom.*

This study was performed to evaluate the effects of pre-treating barley silage-based diet with a fermentation extract derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase; AB Vista, Marlborough, UK) on lactation performance, omasal nutrient flow and digestibility, rumen fermentation characteristics, and rumen pH profile in Holstein dairy cows during early lactation. The dairy trial was conducted using 9 Holstein dairy cows (averaging 46 ± 24 DIM and 697 ± 69 kg BW, 6 cows were fitted with a rumen cannula and 3 were non-cannulated). Two groups of cows were randomly assigned to each of the dietary treatments in a crossover design: control (without FETR supplementation) and supplemented (with 0.75 mL of FETR/kg DM of diet based on our previous study). The pre-treatment was applied to barley silage-based diet one hour before feeding by mixing FETR with the diet. The experiment consisted of 2 consecutive experimental periods of 27 d each. Within each period, the first 18 d were used for adaptation to the treatments, followed by 3 d of milk sampling, 3 d for the collection of the ruminal, omasal, and fecal samples, and the last 3 d for measuring the pH profile using indwelling pH probes. The application of FETR tended to decrease the DM intake compared with control (32.8 vs. 33.7; $P = 0.08$). There was a significant decrease ($P = 0.05$) in milk urea nitrogen by 7% and a numerical decrease ($P = 0.16$) in ruminal ammonia concentration by 14% as a consequence of adding FETR to the diet. In conclusion, dairy cows fed FETR pre-treated barley

silage-based diet display enhanced milk yield with less feed. The positive effect of adding FETR could benefit the dairy industry in Western Canada where barley silage-based diets are common

Key Words: fibrolytic enzyme, milk yield, nutrient flow

M23 Supplemental methionine and lipopolysaccharide alters galectin gene expression in polymorphonuclear leukocytes (PMNL) from Holstein cows in vitro. E. Asiamah*[1], M. Vailati-Riboni[2], M. Worku[1], and J. Loor[2], [1]*North Carolina A&T State University, Greensboro, NC,* [2]*University of Illinois Urbana-Champaign, Urbana, IL.*

Supplementation of methionine (Met) to dairy cows is effective in the optimization of milk production and improvement of health. Isolated PMNL from multiparous Holstein cows were used to evaluate the effect of methionine supplementation on mRNA expression of galectin genes (*LGALS-1*, *-2*, *-3*, *-4*, *-7*, *-8*, *-9*, *-12*) in vitro and on the impact of stimulation with lipopolysaccharide (LPS). Galectins are important immunological mediators of homeostasis and disease regulation. PMNL was isolated from 10 Holstein cows and divided into 2 groups (Group 1: n = 5 and Group 2: n = 5). PMNL from Group 1 were incubated with 3 levels of lysine (Lys) to Met ratios of (3.6:1, 2.9:1, or 2.4:1). PMNL from Group 2 were also incubated with 3 levels of Lys to Met ratios of (3.6:1, 2.9:1, or 2.4:1) and 50 µg/mL of LPS. Cells were incubated at 37°C, with 5% CO_2 for 4 h. Met had no effect on *LGALS* expression in Group 1 cows. In Group 2 cows, however, *LGALS9* expression was reduced after treatment with 2.4 Lys:Met. *LGALS8* transcription was increased when PMNL were treated with 2.9 Lys:Met and LPS (P = 0.0005). *LGALS8* was increased in PMNL treated with 3.6 Lys:Met(P = 0.05). LPS increased the expression of *LGALS4* (P = 0.005) and tended to increase *LGALS12* (0.08) in Group 2. These results shed light on the modulation of galectin expression in cows in response to methyl donors and LPS. The observed modulation of galectins expression by bacterial LPS and methionine may be exploited for the design of anti-inflammatory therapeutics.

Key Words: galectins, methionine, lipopolysaccharide

M24 Aluminosilicate clay reduces the deleterious effects of an aflatoxin challenge on performance in lactating Holstein cows. R. T. Pate*[1], D. M. Paulus Compart[2], and F. C. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*PMI Nutritional Additives, Shoreview, MN.*

Adsorbent use in aflatoxin (AF) contaminated diets is critical in alleviating detrimental effects of AF on dairy cattle performance. The objective of this study was to determine the effects of a commercially available aluminosilicate clay in a traditional lactation diet during an AF challenge on the presence of AF in milk, urine, and feces, and performance parameters of multiparous lactating Holstein cows. Sixteen multiparous, lactating, Holstein cows [BW (mean ± SD) = 758 ± 76 kg; DIM = 157 ± 43 d] were assigned to 1 of 4 treatments in a replicated 4 × 4 Latin Square design: no adsorbent and no AF challenge (CON), no adsorbent and AF challenge (POS), 113 g of aluminosilicate clay top-dressed on TMR (adsorbent; PMI Nutritional Additives, Arden Hills, MN) with AF challenge (F4), and 227 g of adsorbent with AF challenge (F8). For each period, milk was sampled 3 times daily from d 14 to 21, while feces and urine were sampled on d 14, 18, and 21. Statistical analysis was preformed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Fat-corrected milk (POS = 37.2, F4 = 39.2, F8 = 38.9 kg/d) and ECM (POS = 37.3, F4 = 39.3, F8 = 38.9 kg/d) increased (P = 0.05 and 0.04, respectively) as concentration of adsorbent in the diet increased. Lactose yield (kg/d) increased as concentration of adsorbent in the diet increased (POS = 1.67, F4 = 1.89, F8 = 1.74 kg/d; P = 0.05). A quadratic treatment effect was present for protein yield (POS = 1.20, F4 = 1.28, F8 = 1.24 kg/d; P = 0.01). There was a decrease in milk AFM1 concentration (POS = 0.33, F4 = 0.32, F8 = 0.27 µg/kg; P = 0.001) as concentration of adsorbent in the diet increased. A quadratic treatment effect was present for AFM1 transference (POS = 0.45, F4 = 0.49, F8 = 0.39%; P = 0.03). There was a decrease for AFM1 concentration in urine (POS = 2.10, F4 = 1.89, F8 = 1.78 µg/kg; P = 0.04) and feces (POS = 4.68, F4 = 3.44, F8 = 3.17 µg/kg; P = 0.05) as concentration of adsorbent in the diet increased. In conclusion, the adsorbent used in this study had a positive effect on milk production, milk components, and AF excretion in milk, urine, and feces.

Key Words: aflatoxin, urine, adsorbent

M25 A double-stranded RNA, polyinosinic-polycytidylic acid (Poly I:C) enhances the expression of galectins-1, -3, -4, -8, and -9 in cow blood. E. Asiamah*, S. Adjei-Fremah, K. Ekwemalor, B. Osei, and M. Worku, *North Carolina A&T State University, Greensboro, NC.*

Pathogen-associated molecular patterns (PAMP) are highly conserved structural motifs that are recognized by pathogen recognition receptors (PRR) to initiate immune responses. Treatment of cells with double-stranded RNA (dsRNA) mimics viral infection and regulates expression of various genes. Polyinosinic-polycytidylic acid (Poly I:C) is a PAMP for viral infections. It is established that Poly I:C mediates the production of cytokines by binding to its PRR, toll-like receptor 3 (TLR3) on gamma-delta T cells. Because galectins are also involved in immune responses and the outcome of microbial infections, it was important to evaluate whether its production is affected by poly I:C stimulation in whole blood. The effect of Poly I:C, on the expression of galectins in cow blood was assessed in this study. Galectins are multipotent, evolutionarily conserved, carbohydrate-binding proteins that trigger a cascade of transmembrane signaling events such as cell activation, cytokine secretion, migration, and apoptosis. Blood was taken from 5 multiparous Holstein cows and incubated with 12.5 µg/mL of Poly I:C at 37°C, with 85% humidity and 5% CO_2 for 30 min. Total RNA was isolated, reverse-transcribed to cDNA, and then used in real-time PCR experiments with the use of commercially sequenced cow specific primers. GAPDH and β-actin served as internal controls. Fold change in transcript abundance was calculated using the Livak method. Where $\Delta Ct = (\text{Target genes}_{treat} - \text{GAPDH}/\beta\ \text{actin}_{treat}) - \Delta Ct\ (\text{Target genes}_{PBS} - \text{GAPDH}/\beta\ \text{actin}_{PBS})$. Fold change = $2^{(-\Delta\Delta Ct)}$. Concentrations of Galectins in plasma were measured using bovine Enzyme-linked Immunosorbent Assay(ELISA). Galectin concentration was analyzed with Proc GLM in SAS 9.4. Poly I:C increased the transcription of *LGALS1*, *LGALS4,* and *LGALS8* (1.78,1.88, and 1.73 folds respectively). Secretion of Gal-1, -3, -8, and -9 were increased in treated samples compared with control (P = 0.0.10, P = 0.10, $P < 0.0001$, $P < 0.0001$, respectively). The results demonstrate that Poly I:C differentially modulates transcription of mRNA and secretion of galectins in cow blood. Elucidation of the relationship between PAMPS and galectin expression may help to define their roles in viral diseases as well as aid in drug design in the dairy industry.

Key Words: galectin, polyinosinic-polycytidylic acid (Poly I:C), pathogen-associated molecular patterns (PAMP)

M26 The effect of ruminal administration of 5-hydroxytryptophan on circulating serotonin in the dairy cow. M. K. Connelly*[1], A. M. Marshall[2], and L. L. Hernandez[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Amelgo LLC, Covington, KY.*

Degradation of amino acids by rumen microbes poses a challenge as the true benefit of amino acid supplementation is only exploited when the amino acid is presented to the small intestine for absorption. Our laboratory has previously demonstrated that prepartum intravenous (IV) infusions of 5-hydroxytryptophan (5-HTP), the immediate precursor to serotonin, increases circulating total calcium concentrations. This suggests potential for 5-HTP as a therapeutic management strategy for hypocalcemia prevention. However, whether 5-HTP can escape the rumen and be absorbed is unknown. The objective of this study was to determine if ruminal administration of 5-HTP increases circulating serotonin concentrations. The experiment was conducted as a 4 × 4 replicated Latin square using 4 nonlactating, nonpregnant, ruminally fistulated multiparous dairy cows. Experimental dosings of 5-HTP were administered on 2 consecutive days and given on a mg/kg of body weight basis. The resulting 4 treatments were saline infusion of 0 mg/kg 5-HTP (CON), IV infusion of 1 mg/kg 5-HTP (IV), 1 mg/kg intraruminal 5-HTP, and 2 mg/kg intraruminal 5-HTP. Whole blood was collected relative to administration of the second experimental dose for 3 continuous days with a 7-d washout period between treatment periods. Data were analyzed using the MIXED procedure of SAS with repeated measures. The ruminal 2 mg/kg 5-HTP treatment increased ($P = 0.03$) serotonin compared with the 0 mg/kg 5-HTP dose for 8 h after the second treatment. However, on d 3 and 4 of treatment, there were no differences between the CON ($P > 0.05$) and the 2 mg/kg 5-HTP administered intraruminally. The IV dose increased serotonin concentrations on d 2 compared with the 0 mg/kg 5-HTP dose, the ruminally dosed 1 mg/kg and 2 mg/kg 5-HTP treatments ($P < 0.0001$, $P < 0.0001$, and $P = 0.0016$, respectively). These data reveal that 2 mg/kg 5-HTP administered intraruminally acutely increased circulating serotonin concentrations. Future feeding experiments need to be done to determine the optimal 5-HTP dose for potential commercial use as a mitigation tool for hypocalcemia.

Key Words: serotonin, 5-hydroxytryptophan (5-HTP), rumen

M27 Effects of feeding more milk on periprandial plasma glucagon-like peptide-2 (GLP-2) concentrations in dairy calves. J. L. Haisan*[1], M. Oba[1], and T. Sugino[2], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*Hiroshima University, Higashi-Hiroshima, Japan.*

The objective of this experiment was to determine the effects of providing an increased amount of milk on periprandial glucagon-like peptide-2 (GLP-2) concentrations in pre-weaned dairy calves. Nineteen female Holstein calves were randomly assigned to 1 of 2 treatments on d 2 after birth; HIGH (10 L/d; n = 9) or LOW (5 L/d; n = 10) amount of pasteurized whole milk. All calves were allowed 2.5 L of milk per meal until d 50 before a 10-d weaning transition began. Calves were housed in individual pens for the first 21 ± 3 d, before being moved to a group pen and fed using an automated calf feeder. Calf starter was provided ad libitum from d 21 ± 3 d. At wk 3 (before being moved to the group pen), wk 5, and wk 7 (before the weaning transition) of life, a series of blood samples were collected relative to their morning milk meal, which was at least 6 h after a previous milk meal. Overall, HIGH calves tended to have increased mean plasma GLP-2 concentrations on wk 3 ($P = 0.10$) and wk 5 ($P = 0.08$), but no difference was observed on wk 7. Similarly, pre-meal concentration of plasma GLP-2 tended to be greater for HIGH calves compared with LOW at wk 3 (1.05 vs. 0.77 ng/mL; $P = 0.11$), and was greater at wk 5 (0.76 vs. 0.44 ng/mL; $P = 0.04$), however no difference was observed at wk 7 (0.56 vs. 0.47 ng/mL; $P = 0.77$). Although starter intake of HIGH calves tended to be lower than LOW calves (241 vs. 413 g/d; $P = 0.06$) for wk 4 to 7, there was a negative correlation between starter intake and pre-meal GLP-2 concentrations ($r = 0.30$; $P = 0.02$) at wk 5, indicating that plasma GLP-2 concentration of pre-weaned dairy calves can be increased to a greater extent by milk intake than starter intake. Plasma glucose concentrations were not different among treatments at any measured time point; however plasma insulin concentration tended to be higher for HIGH calves on wk 3 ($P = 0.08$). These results suggest that feeding more milk early in life can increase plasma GLP-2 concentrations in pre-weaned dairy calves.

Key Words: glucagon-like peptide-2 (GLP-2), starter intake, dairy calves

M28 Effects of citrus oil components on *Escherichia coli* P4 growth and on bovine neutrophils. C. M. Scholte*[1], T. H. Elsasser[2], S. Kahl[2], D. Biswas[1], and K. M. Moyes[1], [1]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD,* [2]*Animal Biosciences and Biotechnology Laboratory, USDA-Agricultural Research Service, Beltsville, MD.*

Citrus oils (CO) have known antimicrobial properties and as such may serve as alternatives to conventional drug mastitis treatments; however, it is unknown how these oils affect environmental mastitis pathogens and the cow's cellular immune response. The objectives of this study were to (1) determine the minimum inhibitory concentrations (MIC) of CO against *Escherichia coli* P4, and (2) evaluate CO cytotoxicity and their acute effect on oxidative response of bovine blood polymorphonuclear leukocytes (PMN). Citrus oils and its components, citral, linalool, valencene and limonlene, (0–10 µL/mL) were dissolved in ethanol and phosphate buffer solution with a 7:1 ethanol to oil ratio to maintain oil solubility. A control treatment of ethanol-only was also tested. Milk and blood were obtained from 12 healthy, mid-lactation dairy cows. Bacteriostatic MIC were determined through broth and milk microdilution. Bovine PMN were isolated from blood and incubated with varying citral, linalool, and ethanol concentrations. Following incubation, oil and ethanol cytotoxicity and PMN oxidative response were determined by quantifying PMN lactate dehydrogenase release into media and reactive oxidative species production, respectively. Cytotoxicity and oxidative burst response data were analyzed by ANOVA using the MIXED procedure of SAS 9.4. Of the CO components, citral and linalool had the lowest MIC and were unaffected by the presence of ethanol. Citral was the most effective at inhibiting (0.4 µL/mL in broth; 0.8 µL/mL in milk) *E. coli* P4 growth. No citral, linalool, or ethanol concentrations affected PMN oxidative burst response ($P > 0.05$); however, citral and linalool concentrations (0.1–0.8 µL/mL) were more toxic to PMN than control (0 µL/mL; $P < 0.01$). The ability of citral and linalool to inhibit proliferation of *E. coli* P4 highlight their potential as alternative antimicrobial therapies for bovine mastitis. Increased in vitro cytotoxicity suggests that further tests may be needed to optimize treatment strategy.

Key Words: mastitis, alternative therapy, *Escherichia coli*

M29 Evaluating the effects of a rumen and hindgut starch challenge on the inflammatory immune response in Holstein cows. A. M. Barnard*, M. Conklin, B. Aylward, R. Dyer, R. Arsenault, and T. F. Gressley, *Department of Animal and Food Sciences, College of Agricultural and Natural Resources, University of Delaware, Newark, DE.*

Grain induced subacute ruminal acidosis has been linked to systemic inflammation. We hypothesized that a rumen and hindgut starch challenge would stimulate an inflammatory response in intestinal tissue. Six rumen cannulated nonlactating nonpregnant Holstein cows were assigned according to a randomized block design to a control diet (CON) or CON top-dressed with 20% ground barley (STARCH). Diets were restricted fed to achieve weight gains of ~45 kg by the end of the 20-wk experiment. STARCH cows also received abomasal infusions of corn starch (4 g/kg BW per day) and CON cows received abomasal infusions of water pulse dosed twice a day during wk 8, 12, 16 and 19–20. Rumen fluid, feces and blood were collected weekly (non-infusion weeks) or 3 times weekly (infusion weeks). Rumen fluid and feces were analyzed for pH, lactate and VFA and blood samples were analyzed for haptoglobin (Hp) and serum amyloid A. At wk 20, cows were euthanized and mesenteric adipose, colon and jejunum were collected for determination of immune cell phenotype. Weekly data were analyzed using a repeated measures Glimmix model in SAS that included fixed effects of diet, month, infusion, and 2 way interactions and the random effect of cow. Immune cell phenotype data were analyzed with a model including effect of diet only. Diet × infusion tended to affect Hp ($P = 0.08$) and fecal pH ($P = 0.10$), lactate ($P = 0.08$) and butyrate ($P = 0.09$). Interactions were due to effects observed during infusion periods, when Hp and fecal pH tended to be lower in STARCH cows. Fecal lactate and butyrate tended to be higher in STARCH cows. Diet did not affect immune cell phenotype. Overall means (% total) of dendritic cell markers MHCII, CD40, CD80 and CD86 were 25.1%, 1.1%, 0.9%, 0.2% (adipose), 25.2%, 9.3%, 26.4%, 3.3% (colon) and 13.7%, 3.3%, 14.2%, 1.7% (jejunum), respectively. Means of effector T-cell markers CD4 and CD8 were 14.6%, 22.6% (colon) and 4.2%, 12.2% (jejunum), respectively. Although replication was low, the rumen and hindgut starch challenge did not appear to induce systemic inflammation or inflammatory cell infiltration into the intestines.

Key Words: hindgut, starch, inflammation

M30 Body measurements comparison between slick and wild type-haired Puerto Rican Holstein cows. G. A. Soriano-Varela*, M. Mercado-Ayala, E. Matos-Padilla, G. Muñiz-Colón, K. Domenech-Pérez, and H. L. Sánchez-Rodríguez, *University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico.*

Differences between the growth curves of slick (SLICK) and wild type-haired (WT) Puerto Rican Holstein heifers were reported in a preliminary study, suggesting that the first group reaches maturity at a smaller body size. Thus, the present study aimed to evaluate possible differences in body size-related measurements between SLICK and WT cows. For this, 24 SLICK and 54 WT lactating Puerto Rican Holstein cows (at the Agricultural Experimental Station dairy farm, Lajas, P.R.) were compared in terms of milk yield (MY), parity, and days in milk (DIM) (all 3 to verify data balance), as well as body weight (BW), withers height (WH), hip height (HH), thoracic perimeter (TP), barrel (BA), and shoulder to pin bone distance (SP). Also, the ratios of BW over the body size variables (BW/WH, BW/HH, BW/TP, BW/BA and BW/SP) were evaluated. Data were analyzed using the GLIMMIX procedure of SAS. There were no differences in MY ($P = 0.9189$), parity ($P = 0.3660$), and DIM ($P = 0.6197$) between SLICK and WT cows. Neither were differences in BW ($P = 0.4247$), WH ($P = 0.4718$), HH ($P = 0.8833$), TP ($P = 0.5125$), BW/WH ($P = 0.3181$), BW/HH ($P = 0.4958$), BW/TP ($P = 0.5339$), nor BW/BA ($P = 0.9258$) between SLICK and WT cows. However, SLICK cows had a larger BA (236.54 ± 2.68 vs. 231.06 ± 2.29 cm; $P = 0.0363$) and BW/SP (3.51 ± 0.09 vs. 3.32 ± 0.08 kg/cm; $P = 0.0218$), as well as a smaller SP (180.07 ± 1.82 vs. 185.20 ± 1.56 cm; $P = 0.0059$) than their WT counterparts. This indicates that SLICK-haired cows have a shorter body with a deeper barrel than the WT ones. Future studies should determine if such differences are related to metabolic efficiency, heat tolerance capacity, or both.

Key Words: slick-haired Puerto Rican Holstein cow, wild type-haired Puerto Rican Holstein cow, body measurement comparison

M31 Modification of rumen microbiome by supplementing the dairy cow diet with a blend of probiotics and evaluation of changes in energy balance and milk quality. H. L. Mulchay*[1], B. Jensen[1], Z. Evangelo[1], R. Silacci[1], C. Kitts[2], and C. K. Yeung[1], [1]*Animal Science Department, California Polytechnic State University, San Luis Obispo, CA,* [2]*Biological Sciences Department, California Polytechnic State University, San Luis Obispo, CA.*

The incorporation of direct-fed microbials (DFM) into dairy cow diets may enhance overall lactation performance. In this study, a novel blend of probiotics was added to the diet of Holstein cows to modify the rumen microbiome, and changes in energy balance and milk quality were evaluated. Ten cows in early lactation, blocked by their days in milk, were assigned to 1 of 2 groups (control or DFM, n = 5). All cows received the same basal diet during a 14-d acclimation period. On d 15, the DFM group diet was topdressed with calf grains mixed with a blend of probiotics at 5 g DFM per cow per day (*Pediococcus acidilactici*, >1.0 × 10^8 cfu/g; *Pediococcus pentosaceus*, >1.0 × 10^8 cfu/g; *Lactobacillus plantarum*, >1.0 × 10^8 cfu/g; *Bacillus subtilis,* > 1.0 × 10^7 cfu/g). The control group diet was topdressed similarly, but without probiotics. The cows remained on their respective treatment diets for 21 d, followed by a 14-d transition period in which both groups returned to the same basal diet. Blood and milk samples were collected weekly during the trial (on d 0, 7, 14, 21, 28, 35, 42, and 49). Data were analyzed by the *t*-test with statistical significance indicated by $P < 0.05$. There were no differences in blood serum β-hydroxybutyrate or nonesterified fatty acids between the 2 groups at any time points. For milk quality, there were no differences in fat content, protein content, somatic cell count or urea nitrogen. However, on d 35, both lactose content (5.08 ± 0.06% vs. 4.79 ± 0.07%; $P = 0.024$) and solids-not-fat (8.98 ± 0.16% vs. 8.28 ± 0.09%; $P = 0.009$) were significantly higher in the DFM group compared with the control. These increases were not observed after the cows returned to the basal diet. Preliminary data obtained by analyzing the rumen fluid DNA from 4 cannulated experimental cows showed a small but significant change in the rumen microbiome ($P = 0.017$) on the family taxonomic level from d 28 to 42. Our results suggest that a novel blend of probiotics could mildly modify the rumen microbiome, but any resulting effects on milk quality are transient. Further investigations on milk production and underlying factors on the increase in lactose are highly warranted.

Key Words: probiotics, milk, microbiome

M32 Effect of calf hutch type on calf performance and calf hutch temperature-humidity index. K. J. Reuscher*[1], B. W. Jones[1,2], and E. R. Jordan[3], [1]*Tarleton State University, Stephenville, TX,* [2]*Texas A&M AgriLife Research, Stephenville, TX,* [3]*Texas A&M AgriLife Extension, Dallas, TX.*

The objective of this study, conducted at a heifer grower operation in the Texas panhandle from July 10 to October 12, 2017, was to evaluate 4 different calf hutch designs to determine if differences exist in calf performance. Female Holstein calves (n = 120) sourced from 3 different operations were housed in 4 types of Calf-Tel polyethylene hutches (Hampel Corp., Germantown, WI). Eleven calves initially enrolled were removed due to low IgG absorption levels or death. Hutch A (n = 31) had vents on the top of the rear wall. Hutch B (n = 27) had an adjustable rear ventilation door. Hutch C (n = 25) was similar to Hutch B but had 2 added circular vents on the rear wall of the hutch. Hutch D (n = 26) was the same design as Hutch A but was elevated 15.24 cm in the rear by a custom lift. A HOBO external data logger (Onset, Bourne, MA) was placed inside one of each hutch type as well as one placed at calf level outside to record ambient temperature and relative humidity every 10 min. Average calf weight entering hutches was 31.31 ± 2.65 kg and average weight leaving hutches was 96.14 ± 9.20 kg. The MIXED procedure of SAS (Cary, NC) was used to evaluate fixed effects of hutch type, farm origin, and initial calf weight and their 2-way interactions on weight gain. The GLM procedure in SAS was used to evaluate the fixed effect of hutch type on temperature-humidity index (THI). Stepwise backward elimination was used to remove non-significant interactions ($P \geq 0.05$). All main effects were kept in the model regardless of significance. No significant differences ($P \geq 0.05$) existed for weight gain between hutches A, B, C, and D (60.32 ± 1.76, 59.93 ± 1.93, 57.09 ± 1.95, and 57.31 ± 1.98 kg, respectively). No significant differences ($P \geq 0.05$) existed for weight gain between farm 1, 2, and 3 (59.69 ± 1.39, 58.28 ± 2.79, and 58.01 ± 1.28 kg, respectively). When outside temperature humidity index (THI) was above 77, the THI was significantly different ($P < 0.05$) between hutches A, B, C, and D (80.38 ± 0.06, 78.78 ± 0.09, 79.21 ± 0.06, 78.33 ± 0.07, respectively). Results indicate no differences in calf weight gain when housed in different calf hutches.

Key Words: heat stress, hutch, calf

M33 Ultra-short spontaneous cure rates of intramammary infection among mastitis pathogens in dairy cattle. A. Srithanasuwan*, N. Pangprasit, M. Intanon, and W. Suriyasathaporn, *Faculty of Veterinary Medicine, Chiang Mai University, Mueang, Chiang Mai, Thailand.*

The innate immune system represents the first line of defense to intramammary infection (IMI) and is poised to immediately recognize and respond to the earliest stages of infection. The inherent capability of the innate immune system is mediated by its ability to recognize highly conserved motifs shared by diverse pathogens. Therefore, the objectives of this study were to determine the ultra-short spontaneous cure rates (uSCURE), the cure occurred from the immune system within 1 wk after IMI, among mastitis pathogen in the early stage of infection. Quarter milk samples were aseptically collected from all quarters of 26 lactation cows once a wk for 12-wk duration. After collection, milk samples were used for bacterial identification and the samples with positive bacterial results were chronologically sorted for determining the episodes of IMI. After starting episodes, the uSCURE were determined when the episodes was ended within 1 wk of infection by no positive bacteria was found for at least 3 following wks. Fisher's Exact Chi-squared tests were used to determine the differences of uSCURE among mastitis pathogens. From 1,014 collected quarter milk samples, the pathogens were isolated from 455 quarters accounting for 181 IMI episodes. The episodes included 103, 6, 27, 14, 10 and 21 for coagulase-negative staphylococci (CNS), *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and other streptococci, respectively. The uSCURE of CNS (29.1%) was higher than that of *Strep. uberis* (3.7%) at $P < 0.05$ and had a tendency of higher than *Strep. agalactiae* (7.1%) at $P < 0.15$. The differences of uSCURE were not found between CNS and *Staph. aureus* (16.7%), *Strep. dysgalactiae* (10.0%) and other streptococci (19%). In conclusion, CNS has the highest rate of ultra-short spontaneous cure rate compared with other pathogens.

Key Words: ultra-short spontaneous cure rate, intramammary infection

M34 Effects of anti-inflammatory treatment and milking frequency on mRNA abundance of adipose tissue from early lactation cows. M. Riley*, M. Garcia, C. Ylioja, L. K. Mamedova, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

We hypothesized that at the onset of lactation, inflammation may contribute to a temporary adaptive insulin resistance to protect against hypoglycemia and to promote fatty acid mobilization, but infrequent milking may reduce nutrient demand to the extent that this adaptation is unnecessary. In this study, 33 multiparous Holstein cows were used to evaluate whether treatment with the anti-inflammatory drug sodium salicylate (SS) alters plasma nonesterified fatty acid (NEFA) concentrations and mRNA profile of adipose tissue. Cows were randomly assigned to frequent (3×/d) or infrequent milking (1×/d, decreasing milk yield by 24%), and SS (2.3 g/L in drinking water) or control treatments in a 2 × 2 factorial design, beginning ~24 h postpartum. Blood was drawn daily before the morning feeding, and on d 5 of treatment, ~1 h after the conclusion of a hyperinsulinemic-euglycemic clamp procedure, adipose tissue biopsy was performed. Data were analyzed as repeated (NEFA) or non-repeated (mRNA abundance, reported as fold change ± SEM) measures with mixed models. Plasma NEFA concentrations were greater in SS cows (605 vs. 476 ± 34 μ*M*, $P = 0.01$) but did not differ with milking frequency. Regardless of milking frequency, SS tended to reduce the abundance of *INSR* (−1.92 ± 0.48, $P = 0.06$). For cows milked 1×, SS reduced the abundance of *IRS1* (−2.98 ± 1.35, $P = 0.05$). Abundance of *TNFA* tended to decrease with SS (−2.03 ± 1.69, $P = 0.08$) but only for cows with ≥3 parities. The effect of milking frequency on mRNA abundance depended on parity. Within parity 2, cows milked 3 × had greater abundance of the inflammatory transcripts *TNFA* (6.52 ± 2.41, $P < 0.01$) and *MCP1* (4.80 ± 2.50, $P = 0.02$), and the antioxidant regulator *NFE2L2* (2.19 ± 0.464, $P = 0.05$). Within cows milked 3×, second-parity cows had greater abundance of *IRS1* (3.20 ± 1.44, $P = 0.04$) and *NFKB1* (1.70 ± 0.62, $P = 0.03$). Results suggest that SS promotes adipose tissue insulin resistance and NEFA mobilization in early lactation, and that second-parity cows milked 3 × may have exacerbated adipose inflammation, possibly due to greater metabolic requirements during early lactation.

Key Words: inflammation, transition cow, fatty acid

M35 Does considering immunoglobulin G concentration alone constitute a physiology-based colostrum management program? O. M. Reiff*, K. M. Schalich, L. Furman, and V. Selvaraj, *Cornell University, Ithaca, NY.*

Feeding colostrum to calves within 24 h after birth is vital for passive transfer of immunity. Within the US dairy industry, decisions made in colostrum management programs have increasingly hinged upon the idea of colostrum being of "good quality" or "poor quality." In the United States, the Brix refractometer, designed to measure sugars in solution, has been widely adapted as an on-farm tool to measure colostrum IgG concentration, with a score below ~22% Brix (50 g/L IgG) generally considered not acceptable for heifer calves. As such, current colostrum management decisions might benefit from application of knowledge of mammary gland physiology. The objective of this study was to (1) determine the relationship between volume produced, Brix, total protein, IgG in colostrum, and to investigate the influence of lipids on Brix scores, and (2) use such relationships to suggest a more physiology-based colostrum management program. In this study, colostrum samples were obtained from primiparous and multiparous Holstein cows of varying ages at +1, +4, +12, +20, and +28 h post-parturition. Volume produced for the first milking varied for n = 32 primiparous cow (5.5 ± 2.79 L) and n= 67 multiparous cows (6.33 ± 4.42 L). Brix values were determined by an optical refractometer and found to average 22.25 ± 1.26 Brix for primiparous and 25.1 ± 5.74 Brix for multiparous cows. Average total protein content (measured by BCA) also varied amongst samples (n=5) and did not significantly change after fat removal by centrifugation (−2.43 μg/μL ± 23.97 μg/μL). We used quantitative fluorescent Western blot analysis to measure kinetics of IgG levels in colostrum post-parturition, to emulate physiological availability to a suckling calf. Based on the variability in colostrum volume produced per cow and milking, our findings suggest that adjusting volume of colostrum fed to calves based on IgG concentration to reach a threshold IgG delivery would best mimic the natural transfer of passive immunity. This physiology-based colostrum management would likely aid in improving calf health and growth by ensuring optimum IgG supply.

Key Words: colostrum, immunity, calf

M36 Analysis of bulk tank milk differential bacteria tests and pipeline cleaning cycle temperatures on 4 Michigan dairy farms. J. S. Myers*, L. L. Timms, and S. Clark, *Iowa State University, Ames, IA.*

Objectives of this study were to analyze (a) differential bacteria tests on bulk tank milk; and (b) pipeline cleaning cycle temperatures on 4 Michigan dairy farms. Individual bulk tank milk samples were taken on randomly selected separate days (n = 3) over a 2-wk period and

submitted to Eastern Lab Services (Medina, OH). Milk differential bacterial tests included standard plate (SPC), preliminary incubation (PI), laboratory pasteurization (LPC), coliform counts (CC), and bulk tank milk cultures (only once). Bacteria counts were compared with industry quality benchmarks (SPC <5,000 cfu/mL; PI <10,000 cfu/mL; LPC <100 cfu/mL; CC <100 cfu/mL) with single milk culture test used to rule out mastitis-causing bacteria or poor pre-milking teat sanitation bacterial sources. Milking pipeline cleaning cycle temperatures were recorded on 1 complete cleaning sequence following milking on test days using a sterilized Thermocron iButton, sealed inside each milking system wash vat before start of a complete cleaning cycle. Recorded temperatures were compared with recommend cleaning cycle temperatures, especially detergent cycles (>120°F at cycle end). Farms A and C had all differential bacteria counts better than industry quality benchmarks. Farm B showed elevated SPC on 1 d (18,000 cfu/mL) and elevated PI counts on 2 d (1,421,000 and 14,000 cfu/mL) while farm D had one elevated SPC (7000 cfu/mL) and one elevated PI (374,000 cfu/mL). LPC on both farms (B & D) were <100 cfu/mL, indicating clean milking equipment. Milk culture tests indicated elevated levels of Strep. non-ag. and Staph. species at Farms B (5000 and 3500 cfu/mL) and D (1500 and 400 cfu/mL) indicating potential issues with proper teat pre-milking sanitation. All farms met industry standards for initial rinse and acid rinse cycles (85–95°F) and detergent cycles (<160°F at start and >120°F at end). Overall, this study found excellent differential bacteria counts on 2 farms over a 2-wk period, while 2 farms showed elevated SPC and PI. Further differential tests showed low LPC (clean equipment) and pre-milking sanitation issues as the most likely problem (milk culture). All farms had proper detergent cycle cleaning temperatures that correlated with low LPC.

Key Words: standard plate count (SPC), laboratory pasteurization count (LPC), preliminary incubation (PI)

M37 Validation of an automated body condition scoring camera. I. Mullins*[1], C. Truman[1], J. Bewley[2], and J. Costa[1], [1]*Dairy Science Program, Department of Animal and Food Sciences, University of Kentucky, Lexington, KY,* [2]*CowFocused Housing, Bardstown, KY.*

Body condition scoring (BCS) is the management practice of visually estimating subcutaneous fat reserves in dairy cattle. High and low BCS can negatively affect milk production, disease, and reproduction. Manually scoring BCS has proven beneficial yet can be difficult to implement. The desirable range for dairy cows varies and should be monitored at multiple time points throughout lactation for the most impact. A commercial automatic BCS technology is available for dairy cattle (DeLaval International AB, Tumba, Sweden). The objective of this study was to evaluate the agreement of the automated scores in comparison with conventional manual scoring. The study was conducted on a commercial farm in Indiana in April 2017. Three trained researchers scored cows manually, using a 1 to 5 BCS scale, with 0.25 increments. All data analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC). Pearson correlation was calculated to assess interobserver reliability, the correlations being 0.85, 0.87, and 0.86. The automated scores were compared with 2 data sets of manual scores. One data set (MAN1) consisted of cows with ≥ 2 manual scores averaged by cow into one score per cow (n = 343). The second data set (MAN2) included cows that ≥ 2 scorers agreed on the score (n = 237). The mean was 3.38 ± 0.48 and 3.38 ± 0.78 (mean ± SD) for MAN1 and MAN2, respectively. The average automated score was 3.27 ± 0.27. Correlations were calculated for MAN1 and MAN2. Resulting coefficients were 0.78 and 0.77 ($P < 0.001$). The results from the *t*-test demonstrated equivalence between automated and manual data sets ($P < 0.001$). When scores were separated into manual scores: low (<3.00), average (3.00 to 3.75), and high (>3.75), the low and high BCS categories were shown to not be equivalent ($P < 0.001$). A Bland-Altman plot was constructed and demonstrated that the technology tended to underscore cows as their manual score increased. The system tends to be inaccurate at determining the extreme low and high BCS, although these categories represent a small proportion of cattle. An automated system may encourage more producers to adopt BCS into their practices.

Key Words: automatic measurement, technology, precision dairy farming

M38 The role of serotonin in systemic immune response during mid to late lactation in dairy cows. H. P. Fricke*, M. K. Connelly, and L. L. Hernandez, *University of Wisconsin-Madison, Madison, WI.*

It has been previously established that dairy cows are immunosuppressed during early lactation. Serotonin has been shown to influence immune function in other species. Therefore, we hypothesized that infusions of a serotonin precursor during lactation would have an impact on immune function in lactating dairy cows. The objective of this study was to evaluate if intravenous (IV) infusions of 5-hydroxytryptophan (5-HTP), in mid- to late-lactation cows will influence immune response. This study utilized 12 multiparous Holstein cows, blocked by parity in a randomized complete block design. The cows were administered either IV 1.5 mg/kg 5-HTP (n = 6) or saline (n = 6) once a day for 3 consecutive days. Baseline blood samples were collected before treatment, as well as 0, 8, and 24 h after the final 5-HTP infusion. Blood samples were analyzed via qPCR to evaluate mRNA expression of general circulating markers of the innate and adaptive immune response. The genes analyzed were: cluster of differentiation 19 (CD19), protein-tyrosine phosphatase receptor type C (CD45), cluster of differentiation 3 gamma (CD3-G), cluster of differentiation 3 delta (CD3-D), cluster of differentiation 3 epsilon (CD3-E), and low affinity immunoglobulin gamma Fc region receptor III-A (CD16-A). There was no difference in gene expression of CD16-A, CD45, CD3-G, CD3-D, or CD3-A expression between treatment groups ($P = 0.27$, $P = 0.22$, $P = 0.78$, $P = 0.27$, or $P = 0.27$, respectively). However, CD19, a circulating marker of adaptive immunity, had increased expression in 5-HTP treated cows compared with control cows ($P = 0.0025$). Milk yield data were collected throughout the treatment, and there was no difference between treatment groups ($P = 0.57$). Taken together, this data suggests a lack of an innate immune response to infusions of 5-HTP. Further experiments should be conducted to determine the effect of 5-HTP on the adaptive immune response in the dairy cow during lactation.

Key Words: lactation, immunity, serotonin

M39 In vitro fermentability of rumen fluid from heat-stressed or cooled cows fed high or low dietary crude protein. C. M. Johnson*, J. D. Kaufman, H. R. Bailey, A. M. Devolk, C. A. Steren, F. E. Loeffler, and A. Ríus, *University of Tennessee, Knoxville, TN.*

Heat stress (HS) and dietary crude protein (CP) affect dairy cattle production through alterations of rumen microbial fermentation. The objective of this study was to determine in vitro fermentability, pH, and volatile fatty acid and gas production of rumen content from donor cows exposed to environmental and dietary treatments. A completely randomized design with a 2 × 2 factorial arrangement of treatments was conducted with 12 multiparous Holstein cows (215 ± 8 d in milk) for

21 d. Donor cows received treatments that consisted of HS or cooling with fans and sprinklers (CO) and high (17.9%, HCP) or low (14.2%, LCP) CP diets on a DM basis. Cows were exposed to prevailing July and August weather in Tennessee. Rumen fluid was collected by stomach tubing from each animal (3 cows/treatment) to determine individual in vitro fermentability. Data presented herein were determined at the end of 48 h in vitro incubation (ANKOM Technology, Macedon, NY). Metabolite concentrations were measured using HPLC, ^{1}H nuclear magnetic resonance, and ion chromatography. Treatment effects were tested using the Mixed procedure in SAS (LSM ± SEM). In vitro concentrations of volatile fatty acids did not change. At 48 h in vitro incubation, there was an interaction ($P < 0.01$) showing a greater reduction in gas production from LCP vs. HCP diet under CO (96.8 vs. 63.7 ± 10.9 mL/g of DM) relative to that under the HS treatment (19.2 vs. 17.7 ± 10.9 mL/g of DM). There was an interaction ($P = 0.04$) showing a greater reduction of ammonium concentrations in vitro from LCP vs. HCP diet under HS (12.7 vs. 1.45 ± 1.19 m*M*) relative to that under the CO treatment (10.4 vs. 4.17 ± 1.19 m*M*). Fluid incubation from HS cows increased ($P < 0.01$) rumen pH compared with that from CO cows (6.99 vs. 7.15 ± 0.03). Compared with HCP, LCP fluid showed an increase ($P = 0.02$) in pH (7.02 vs. 7.12 ± 0.03) and a decrease ($P < 0.01$) in methane concentration (20.9 vs. 15.4 ± 1.20 g/mL). In summary, results revealed that rumen content from cows exposed to HS and a low CP diet affected metabolite production in vitro.

Key Words: dairy cow, metabolite, rumen fluid

M40 Effect of rumen-protected methionine supplementation on milk production in dairy cows. H. R. Bailey[1], E. M. Belanger*[1], J. D. Kaufman[1], B. Barton[2], C. Zimmerman[2], K. Estes[2], and A. G. Ríus[1], [1]*University of Tennessee, Knoxville, TN,* [2]*Balchem Corp., New Hampton, NY.*

Methionine (Met) is essential to support milk and milk protein synthesis. A study was conducted to evaluate the effects of a rumen-protected Met (RP-Met) prototype on milk production and plasma metabolite concentrations in dairy cows. Ten multiparous Holstein cows (76 ± 17 d in milk) were used in a 5 × 5 duplicated Latin square with 18-d treatment periods. Cows were randomly assigned to either a Met-deficient control diet (−16.4 g/d of MP-Met; −CTL), −CTL supplemented with Smartamine-M (28.7 g/d of RP-Met; SA), or 1 of 3 levels of a RP-Met prototype (Balchem Corp., New Hampton, NY) to provide 1.0 (31.6 g/d of RP-Met; 1.0Met), 1.25 (38.9 g/d of RP-Met; 1.25Met), or 1.5 (47.3 g/d of RP-Met; 1.5Met) times the amount of DL-Met supplied by Smartamine-M. The prototype contained 69% Met. Feed ingredients and milk and blood samples were collected and analyzed. Data were analyzed using the Mixed procedure of SAS 9.4 and reported as least squares means ± SEM. Compared with −CTL, SA, 1.25Met, and 1.5Met decreased ($P = 0.02$) lactose percent by 1.3%. Relative to −CTL, SA tended to increase milk protein content ($P = 0.07$). Relative to −CTL and SA, 1.0Met, 1.25Met, and 1.5Met treatments tended to maintain milk protein content. Relative to −CTL, SA and 1.5Met increased plasma Met concentrations (27.9 vs. 38.1 and 32.6 μ*M*, respectively; $P < 0.01$). Compared with SA, 1Met, 1.25Met, and 1.5Met treatments support similar plasma methionine concentrations. In summary, SA and 1.5Met treatments increased plasma methionine concentrations. Smartamine-M tended to promote milk protein content; however, protein yield was not affected.

Key Words: dairy cow, milk protein, rumen-protected methionine

M41 The effects of comfortable or stressful housing environments on short-term behavior, milk quality, and milk production following group transition for Holstein dairy cattle. A. Bradtmueller*[1], M. Borchers[1], and J. Bewley[2], [1]*University of Kentucky, Lexington, KY,* [2]*CowFocused Housing, Bardstown, KY.*

The objective of this study was to determine how behavior, milk quality, and milk production of cows differs on a short-term basis following movement into a high comfort, low stress (HC) or a low comfort, high stress (LC) environment. The study was conducted at the University of Kentucky Coldstream Research Dairy from July to September 2017. Lactating Holstein dairy cows (n = 30) were balanced for DIM (>100 DIM) and parity. Cows were assigned to either HC or LC housing for the first 28 d of the study. At the midpoint, cattle were crossed-over to the alternate housing for the remaining 28 d. Quarter-level SCC and culture samples were collected every 24 h following movement into the new environments. Quarters with no clinical symptoms, a SCC ≥200,000 cells/mL, and a positive culture were considered subclinically infected. Daily milk yield, fat, protein, and solids were recorded by the AfiLab System (Afimilk, Kibbutz Afikim, Israel). AfiTag II leg-bands (Afimilk) monitored lying time and CowManager ear tags (Agis, Harmelen, the Netherlands) monitored activity, rumination, and feeding behaviors. Statistical analyses were performed using SAS 9.3 (Cary, NC). Univariate models treating activity, lying time, composite SCS, rumination, feeding behavior, and energy-corrected milk (ECM) as dependent variables were developed. Baseline values for each predicted variable (3-d moving average before transition), day after transition (d 1 to 7), and housing type were treated as independent variables. Univariate models determined independent variables for multivariate models. Cow within housing type served as a repeated effect. Housing significantly affected activity (HC = 224.0 and LC = 260.8 min; $P < 0.01$), lying time (HC = 684.3 and LC = 516.8 min; $P < 0.01$) composite SCS (HC = 1.3 and LC = 1.7; $P = 0.02$), rumination (HC = 571.9 and LC = 498.7 min; $P < 0.01$), feeding behavior (HC = 106.4 and LC = 90.1 min; $P < 0.01$), and ECM (HC = 33.2 and LC = 31.8 kg; $P < 0.01$). Transitions to new environments had a greater impact in the LC environment than the HC environment, indicating producers should place greater emphasis on cow comfort at these times.

Key Words: somatic cell count, subclinical infection, health

M42 Preference of flavored concentrate premixes by young ruminants. K. Nedelkov[1], M. T. Harper*[2], A. Melgar[2], X. Chen[3], S. Räisänen[2], C. M. M. R. Martins[4], E. H. Wall[5], and A. N. Hristov[2], [1]*Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria,* [2]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [3]*College of Pastoral Agriculture Science and Technology, Lanzhou University, Gansu, China,* [4]*School of Veterinary Medicine and Animal Science, University of Sao Paulo, Pirassununga, Brazil,* [5]*Pancosma, Geneva, Switzerland.*

Three cafeteria design experiments were conducted to test whether young ruminants have flavor preferences. Exp. 1 was with 11 Dorset × Suffolk weaned lambs of both sexes, aged 5 mo and averaging 47.5 (SD = 5.8) kg body weight. The lambs were offered a choice of 5 flavored concentrate premixes (FCP) with 5 synthetic flavors (vanilla, milky, spicy/fenugreek, red summer fruits, and molasses) and an unflavored control for 5 min, 4 times over 10 d at flavor inclusion rates of 200 to 300 g/t (as-is basis). A similar proportion of lambs consumed neutral (control) (84.5%), vanilla (72.1%), and milky (68.2%) flavors. The consumption rate of FCP were similar among treatments ($P = 0.26$). Lambs spent more time ($P \leq 0.002$) eating milky and control at 123 and 144 s/offering, respectively, compared with all other FCP (average 64.8 s/offering). In Exp.2, 12 weaned female Holstein calves (56 to 68 d of age) with 75.8 kg (SD = 8.45) body weight were offered a choice of 4 FCP (vanilla, milky, spicy/fenugreek, and red summer fruits) and unflavored control for 5 min, 4 times over 10 d at inclusion rate of 150 to 200 g/t (as-is basis). There were no differences in consumption rate among FCP ($P = 0.40$). In Exp. 3, a total of 12 weaned female Holstein calves (47 to 62 d of age) with 65.3 kg (SD = 7.91) body weight were offered a choice of 4 FCP with 2 different flavor combinations, vanilla – fenugreek and milky – vanilla type included at 75 g/t (as-is basis; Low) and 150 g/t (High). FCP were offered daily for 14 d for 30 to 60 min/d. The low dose of the vanilla-fenugreek combination was consumed less ($P \leq 0.008$) at 72.1 g/offering compared with the other FCP (average 111 g/offering). There were no other differences among FCP in Exp. 3. Overall, compared with the control, flavors used in the present experiments did not affect feed intake of weaned lambs and calves. Other factors, such as taste, sight, texture of the feed, and their interactions may also play a role in flavor preferences of young ruminants.

Key Words: flavored premix, lambs, calves

M43 Impact of a reduction in dietary energy density on feeding behavior, intake, and production of dairy cows. S. M. Moore* and T. J. DeVries, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

A study was conducted to test the hypothesis that cows fed a diet that does not meet nutrient requirements for production will alter their behavior to maximize nutrient consumption, particularly when fed a more easily sorted diet. Holstein cows (n = 30) were followed for a 2-wk baseline period where they were fed (on a DM basis) a lactating diet (NE_L = 1.66 Mcal/kg; 68% forage), and then exposed for 3 wk to 1 of 2 treatment diets: a TMR, formulated for a 12% reduction in energy available for milk (NE_L = 1.56 Mcal/kg; 73% forage), containing 17% straw chopped to: 1) 2.54 cm (Short; n = 15) or 2) 10.16 cm (Long; n = 15). TMR samples (fresh and orts) were collected every 3 d and separated into 4 fractions: long (>19mm), medium (<19mm, > 8mm), short (<8mm, > 4mm), and fine (<4mm) particles. Feed sorting was calculated as: actual intake of each particle fraction expressed as a % of its predicted intake. Data were analyzed in repeated measures, mixed-effect linear regression models. Cows consumed 25.3 ± 1.1 kg/d during the baseline. DMI decreased ($P < 0.01$) on the experimental diets; DMI was greater for the Short diet as compared with the Long diet (23.2 vs 22.4 kg/d; SE = 0.5; $P = 0.04$). During the baseline period, cows sorted against long particles (95.4 ± 2.0%), did not sort medium particles (99.8 ± 0.23%), and sorted for short (101.1 ± 0.4%) and fine (101.9 ± 0.6%) particles. Cows did not change ($P = 0.99$) sorting of long particles on the Short diet (95.1 ± 1.0%), but increased ($P < 0.01$) sorting against these on the Long diet (89.4 ± 1.0%). Medium particle sorting did not change ($P > 0.8$) on the Short (100.1 ± 0.2%) and Long (99.9 ± 0.2%) diets. On the Short diet cows did not change ($P > 0.2$) their sorting of short (100.9 ± 0.9%) and fine (100.6 ± 0.9%) particles. On the Long diet, cows increased ($P < 0.01$) sorting for short (103.3 ± 0.9%) and fine (104.5 ± 0.9%) particles. During baseline, cows produced 44.6 ± 1.2 kg/d of milk; this decreased ($P < 0.01$) on the experimental diets. Despite differences in DMI, milk yield remained similar between treatments (Short = 38.7, Long = 39.5 kg/d; SE = 1.9; $P = 0.5$), possibly due to sorting. This suggests that cows exposed to a diet that does not meet their nutrient requirements will sort that diet, when possible, to maximize nutrient consumption.

Key Words: sorting, energy density, behavior

M44 Impact of dietary transition at dry off on the feed sorting behavior of dairy cows. K. M. Dancy, E. S. Ribeiro, and T. J. DeVries*, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to assess the impact of the magnitude change in ration nutrient density at dry off on the feed sorting behavior of dairy cows. Forty-eight cows (2.5 ± 0.7 lactations) were dried off over 5 d, approximately 60 d before their expected calving dates. At the start of dry off (d 1), cows were randomly assigned, for 26 d, to 1 of 2 dry cow TMR: higher nutrient density (HND; 1.55 Mcal/kg, 12.9% CP and 38.7% NFC) or lower nutrient density (LND; 1.48 Mcal/kg, 12.2% CP and 34.7% NFC). During dry off, cows were milked 1x/d on d 1, 2, 3, and 5. Before the start of dry off, cows were producing 26 ± 5.6 kg/d, milking 2x/d, and consuming a TMR (1.66 Mcal/kg, 14.7% CP and 48.5% NFC). Fresh feed and refusal samples were collected to assess feed sorting. A particle separator was used to separate feed samples into 4 fractions: long (>19 mm), medium (8–19 mm), short (4–8 mm), and fine (<4 mm) particles. Feed sorting was calculated as follows: actual DMI of each particle fraction divided by the predicted DMI of each fraction (expressed as a %). Data were summarized by cow and day and analyzed in repeated-measures linear mixed-effect regression model. Cows sorted against ($P < 0.001$) long particles before dietary change (85.8 ± 1.3%) and similarly ($P > 0.5$) afterward (LND: 82.5%; HND: 85.6%; SE = 1.8). Sorting of long particles did not vary between treatments ($P = 0.11$). Before dietary change, cows tended ($P = 0.1$) to sort in favor of short particles (100.7 ± 0.45%), but this occurred to a greater extent ($P \leq 0.04$) after dietary change in both treatments (LND: 103.1%; HND: 102.5%; SE = 0.3). A treatment × day interaction indicated that LND cows sorted for short particles more than HND cows on d 1 ($P = 0.03$) and tended to do so more on d 6, 13, 16, and 25 ($P < 0.10$). Before dietary change, cows sorted ($P = 0.002$) in favor of fine particles (101.5 ± 0.5%), but this occurred to a greater extent ($P = 0.03$) after dietary change in LND cows (105.4 ± 0.5%).

A treatment × day interaction indicated that LND cows sorted for fine particles more ($P = 0.04$) than HND cows (102.9 ± 0.5%), except on d 13 and 25 ($P > 0.20$). Thus, sorting behavior likely resulted in greater nutrient intake than predicted during the early dry period when a ration of lower nutrient density was fed.

Key Words: dietary transition, feed sorting, dry off

M45 Effects of different feed type exposure in early life on performance, rumen fermentation and feed preference of dairy calves. Y. J. Wang*, J. Xiao, S. Li, Z. Cao, and G. M. Alugongo, *China Agricultural University, Beijing, China.*

The aim of the study was to evaluate the effects of early feed exposure (EFE) in dairy calves to different feedstuffs on feed preference once fed a free-choice diet and a TMR later in life. Thirty female calves were randomly assigned to one of the 3 EFE treatments including CON (only concentrate), HAY (only hay) and COH groups (both concentrate and hay) from d 2 to 56. All calves were offered both concentrate and hay in different bucket from d 57 to 70 to allow them free choice between the 2 feedstuffs. Calves were then fed TMR from d 71 to 196. Feed intake was recorded daily from d 2 to 70 to determine the feed preference before and after weaning. Upon transition to a TMR, feed sorting was calculated from d 190 to 196 as the actual intake of each fraction expressed as a percentage of predicted intake by using a particle separator. BW, ADG, glucose, total protein (TP), ruminal pH and VFA concentrations were analyzed throughout the experiment. Early feed exposure did not significantly affect the feed intake, BW, ADG, blood glucose and structural growth before and after weaning, but did affect feed preference and rumen fermentation. After transition to a free-choice diet, HAY calves consumed more hay (550.2 g/d) on the first day after transition than CON (177.4 g/d, $P < 0.01$) and COH (396.4 g/d, $P < 0.01$), but this effect lasted for only a day. Provision of both concentrate and hay early in life resulted in greater intake of hay and higher ratio of hay to total solid (35.6%) than provision of either concentrate (17.7%, $P = 0.03$) or hay (16.5%, $P = 0.02$), giving rise to a higher rumen pH (CON:5.59, HAY:5.44 and COH:5.84, $P = 0.01$) and numerically lower total VFA (147.5 mmol/L, 163.7 mmol/L and 135.8 mmol/L, $P = 0.09$) and blood glucose (3.78 mmol/L, 3.85 mmol/L and 3.62 mmol/L, $P = 0.59$) at d 70. Upon transition to a TMR, a similar sorting pattern was exhibited, sorting against the long particle (CON: 97.1%, HAY: 97.7% and COH: 99.3%, $P = 0.12$) and for the fine particle fraction (104.3%, 104.2% and 103.9%, $P = 0.15$). Therefore, our results suggested that EFE could influence choice of feed immediately after weaning, but did not have a long-lasting effect on feed preference in calves.

Key Words: dairy calf, feed preference, early experience

M46 Effects of feeding corn stover pelleted with soybean meal or distillers grains on chewing activity and ruminal pH of dairy cows. A. J. DuMez[1], B. C. Dooley*[1], E. H. Branstad[1], C. S. McCarthy[1], G. M. Bonetto[3], R. Zeeck[2], A. G. Carpenter[4], J. O. Sarturi[5], and H. A. Ramirez-Ramirez[1], [1]*Iowa State University, Ames, IA,* [2]*Pellet Technology USA, Gretna, NE,* [3]*Instituto Nacional de Tecnología Agropecuaria, Manfredi, Córdoba, Argentina,* [4]*University of Guelph, Ridgetown, ON, Canada,* [5]*Texas Tech University, Lubbock, TX.*

This study aimed at determining the effects of pelleted corn stover (CS) on chewing activity and ruminal pH of lactating dairy cows. A total of 45 cows (30 primiparous, 15 multiparous; 118 ± 33 DIM and 604 ± 70 kg BW), were used in replicated 5 × 5 Latin squares with 28-d periods, using a 2 × 2 + 1 augmented factorial arrangement of treatments. Rumen pH was measured every 2 h for 24 h (n = 5, rumen cannulated cows) during d 21, behavior was visually recorded every 5 min for 24 h (n = 10, same cows for all periods) on d 27. Diets contained 10 or 15% (DM basis) corn stover-based pellets processed with soybean meal (CS-SBM) or distillers grains (CS-DG) as protein sources. Treatments were (1) Control (CON); (2) 10% CS-SBM; (3) 15% CS-SBM; (4) 10% CS-DG; and (5) 15% CS-DG. Data were analyzed with the MIXED procedure of SAS (fixed effects: protein source, inclusion level, source by inclusion interaction, square, and period within square; random effect: cow within square); pH data were analyzed as repeated measures. Overall, cows on CON diet had the greatest DMI ($P < 0.01$), 29.4 ± 1.32 kg/d, compared with 24.7, 23.6, 26.5 ± 1.32 kg/d for 10% CS-SBM, 15% CS-SBM, 10% CS-DG, and 15% CS-DG, respectively. Cows on CON spent the least amount of time per kg of DM consumed (8.0 ± 0.80 min/kg of DMI) compared with all other diets (9.7 and 11.8 min/kg DMI for 10 and 15% inclusion, respectively). Within pellet formulations, protein source did not affect eating time ($P = 0.28$), whereas cows consuming 15% CS-SBM or CS-DG spent more time eating compared with 10% inclusion (264 vs 238 ± 14 min/d; $P = 0.02$). Ruminating time was similar with either CS-SBM or CS-DG regardless of inclusion level ($P \geq 0.17$), averaging 409 ± 17 min/d. Despite differences in DMI and chewing activities, mean rumen pH was similar for all diets (5.87 ± 0.08; $P = 0.13$). Feeding corn stover pelleted with SBM or DG affected eating behavior and increased eating time per kg DM, which resulted in reduced dry matter intake. Further refinement of pellet size, hardness, or inclusion level may be warranted to effectively incorporate pelleted corn stover in dairy rations.

Key Words: rumination, crop residue, behavior

M47 Providing environmental enrichment during the milk-feeding stage influences cognition of group-housed dairy calves. K. N. Gingerich*, K. C. Horvath, and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Environmental complexity influences cognitive development and may benefit group-housed calves. We hypothesized that environmental enrichment would improve success in a cognitive task. Holstein heifer calves were randomly assigned to different group pens with access to either hay (H; n = 6), a rotating brush (B; n = 6), or no additional enrichment (C; n = 5). All calves received milk replacer (8 L/d) via an automated milk feeder and grain concentrate ad libitum. At wk 5 of age, calves were tested in a T-maze with a reward (0.2 L milk). Calves were assessed first in initial spatial learning and then in reversal learning, where the reward location was changed to the opposite arm. Calves received 5 sessions/d for 5 d until they met a passing criteria (moving directly to correct side in 3 consecutive sessions) or reached a maximum of 20 sessions. We recorded the time to find the reward (completion time) for individual sessions and the total number of sessions required to pass both learning stages. Data were analyzed by stage in a generalized linear mixed model, with session as a repeated measure for completion time. All B and H calves passed initial learning and 80% (n = 4) C calves passed. Number of sessions required to pass initial learning did not differ (5.9 sessions; SE = 2.38; $P = 0.52$). In reversal learning, there was an effect of enrichment on number of sessions required to pass ($P < 0.001$), with C calves requiring more sessions than H calves ($P = 0.005$) and B calves having an intermediate outcome (13.0 vs. 9.83 vs. 6.83; C vs. B vs. H; SE = 1.22). Completion time did not differ between treatments during initial (50.2 s/session; SE = 18.2; $P = 0.40$) or reversal (75.4 s/session; SE = 21.4; $P = 0.59$) stages, but decreased over sessions ($P < 0.001$). These results suggest that environmental enrichment, par-

ticularly nutritional enrichment, may improve behavioral flexibility of group-housed calves.

Key Words: dairy calf, enrichment, cognition

M48 Individual differences in responses to weaning in dairy calves. H. W. Neave*, J. Benetton, J. H. C. Costa, D. M. Weary, and M. A. G. von Keyserlingk, *University of British Columbia, Vancouver, BC, Canada.*

Little is known about individual differences in responses to weaning and how these relate to other individual traits. The aim of this study was to associate measures of early-life vitality, learning ability, and personality of dairy calves with time to wean, weight gains, and feed intake over the weaning period. Calves (n = 40) were scored for calf vitality (score 1–10), suckle strength (score 1–4), and milk intake at first meal and in the first week. Calves were moved to group pens at d 1 of age and scored for number of assists required to learn to drink from the milk feeder. Calves received 12 L/d of milk until d 30. Milk was then reduced by 25% relative to the individual's previous 3-d intake average. Milk was reduced by another 25% at each of 2 starter intake targets: 225 and 675 g/d (based upon 3-d rolling averages), and calves were completely weaned at the target of 1300 g/d. Feed intakes and weight gains were summarized over 4 experimental periods: pre-wean (d1–30), weaning (d31 until weaned), post-wean (weaned to d105), and total experimental period (d1–105). Personality measures were scored individually in novel arena, novel human, and novel object tests at d 21. A principal component analysis identified 5 factors that explained 68% of the variance. Factor 2 (active and playful in novel arena) was associated with greater pre-wean milk intake ($P = 0.08$) and pre-wean ($P = 0.1$) and post-wean ($P = 0.01$) ADG, Factor 3 (reduced vitality, increased inactivity) with reduced pre-wean milk intake ($P = 0.05$) and reduced final weight ($P = 0.06$), and Factor 4 (reduced first-week milk intake, slow to learn to drink milk) with reduced starter intake across all periods ($P = 0.01$), reduced pre-wean ADG ($P = 0.1$), and delayed age to complete weaning ($P = 0.04$). Factor 1 (high milk intake at first meal, strong suckle score, quick to learn to drink milk) and Factor 5 (less exploratory, longer to touch the novel object) were not associated with any weaning outcome. These results suggest that measures of early-life vitality, learning ability and personality are associated with feed intake and weight gains during the milk-feeding period, and calves at risk for delayed weaning may be identified by poor milk intake and learning ability.

Key Words: temperament, precision dairy, development

M49 Influences of environmental enrichment on activity and performance of group-housed dairy calves. K. C. Horvath* and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Providing different forms of enrichment can influence behavioral development, performance, and animal welfare. We examined the effects of environmental enrichment in group housed dairy calves on activity and performance. Holstein calves (n = 48) were group-housed (4/pen) and randomly assigned to receive either hay (H), a rotating brush (B), or no additional enrichment (C). All calves received milk replacer (8 L/d) through an automated milk feeder and grain concentrate. Calves were weaned over 10 d beginning at wk 7 of life. We recorded daily feed intake, weekly bodyweight and coat cleanliness (0 = clean; 1 = dirty), and standing time using electronic data loggers. Data were analyzed by stage (preweaning and weaning) in a generalized linear mixed model with week as a repeated measure. Cleanliness score was analyzed by week using Fisher's Exact Test. During preweaning, concentrate intake was similar between pens (0.58 kg DM/d; SE = 0.14; $P = 0.24$), but total solid feed intake (including hay) tended to evolve differently over time ($P = 0.07$) and calves offered hay had greater total intake in wk 6 (1.6 vs. 0.95. vs. 0.94 kg DM/d; H vs. C vs. B; SE = 0.17; $P = 0.02$). Preweaning ADG was similar between treatments (0.81 kg/d; SE = 0.04; $P = 0.19$). Standing time (402.2 min/d; SE = 9.7; $P = 0.60$) and standing bout frequency (19.2 bouts/d; SE = 0.85; $P = 0.28$) did not differ before weaning. During weaning total solid feed intake (3.23 kg DM/d; SE = 0.37; $P = 0.79$) and ADG (0.67 kg/d; SE = 0.34; $P = 0.79$) were similar. Daily standing time did not differ during weaning (439.4 min/d; SE = 9.7; $P = 0.80$) but B calves had more frequent standing bouts (B: 17.5 vs. C: 14.6 vs. H: 14.0 bouts/d; SE = 0.84; $P = 0.03$). Calves provided a brush were cleaner during weaning (B: 50% vs. C: 6% vs. H: 0% clean in wk 8; $P < 0.001$). These results suggest positive effects of enrichment for group-housed calves, with hay access stimulating intake and brush access influencing activity and improving cleanliness.

Key Words: dairy calf, enrichment, performance

M50 Acquired immunity and performance of single versus pair housed Holstein dairy calves. C. B. Kesterson*[1], L. G. Schneider[1], M. Caldwell[2], P. D. Krawczel[1], and G. M. Pighetti[1], [1]*The University of Tennessee, Knoxville, Knoxville, TN,* [2]*College of Veterinary Medicine, The University of Tennessee, Knoxville, TN.*

Our objective was to determine the effects of pair vs individual housing on acquired immunity and body weight (BW) of pre-weaned Holstein calves. Calves with successful passive transfer of immunity (STP >5.5 g/dL) were enrolled into pair (n = 28) or individual (n = 14) housing on 5 ± 1 d postpartum. Regardless of treatment, 2.1 m^2 of housing space/calf was available. In the paired treatment, only 1 calf was used for data collection. Milk replacer (protein 26: fat 20; 3L) was fed 2x/d, and grain and water was provided ad libitum. On 7 ± 3 d postpartum, calves were injected with 1 mL of keyhole limpet hemocyanin (KLH; 0.1 mg), Quil-A adjuvant (0.5 mg), and nonpyrogenic saline. A secondary injection was given on d 21 ± 3 including KLH (0.1 mg), Quil-A (0.5 mg), and heat-killed *Candida albicans* (CA, 2×10^6 cells) in nonpyrogenic saline. Serum samples were collected 0, 7, and 14 d after injections. IgG from each collection day were evaluated by ELISA. On d 28 ± 3, calves were inoculated with intradermal injections of CA (2×10^6 cells) or saline in the neck to evaluate cell-mediated immunity. Injection sites were measured with calipers at 0, 6, 24, and 48 h post-injections. A stimulation index (SI) was calculated by mean CA response over saline response in cm. BW was measured weekly, at weaning, and 1 wk-post-weaning. Separate linear MIXED models (SAS 9.4, Cary, NC) were used to evaluate the effects of housing treatments on CA, IgG, and BW. The SI was similar in both housing treatments ($P = 0.81$). However, SI increased over time ($P < 0.01$), and peaked at 24 and 48 h compared with 0 and 6 h ($P < 0.01$; h 0 = 1.10, h 6 = 1.18, h2 4 = 1.58, h 48 = 1.58 ± 0.08 cm). IgG concentrations to KLH were similar between both housing treatments ($P = 0.78$). IgG concentrations at d 7 were lower than d 0 and 14, regardless of treatment ($P < 0.01$; d0 = 0.77, d7 = 0.56, d14 = 0.71 ± 0.06 ELISA units/d). Maternal antibodies may have interfered with binding of KLH. ADG ($P = 0.35$) and BW gain 1 wk post-weaning were similar between treatments ($P = 0.67$). Regardless of housing type, calves with successful maternal antibody transfer had similar adaptive immune responses and BW gains.

Key Words: calf, immunity, pair housing

M51 Using behavior as an early predictor of calf's health disorder. M. A. Belaid*[1], M. Rodriguez-Prado[1], D. V. Rodriguez-Prado[2], E. Chevaux[3], and S. Calsamiglia[1], [1]*Animal Nutrition and Welfare Service, Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, Bellaterra, Spain,* [2]*Universitat Pompeu Fabra, Barcelona, Spain,* [3]*Lallemand Animal Nutrition, Blagnac, France.*

Few studies have focused in calf behavior as an early predictor of health disorders. The objective of this study is to use calf behavior as early predictor of health disorders. A total of 325 calves (male calves from 30 to 90 d of life) were fitted with pedometers (Trackacow, ENGS system, Israel) to measure calves steps, lying time, standing time, changing position from lying to standing (swaps) and access to the feed bunk (number of times and time per access). The incidence of health problems, weaning time and preventive treatment time (vaccine and antibiotics in water) were recorded. Data were analyzed by using GLIMMIX procedure of SAS (version 9.4) and significance was declared at $P < 0.05$. On average, calves spent 16.3 h/d lying, 4.7 h/d standing, changed position from lying to standing 19.3 times/d, and did 1,468 steps/d. They attended to the feed bunk 10.4 times/d and spent a total of 3.0 h/d (average of 17.3 min/visit). Sick calves (n = 24, 80% respiratory diseases) versus healthy calves spent more time lying (+5.0%), less time eating (−5%) and did less steps (−5.0%) during 3 to 4 d before being diagnosed. These changes in behavior allow predicting the occurrence of health disorder with 73% accuracy 3d before actual diagnostic. Moreover, weaning increased eating time (+15%), the number of visits to the feed bunk (+13%) and the number of steps (+7.0%); and preventive treatments reduced intake time (−20%), the number of visits to the feed bunk (−5%) and the number of steps (−11%), and increased the number of swaps (+9%). These effects have to be considered in the interpretation of results to avoid false alarms. Calf behavior monitored with pedometer can help to predict sick calves before they manifest clinical symptoms allowing early treatment.

Key Words: calves, behavior, pedometer

M52 Influences of disbudding on feeding behavior and brush use in group-housed dairy calves. C. L. Hixson* and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Disbudding of dairy calves, a common on-farm practice, results in pain-associated behavioral changes. The objective of this study was to determine the effects of disbudding on feeding behavior and use of a rotating brush in group-housed dairy calves. Holstein calves (n = 7) were placed in existing group pens at 2 wk of age (4 calves/group) and provided milk replacer (8 L/d) via an automated milk feeder and received grain concentrate and water ad libitum. Calves were disbudded by hot iron at 29.7 ± 3.3 d of age (mean ± SD). Milk intake and feeding behavior data were obtained from the automated milk feeder and brush usage was recorded continuously from video for 72 h encompassing the day before (d −1), day of (d 0), and day after (d 1) disbudding. Data were analyzed in a general linear mixed model with day as a repeated measure. Milk intake (7.2 L/d; SE = 0.44; $P = 0.30$) and frequency of rewarded visits to the feeder (6.2 visits/d; SE = 1.31; $P = 0.30$) did not change following disbudding. The frequency of unrewarded visits decreased on the day of disbudding (0.67 vs. 5.0 visits/d; d 0 vs. d −1; SE = 1.18; $P = 0.036$), but promptly returned to baseline (d −1 vs. d 1; $P = 0.73$). The frequency of using the brush to rub the head did not change after disbudding (32.0 times/d; SE = 4.7; $P = 0.90$), but calves rubbed their head for longer during each visit on the day of disbudding (46.1 vs. 27.1 s/visit; d 0 vs. d −1; SE = 3.23; $P = 0.009$). Brush usage returned to baseline on the following day (d −1 vs. d 1; $P = 0.26$). Disbudding did not influence the frequency (15.6 times/d; SE = 4.4; $P = 0.26$) or duration (16.0 s/visit; SE = 2.3; $P = 0.17$) of using the brush to rub parts of the body other than the head. These results reflect behavioral changes possibly associated with pain related to disbudding and suggest that access to a brush may provide comfort following disbudding.

Key Words: dairy calf, disbudding, behavior

M53 Round-day behavior of ewe-lambs at grazing. E. González-García*[1], M. Alhamada[1], A. C. C. Souza[2], Z. Holubová[3], and G. Bishop-Hurley[4], [1]*SELMET (Systèmes d'Élevage Méditerranéens et Tropicaux), INRA, Montpellier SupAgro, CIRAD, Univ Montpellier, Montpellier, France,* [2]*Universidade Estadual de Londrina (UEL), Londrina, Brazil,* [3]*Czech University, Prague, Czech Republic,* [4]*CSIRO, Queensland, Australia.*

An experiment was conducted in the springtime with the objective of characterizing the behavior of Romane ewe lambs under the changing grazing and weather conditions of La Fage INRA Farm (France). Fifteen ewes (38.1 ± 2.2 kg BW; 11 mo) were submitted to a rotational grazing system during 2 mo (stocking rate: 15 ewes/ha). The 1 ha pastureland (mix of grass-legume; Table 1), was divided in 27 paddocks (~290 m^2) with grazing stays of 2 to 3 d/paddock. The biomass availability and its nutritive value (NV) increased and decreased with time, whereas average temperature and rainfall were 14.7°C and 2.24 mm/d, respectively. Four equidistant days were scheduled to record the time spent for grazing (GRZ), ruminating (RUM), walking (WALK), resting (REST), standing (STD), urinating (URI) or defecating (DEF). Visual observations were made by 2 operators every 10 min. during 24-h. Notations combined 2 behaviors if required (e.g., GRZ while WALK) and were based on the % of animals in one or another activity each time. The MIXED procedure, with repeated measures, of SAS was used for data analyses. Ewe behavior was significantly modified by biomass quality. As the experiment advanced, NV was affected and more time was spent for GRZ, which normally started after dawn (~0500 h), with peaks at 0540, 0930, 1550, 1740 h, and finally from 1920 to 2100 h. The GRZ-REST cycles were coordinated. The 100% ewes were in REST from midnight until dawn. The REST was significantly related to RUM, mainly during

Table 1 (Abstr. M53). Herbage quality (% DM) at the entrance (In) and exit (Out) from each paddock

	DM		OM		CP		NDF		ADF		ADL		DMD	
Test day	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
D1	22	23.5	93.0	92.7	13.3	9.8	48.8	48.9	24.5	24.5	3.7	3.0	68.3	67.1
D2	29	51.0	95.2	94.5	8.6	7.5	58.9	60.4	31.3	34.1	4.7	6.2	56.5	51.4
D3	35	56.0	91.6	94.4	7.7	4.7	52.6	59.6	30.0	33.3	5.9	5.3	57.9	51.7
D4	43	27.0	92.3	94.7	6.4	6.2	54.8	63.2	31.2	35.1	5.9	6.6	56.3	47.6

Table 1 (Abstr. M54). Water intake behavior pre- and postpartum by breed

	Prepartum				Postpartum			
Item	Holstein	Gir	Crossbreed	SEM	Holstein	Gir	Crossbreed	SEM
Total daily water intake (kg)	36.4[a]	12.3[c]	22.9[b]	2.6	57.8[a]	20.0[c]	39.3[b]	3.6
Daily time spent drinking (min)	77.1[a]	12.1[c]	32.9[b]	5.0	89.0[a]	13.2[b]	26.8[b]	3.7
Visits to the drinker (no.)	4.4[a]	2.2[b]	4.3[a]	0.4	5.2[a]	5.5[a]	4.9[a]	0.5
Duration of visits (min)	17.8[a]	4.1[c]	8.8[b]	1.2	14.4[a]	2.7[b]	5.1[b]	2.1

[a,b,c]Means in the same row followed by different letters are distinct ($P < 0.05$).

the morning and afternoon. The URI and DEF peaks were at noon and during the night. These results are original as minimal literature is available regarding detailed 24-h diurnal behavior of grazing sheep. These data provide new opportunities for improving flock management and the efficiency of using the available biomass based on the combined dynamics of individual and mob-based grazing behaviors during the day.

Key Words: coordinated behavioral activity, grazing and ruminating, biomass and environment

M54 Water intake behavior of lactating heifers during the transition period. S. C. B. Stivanin[1], E. F. Vizzotto[1], V. Fischer*[1], F. S. Machado[2], M. M. Campo[2], T. R. Tomich[2], and L. G. R. Pereira[2], [1]*Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil,* [2]*Empresa Brasileira de Pesquisa Agropecuaria, Juiz de Fora, MG, Brazil.*

This study aimed to evaluate the water intake behavior, during the transition period, of Holstein, Gir and Holstein-Gir crossbreeds heifers receiving different TMR allowances. Thirty-six heifers with BW of 715 ± 64.5 kg, and BCS of 4.2 ± 0.3 were randomly assigned to 1 of 2 TMR allowances: 1.69 or 1.89% of BW (values expressed as TMR dry matter) during the prepartum period. Forage to concentrate ratio of the basal TMR was 82:18. Forage was composed of corn silage and sorghum silage, concentrate was based on ground corn and soybean meal. Automatic drinkers weighed and registered the amount of ingested water, time spent at the water trough, number of drinking events, mean duration of visits to the water trough and water ingestion rate. Data were submitted to variance analysis, using the Mixed procedure of SAS according to a completely randomized design and repeated measurements, considering the effect of diet allowance, genotype, day of measurement and their interactions, days as repeated measurements; BW and BCS were used as covariates. Number of events was analyzed using nonparametric statistics (Wilcoxon test). No interaction between genotype, TMR offer and day were detected except for water ingestion rate in the prepartum. Holstein and crossbreed heifers showed lower intake rates than Gir when fed TMR at 1.89% of BW, 0.5 ± 0.2, 0.9 ± 0.2 and 2.7 ± 0.2 kg/min, respectively. Genotype did not affect water intake rate in the postpartum (2 ± 0.5 kg water/min). Holstein heifers had higher water intake, spent more time drinking, had more visits to the water trough, and spent more time per visit drinking than Gir (Table 1). Genotype was associated with the amount of ingested water and behavior related to water ingestion.

Key Words: behavior, *Bos indicus*, *Bos taurus*

M55 Validating a novel precision dairy monitoring technology recording activity, rumination, and feeding behaviors in dairy cattle. S. Gavigan*[1], M. Borchers[1], and J. Bewley[2], [1]*University of Kentucky, Lexington, KY,* [2]*CowFocused Housing, Bardstown, KY.*

The purpose of this research project was to evaluate the Nedap Smarttag Neck 2.0 (Nedap Identification Systems, Groenlo, the Netherlands) for dairy cow behavior measuring accuracy. The behaviors measured were time inactive, ruminating, feeding, and miscellaneous activity or movement. Fifty lactating Holstein dairy cows were randomly selected from the University of Kentucky's Coldstream Dairy Research Herd and fitted with the technology. The same observer observed each cow on a separate day for a total of 4 h, including 2 h after the morning milking (0800 h) and 2 h after the evening milking (2000 h). The observer recorded the time behaviors occurred using a synchronized watch (CASIO, CASIO America, Inc., Dover, NJ). The hour, minute, and second of the day each behavior occurred was compared with corresponding technology measurements. Concordance correlation coefficients (CCC; epiR package; R Foundation for Statistical Computing, Vienna, Austria) and Pearson correlation coefficients (r; CORR procedure; SAS Institute Inc., Cary, NC) were used to calculate association between visual observations and technology-recorded behaviors. Results are shown in Table 1. Visually recorded inactive time and rumination time were moderately correlated with technology data. Visually recorded feeding behaviors were strongly correlated with technology data but visually recorded miscellaneous activity behaviors were poorly correlated. The Nedap Smarttag Neck 2.0 accurately monitored activity, feeding, and ruminating behaviors and would be effective in monitoring these behaviors in a commercial farm setting.

Table 1 (Abstr. M55). Results of a validation study between technology or visual observation measured behaviors in Holstein dairy cattle

	Pearson correlation		Bias	Location	Scale shift	
Variable	(r)	*P*-value	correction	shift (V)	(μ)	CCC
Ruminating	0.86	< 0.01	0.97	−0.12	1.22	0.83
Inactive	0.87	< 0.01	0.98	−0.16	0.85	0.85
Eating	0.90	< 0.01	0.99	0.06	0.92	0.89
Other	−0.18	0.43	0.46	1.26	2.35	−0.08

Key Words: correlation, Nedap Smarttag Neck, validation

Table 1 (Abstr. M56). Mean number of steps per day and lying time (h/d) 3 days before, day of estrus (0) and 3 days after estrus and between BCS ≤2.5 (n = 22), BCS 2.75 (n = 81) and BCS ≥3 (n = 35)

		Day								*P*-value		
Activity	BCS	−3	−2	−1	0	1+	2+	3+	SED	BCS	Day	BCS × day
Steps, no./d	≤ 2.5	1472	1468	1756	3982	1768	1410	1392	227.3	0.288	<0.001	0.715
	2.75	1611	1522	1716	4481	1890	1705	1579				
	≥3	1652	1686	1783	4362	1965	1718	1716				
Lying time, h/d	≤ 2.5	11.8	12.3	12.0	10.0	12.1	12.0	12.3	0.547	<0.001	<0.001	<0.001
	2.75	10.2	10.1	9.6	6.1	11.0	10.8	10.4				
	≥3	10.1	9.9	9.7	6.7	11.3	10.9	10.7				

M56 Effect of body condition score on activity of dairy cows in estrus. H. M. H. Zebari*[1], G. L. Charlton[2], M. S. Rutter[2], and E. C. L. Bleach[2], [1]*College of Agriculture, University of Duhok, Duhok, Kurdistan Region, Iraq,* [2]*Department of Animal Production, Welfare and Veterinary Sciences, Harper Adams University, Newport, Shropshire, United Kingdom.*

Poor estrus expression is considered one of the main causes of declining fertility in dairy cows where artificial insemination is used. One of the factors that affects estrus expression is the body condition score (BCS). This study aimed to investigate the effect of BCS on the number of steps and lying time (h/d) during estrus. Lactating Holstein Friesian cows (n = 92) were used from 14 d postpartum with mobility score 2.1 ± 0.5 (mean ± SEM) and milk yield 31.6 ± 9.3 kg/d at the Harper Adams University dairy unit. Cows were housed in a free stall yard with sawdust covered, rubber mattresses, and grooved concrete passageways. They were fed a TMR ad libitum with grass during the summer. Their BCS was measured according to AHDB Dairy (2014). Milk samples were collected 3 d per week for progesterone (P4) ELISA (Ridgeway Science Ltd., UK). Activity was monitored continuously using IceQubes (IceRobotics Ltd., Edinburgh, UK) attached to the back left leg. A cow was considered in estrus when milk P4 concentration was <3 ng/mL, followed by an increase to >15 ng/mL and an IceQube estrus alert was recorded. The data were analyzed by repeated measures ANOVA (GenStat 17th edition). The number of steps were increased (Table 1) and lying times (h/d) were reduced ($P < 0.001$) on the day of estrus (0) compared with 3 d before and 3 d after estrus. On the day of estrus, more steps ($P < 0.001$) were recorded in BCS 2.75 cows compare with BCS ≤ 2.5 and BCS ≥ 3 cows. Lying time were higher ($P < 0.001$) in BCS ≤ 2.5 cows compared with other BCS. The interaction between BCS and time was significantly ($P < 0.001$) affected on lying time. In conclusion, dairy cows that had a BCS 2.75 during estrus spent more time active at the expense of the lying time on the day of estrus.

Key Words: estrus, steps, lying time

M57 Pathologic features of *Staphylococcus aureus*-induced mastitis in dairy cows and isobaric tags for relative and absolute quantitation proteomics analyses. Z. Chen, M. Li, H. Zhang, Y. Mao, and Z. Yang*, *College of Animal Science and Technology, Yangzhou University, Yangzhou, China.*

Mastitis in dairy cattle causes significant economic losses through the decreased production and quality of milk. Pathogenic microbial infection is the main cause of mastitis, with *Staphylococcus aureus* being a major causative pathogen. *Staphylococcus aureus* can colonize and multiply in the mammary gland, and can enter and persist in cells. Most antibiotics cannot enter cells or may be inefficient if their cellular concentration is too low. As with many bacteria, *S. aureus* can develop resistance to the antibiotic being used. Residual antibiotics in milk present significant food safety problems for human health. Therefore, non-antibiotic methods for the prevention and treatment of bovine mastitis are of great interest. We used teat tube perfusion to establish a Chinese Holstein cattle clinical mastitis model. Clinical examination, Beijing mastitis test, microscopy enumeration of somatic cell count, and histopathology confirmed the successful establishment of the mastitis model. The influence of mastitis on the mammary tissue proteomic profile was investigated using isobaric tags for relative and absolute quantitation. Healthy and diseased mammary tissue were screened and differentially expressed proteins were identified. Bioinformatic analysis identified proteins related to inflammation and immunization of dairy cows. In this study, the immune response of dairy cow to *S. aureus* was explored. Western blotting and immunohistochemical staining were used to study the levels of PGLTRP1 and PTX3 proteins in mammary gland tissue and to characterize the changes of these immune-related candidate proteins. The results show that PRLYRP1 and PTX3 play an important role in the development of bovine mastitis and future studies of PRLYRP1 and PTX3 are warranted.

Key Words: *Staphylococcus aureus*, mastitis, dairy cows

M58 BoHV-1 neutralizing antibody response of calves vaccinated with licensed infectious bovine rhinotracheitis (IBR) modified live virus vaccines in field. O. Boix-Mas, M. Baratelli, M. Blanch-Freixa, V. Robles, and J. P. Campillo-Benéitez*, *HIPRA, Amer, Girona, Spain.*

Infectious bovine rhinotracheitis (IBR) is a worldwide endemic viral disease (BoHV-1) of cattle. Various vaccination plans against IBR are implemented in field; however, little is known about their ability to induce neutralizing antibody immune responses. The objective of this study was to evaluate this type of immune responses in calves after they had been vaccinated with several licensed (gE−) modified live virus (MLV) marker vaccines in the field. BoHV-1 gE/gB seronegative calves of 6 to 7 mo of age were selected from a fattening farm. Animals were randomly allocated into groups and vaccinated with either the gE−/tk− MLV HIPRABOVIS IBR MARKER LIVE (G1, n = 15) or 2 other licensed gE− MLV (G2, n = 15; G3, n = 14). One group, G4 (n = 12) did not receive any vaccine. G1 was boost-vaccinated with a further dose of the same vaccine as specified by the manufacturer's instructions. Sera were collected before and 42 d post-vaccination (dpv) in all groups and tested by a virus neutralization assay against "Los Angeles" BoHV-1 strain. Titers higher than 1/2.5 were considered positive. Statistical comparisons were performed using the Mann-Whitney U-test. Neutralizing antibody responses against BoHV-1 were detected at 42 dpv in all vaccinated calves and titers ranged from 1/5 to 1/240. The highest titers were observed in G1 and differences were statistically significant. In particular, a subpopulation of animals (25%) with titers higher than 1/120 was only detected in G1. G2 did not show any statistically significant difference when compared with G3. The licensed MLV marker vaccines studied were able to induce a neutralizing antibody immune response against BoHV-1 providing a complete protection to the selected herds. Strength of the responses varied based on the type of vaccine used, although results showed that HIPRABOVIS IBR MARKER LIVE conferred the highest protection level.

Table 1 (Abstr. M58). Neutralizing antibody responses against BoHV-1 at 42 dpv

Group	Arithmetic Mean	SD	Geometric Mean
1	78.33[a]	60.11	60.89
2	33.57[b]	17.26	29.61
3	25.00[b]	22.66	17.35
4	0.00[c]	0.00	0.00

[a–c]Statistically significant difference between groups ($P < 0.05$).

Key Words: infectious bovine rhinotracheitis (IBR) modified live virus, marker vaccine, neutralizing antibody response

M59 The effect of pegbovigrastim on circulating neutrophil count in dairy cattle. S. J. Van Schyndel*[1], J. Carrier[2], O. B. Pascottini[1], and S. J. LeBlanc[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Elanco Canada Ltd., Guelph, ON, Canada.*

Previous research in various species has shown that granulocyte-colony stimulating factor stimulates the production and release of neutrophils cells from bone marrow. The objective of this study was to characterize the effects of polyethylene glycol-bound bovine granulocyte colony-stimulating factor [pegbovigrastim (PEG); Imrestor, Elanco] on circulating leukocyte counts. Thirty-four Holstein cows were randomly assigned to receive 2 injections of either physiologic saline (n = 16) or PEG (n = 18), 7 d before expected calving (d −7) and within 24 h after calving (d 0). Cows were sampled at d −7, d −6, d 0, d +1, d +7, and d +21, relative to calving. We included only cows for which the interval from the first injection to calving was ≥3 d, such that the interval (mean ± SD) from first treatment to calving was 6.7 ± 1.9 d. Treatment effects were assessed with mixed linear regression models. After the first injection, neutrophil counts ($\times 10^9$/L) in PEG-treated cows increased from 4.3 (95% CI 3.8 to 4.8) at d −7 to 18.2 (CI 16.3 to 20.3) at d −6 ($P < 0.0001$). Their counts then decreased from d −6 to d 0, when the second injection was administered, at a rate of -0.31×10^9 neutrophils/L per day ($P < 0.0001$). After the second injection, neutrophil counts increased from 16.4 (CI 13.7 to 19.6) at d 0 to 32.8 (CI 25.2 to 42.7) at d +1 ($P < 0.0001$), after which counts decreased at a rate of -3.73×10^9 neutrophils/L per day until d +7 ($P < 0.0001$). Counts continued to decrease from d +7 to d +21 at a slower rate of -0.43×10^9 neutrophils/L per day ($P < 0.0001$), until baseline levels were reached. Conversely, in control cows, neutrophil counts were unchanged from d −7 to d −6 ($P = 0.86$) after the first injection and then decreased from 6.1 (CI 5.0–7.3) at d 0, to 3.2 (CI 2.4–4.2) at d +1 ($P < 0.0001$) after the second injection. Neutrophil count was greater ($P < 0.001$) in PEG than control at d −6, 0, +1, and +7. Area under the curve (cells $\times 10^9$/L per 28 d) for neutrophil counts in the PEG group was 429 versus 99 in

the control group ($P < 0.0001$). The sustained increase in circulating neutrophil count around the time of calving may contribute to improved health during the peripartum transition period.

Key Words: transition, immune function, health

M60 Changes in ruminal and fecal microbiota due to the challenges of indigestion, mastitis, and laminitis in Holstein cows. J. J. Liu, S. K. Ji, Y. J. Wang, T. Jiang, C. Y. Guo, H. Yan, Z. J. Cao, S. L. Li, and F. R. Wang*, *State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology China Agricultural University, Beijing, China.*

The objective of this study was to determine the differences in bacterial diversities and compositions of rumen and feces between healthy cows and cows with indigestion, mastitis or laminitis. Forty-five lactating dairy cows including 10 healthy, 11 with indigestion, 13 with mastitis, and 11 with laminitis were enrolled. Bacterial communities were assessed using MiSeq Illumina sequencing of the 16S rRNA gene. One-way variance analysis and LSD multiple comparisons were used to compare microbial compositions and diversity indices between groups. A $P < 0.05$ was considered significant. *Bacteroidetes* and *Firmicutes* were the predominate phyla in both the rumen fluid and the feces. Indigestion reduced the diversity of fecal bacterial community, increased the diversity of ruminal bacterial community, and changed the relative abundance of *Firmicutes* in both the rumen and feces and that of *Bacteroidetes* in the feces. Although they did not influence the diversity, mastitis and laminitis altered the compositions of the ruminal and fecal bacterial community at different taxonomic level. At the phylum level, the relative abundance of *Spirochaetes* and *Fibrobacteres* in the rumen and *Proteobacteria* in the feces were increased ($P < 0.05$), and *Firmicutes* in the feces was reduced by the indigestion challenge; and the relative abundance of *Bacteroidetes* in the rumen was reduced and *Firmicutes* in the rumen was increased ($P < 0.05$) by the laminitis challenge. At the family level, the indigestion challenge increased the proportion of *Porphyromonadaceae* in rumen as well as *Enterobacteriaceae* and *Bacillaceae* in feces, and decreased the proportion of *Lachnospiraceae* in rumen ($P < 0.05$) as well as *Clostridiaceae*, *Peptostreptococcaceae* and *Paenibacillaceae* in feces ($P < 0.05$); the mastitis challenge increased the proportion of *Porphyromonadaceae* and others at lower percentages in rumen ($P < 0.05$), and increased the proportion of *Bacillaceae* in feces; the laminitis challenge increased the proportion of *Prevotellaceae* and reduced the proportion of *Acidaminococcaceae* in rumen ($P < 0.05$). Results from this study show that the fecal or rumen microbial compositions of dairy cows with indigestion, mastitis, and laminitis shifted compared with those in healthy cows.

Key Words: ruminal microbiome, fecal microbiome, challenge

M61 Central administration of an acute phase protein, α-1-acid-glycoprotein, increases rectal temperature in sheep. B. A. Gregg*[1], P. A. Parker[1], M. K. Waller[1], M. Garcia[2], B. J. Bradford[2], J. A. Daniel[3], and B. K. Whitlock[1], [1]*College of Veterinary Medicine, University of Tennessee, Knoxville, TN,* [2]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS,* [3]*Department of Animal Science, Berry College, Mount Berry, GA.*

Bioactive lipids, such as platelet-activating factor, prostaglandins, and leukotrienes, convey febrigenic signals to the brain in response to bacterial infections, predominantly as complexes with carrier proteins, such as α-1-acid-glycoprotein (AGP). Concentrations of acute phase proteins, like AGP, change by >25% in response to inflammation, and are part of the innate immune system. AGP binds and potentially enhances the activity of platelet-activating factor, the most proximal mediator of endotoxin-induced fevers. To determine the rectal temperature dose response of sheep to central AGP administration, nonlactating, non-pregnant, adult (≥1 yr of age) mixed breed black face ewes (n = 4) weighing 79.0 ± 5.0 (SD) kg were ovariectomized and surgically implanted with a cannula into a lateral ventricle of the brain. Ewes were kept indoors in individual pens with a 12-h light/dark photoperiod and approximately 22–24°C. Ewes were fed a diet calculated to meet 100% of daily maintenance requirements and had ad libitum water. Ewes received 1 of 4 treatments [0 (control), 12 (low), 60 (medium), or 300 (high) µg/kg BW AGP (AGP from bovine plasma; Sigma Aldrich Co., Saint Louis, MO)] administered in 500 µL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride into the lateral ventricle. Treatments were tested negative for lipopolysaccharide contamination by the limulus amebocyte lysate assay. Rectal temperature was determined at −72, −48, −24, −2, 0, 2, 4, 6, 8, 12, 24, 36, and 48 h relative to treatment. The study was repeated until all sheep received all treatments with a 10-d washout period between treatments. Data were analyzed using procedures for repeated measures with JMP software (version 10.0.0; SAS Inst. Inc., Cary, NC) and tested for effects of replication, treatment, time, and treatment × time interaction. There was no effect of treatment ($P = 0.54$) or replication ($P = 0.86$), but there was an effect of time ($P < 0.0001$) and treatment × time interaction ($P = 0.002$). Rectal temperatures were greater ($P < 0.05$) with high dose compared with control at 6, 8, and 12 h or low dose at 6 and 8 h. Thus, central AGP administration can increase rectal temperature in sheep.

Key Words: fever, inflammation, sheep

M63 Effects of lameness at dry-off on the incidence of transition period disease of dairy cows. R. R. Daros*, H. Eriksson, D. M. Weary, and M. A. G. von Keyserlingk, *Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada.*

Transition period diseases continue to be a challenge for the dairy industry despite major advancements in dairy management and nutrition. Lameness during the dry period is understudied and may be associated with other transition period diseases. In the current study, we hypothesized that cows entering the dry period as lame would be more likely to become ill. Between May and December 2017, we visited 6 commercial freestall dairy farms in British Columbia. A total of 426 cows were enrolled at dry-off. Gait score at dry-off was assessed for each cow using a 5-point score. Cows scored ≥3 were classified as lame. Cows were assessed for health 4 times per cow between 3 and 18 d in milk, and were diagnosed with subclinical ketosis (SCK) based on blood BHB ≥1.2mmol/L, and puerperal metritis (PM) based on fetid watery vaginal discharge. Assessment of retained placenta (RP) was based upon farm records. Logistic regression multilevel models, using farm as random effect, were used to test the effect of lameness at dry-off on the incidence of SCK, PM, and RP. Parity was included as the covariate in all models. BCS was considered an intervening factor and not included in the models. The incidence of SCK, PM, and RP over the first 18 DIM were 36, 29 and 10%, respectively. The prevalence of lame cows at dry-off was 41% (22% primiparous, 78% multiparous). Primiparous cows were 60% (OR: 0.39; 95% CI: 0.24 – 0.63; $P < 0.01$) less likely to develop SCK postpartum than multiparous cows. No association between parity and PM or RP was found. Being lame at dry-off was not associated with SCK post calving, tended to increase the chances of PM (OR: 1.50; 95% CI: 0.95 – 2.39; $P = 0.08$) and increased the chances of RP by 1.98 times (95% CI: 1.03 – 3.80; $P =$

0.04) compared with non-lame cows at dry-off. This work indicates that lameness is associated with some transition period diseases. Strategies to reduce lameness during the dry period may improve other measures of health in early lactation.

Key Words: hyperketonemia, metritis, retained placenta

M64 Estrogen receptor alpha and progesterone receptor expression in uninfected and *Staphylococcus aureus*-infected quarters. B. D. Enger*[1], H. L. M. Tucker[1], C. L. M. Parsons[1], S. C. Nickerson[2], and R. M. Akers[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*University of Georgia, Athens, GA.*

Intramammary infections (IMI) are prevalent in nonlactating dairy cattle and are known to impact mammary structure and negatively affect the amount of mammary epithelium in the gland. It is currently unknown what chief mechanisms involved with mammary growth are affected by the presence of an IMI. This study's objective was to characterize the expression of estradiol receptor α (ESR1) and progesterone receptor (PGR) in mammary glands that were stimulated to grow and develop in the presence or absence of an IMI. Mammary growth was stimulated in 18 nonpregnant nonlactating dairy cows, using estradiol and progesterone injections, and 2 culture negative quarters of each cow were subsequently infused with either saline (n = 18) or *Staphylococcus aureus* (n = 18). Mammary parenchyma tissues were collected 5 d (n = 9) or 10 d (n = 9) post-challenge and examined using immunofluorescence microscopy. Data were analyzed using a mixed model with treatment and euthanasia day as fixed effects and cow nested within euthanasia day as a random effect. The number of ESR1-positive nuclei, observed across 8 random mammary parenchyma fields of view for each quarter, tended to be greater in saline quarters than challenge quarters (201 vs 163 ± 44 nuclei; $P = 0.1$) and were lower in tissues collected 5 d post-challenge than 10 d (130 vs. 234 ± 59 nuclei; $P = 0.04$). Saline quarters contained a greater number of PGR positive nuclei than challenged quarters (520 vs 440 ± 45 nuclei; $P = 0.024$) but, positive PGR nuclei counts were not influenced by tissue collection day ($P = 0.78$). The area and intensity of nuclear ESR1 and PGR staining was differentially affected by the effects of quarter treatment and day of tissue collection. These results indicate that IMI in nonlactating glands, that were stimulated to grow, affects the number of nuclei expressing ESR1 and PGR and alters expression characteristics of these receptors in the nucleus. Alterations in estradiol and progesterone signaling in mammary parenchyma would likely affect the signaling pathways of these key hormones involved with mammary growth and development.

Key Words: mastitis, dry cow, mammary growth

M65 Development of antibody-conjugated chitosan microparticles selectively targeting Shiga toxin producing *Escherichia coli* in the gastrointestinal tract. Z. Ma*[1,2], M. Kang[1,2], S. Meng[3], Z. Tong[3], A. Adesogan[1], and K. Jeong[1,2], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Emerging Pathogens Institute, University of Florida, Gainesville, FL,* [3]*Department of Agricultural and Biological Engineering, University of Florida, Gainesville, FL.*

The objective of this study was to develop anti-Shiga toxin-producing *Escherichia coli* (STEC) antibody conjugated chitosan microparticles (CM) to control STEC at the pre-harvest level. STEC are important foodborne pathogens, and cattle are the major reservoirs. Several methods have been tried to reduce these pathogens at the pre-harvest level. However, the trials have not been successful. CM, derived from chitosan, have shown great broad-spectrum antimicrobial activity. Here, we report that conjugation of CM and anti-STEC IgY antibodies kill STEC selectively. Lipopolysaccharides (LPS) of 7 STEC serotypes (O26, O45, O103, O111, O121, O145, and O157) were extracted by phenol-water extraction and purified with high performance liquid chromatography (HPLC). Five laying hens were immunized with each LPS respectively, and IgY antibodies were purified from egg yolk. The sensitivity and specificity of IgY were tested by ELISA. The detection limit of IgY ranged between 2 and 3 log cfu/well. Five out of 7 types of anti-STEC IgY were able to recognize the corresponding STEC serotype selectively. CM and the antibodies were linked by stable covalent amide bonds at 10:1, 10:2, and 10:4 ratios respectively. The activity of anti-O157 CM-IgY and CM at 0.05% were examined against *E. coli* O157:H7, *S. enterica* and their combination. Data were analyzed using the GLM procedure of SAS and a statistical model that included different treatments was generated. Compared with CM, CM-IgY (10:2) showed significantly stronger activity against *E. coli* O157 ($P < 0.05$), indicating that IgY enhanced the specificity of CM. When the CM and CM-IgY were applied to only *S. enterica*, no significant difference was found in the bacterial inhibition ($P > 0.1$). In a mixed culture of both bacteria, CM-IgY conjugates decreased the concentration of *E. coli* O157 significantly compared with CM ($P < 0.05$), but did not affect the concentration of *S. enterica*. The same selective activity was maintained in synthetic intestinal fluid and a mixed culture of *E. coli* O157 and *Lactobacillus plantarum*. These results suggested that the CM-IgY conjugates have strong specific antimicrobial activity, and are great candidates to eliminate pathogens selectively in the gastrointestinal tract of animals.

Key Words: chitosan microparticles, Shiga toxin-producing *Escherichia coli*

M66 *Staphylococcus aureus* surface proteins extraction method with immunoproteomics and electron microscopic study. R. D. Abdi*[1], J. R. Dunlap[2], D. B. Ensermu[1], B. E. Gillespie[1], R. A. Almeida[1], S. P. Oliver[1], and O. K. Dego[1], [1]*Department of Animal Science, The University of Tennessee, Knoxville, Knoxville, TN,* [2]*JIAM Microscopy Center and Advanced Microscopy and Imaging Center, The University of Tennessee, Knoxville, TN.*

Staphylococcus aureus is the major contagious bovine mastitis pathogen. It harbors several cell surface proteins that involve in the pathogenesis of mastitis. There is no effective vaccine against *S. aureus* mastitis. Strain variation and lack of common target antigen/s are implicated for absence of effective vaccine. The aim of this study was to extract surface proteins *S. aureus* strains from cases of bovine mastitis and evaluated their immunogenicity. Nine genetically distinct *S. aureus* strains were used in the extraction. We used cholic acid, hexadecane, and biotinylation for surface proteins extraction. After extraction, we evaluated bacterial cells integrity by Gram stain and electron microscopy. The extracted surface proteins were visualized on sodium dodecyl sulfate PAGE (SDS-PAGE) and evaluated for immunogenicity by Western blot. We used descriptive statistics for data analysis. Our results showed that the 3 extraction methods provided comparable number of protein bands on SDS-PAGE. The average visible bands were 19, 21, and 20 by hexadecane, biotinylation, and cholic acid, respectively. Western blot showed over 10 reacting surface proteins, of which 5 (18, 22, 30, 48 and 110 kDa) were conserved immunoreactive surface proteins across the 9 strains tested. Hexadecane and cholic acid induced minor damage to the bacterial cell wall as confirmed by Gram staining and scanning and transmission electron microscopy, implying minimal contamination of the surface proteins by cytosolic proteins. We concluded that

any of the 3-methods are valid for the extraction of surface proteins. However, hexadecane or cholic acid methods are easy, cheap and have minor effects on bacterial cell wall. The 5 conserved immunoreactive surface proteins may serve as potential candidates for vaccine antigen to control *S. aureus* mastitis in dairy cows.

Key Words: bacterial surface-protein, vaccine, bovine mastitis

M68 Effect of yeast-based supplements for S*almonella* prevention in dairy cows: A field study. A. Delafosse[1], B. Poupée[1], and C. Julien*[2], [1]*GDS61, Groupement de Défense Sanitaire du Cheptel Ornais, Alençon, France,* [2]*Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France.*

The objective was to evaluate the interest of yeast probiotic (Actisaf, *Saccharomyces cerevisiae* Sc47-CNCM I-4407, 10^{10}cfu/g DM, Phileo Lesaffre Animal Care, France, 5 g/h/d) and yeast fraction (Safmannan, Phileo Lesaffre Animal Care, France, 10 g/h/d) distributed during 1 year to animals from 10 French commercial dairy farms, with 102 head/farm, on average. *Salmonella* has been identified in bulk milk at least 3 times over the previous year in the 20 selected farms (shedding results higher than 50,000 cfu/g manure) assigned randomly to 2 groups (C, YP) according to *Salmonella* spp. shedding before (visit 0) starting the project: C as control group; YP receiving yeast products daily. Farms were regularly monitored (visits 1 to 4) and analyzed on 1 composite environmental fecal sample (CF) and 6 individual fecal samples (IF) collected on randomly selected cows. Shedding level was evaluated using the most probable number or NPP count method and expressed in cfu/g. Results at visits 1 to 4 were compared with visit 0 using Mann-Whitney test. Multivariable analyses were conducted, using ordinary logistic regression (OLR) model and generalized estimating equation (GEE, family = binomial) model, to account for within-herd correlation. The median CF count was higher in YP (938,200) than C group (125,800) at visit 0. Difference between groups (YP – C) was less at both visits 1 and 4 (+155,000 and +104,343) and was negative at both visits 2 and 3 (−490 and −19,000). However, this trend remained insignificant using OLR analysis. The IF was higher in YP at visit 0 (median 53,000 versus 8,700; i.e., +44,300). Difference was smaller at visits 2, 3, 4 (+3,840, +4,000, +160) and was reversed at visit 1 (−9,800). A significant decrease in shedding was observed at visit 1, compared with visit 0, only in YC (58 cattle, $P = 0.01$). The GEE analysis was conducted on 122 animals presenting at least 3 counts over the 4 control points. The model showed an overall reduction of shedding in YP cattle, but the trend remained insignificant (coeff. −0.448, SD 0.674; adjusted odds ratio 0.64, 95% [0.17–2.39]). Consequently, feeding yeast products could help to reduce excretion of *Salmonella* on dairy farms. Further investigations are needed to confirm the results.

Key Words: *Salmonella*, dairy cow, yeast product

M69 Feed restriction as a model to induce systemic inflammation in dairy cows before calving. O. B. Pascottini*[1], M. R. Carvalho[2], E. Ticiani[2], J. F. W. Spricigo[2], E. S. Ribeiro[2], and S. J. LeBlanc[1], [1]*Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Most dairy cows experience a substantial decrease in feed intake in the 1 to 2 weeks preceding parturition, which has been associated with systemic inflammation (SI). We aimed to assess the association of prepartum decrease in feed intake and the onset of SI, and if present, the ability of anti-inflammatory treatment to mitigate inflammation. Holstein cows (n = 45) were blocked on parity (1.1 ± 1, range 0 to 3) and BCS (3.6 ± 0.3, range 3.25 to 4) and assigned to control (n = 13), untreated feed restriction (FR-U; n = 15), or feed restriction treated with anti-inflammatory (FR-T; n = 17) groups. Daily feed intake was measured from −22 d before expected parturition until 35 d postpartum. All cows received the same total mixed rations during the dry and fresh periods. Control cows were fed ad libitum while FR-U and FR-T cows were reduced to 70% of their average intake for 4 d (−15 to −12 d). FR-T cows received meloxicam (0.5 mg/kg of BW) once/day for 4 d (−13 to −10 d). Blood samples were collected before feeding on d −22, −15, −14, −13, −12, −10, −7, −5, −3, 0, 1, 3, 5, 7, 15, 22, and 35 from calving to measure total protein, albumin, urea, glucose, GGT, AST, GLDH, BHB, NEFA, haptoglobin, and IGF-1. Cows with dystocia or clinical metabolic disease were excluded from the analyses of the postpartum but not from the prepartum period. Treatment effects were assessed by linear regression models treatment, sampling day, and their interactions. All outcomes changed ($P < 0.05$) with time independent of treatment. In both FR groups, concentration of NEFA in serum gradually increased from the first until the last day of restriction, when it was higher ($P = 0.02$) than the control group (control 0.28 ± 0.03, FR-U 0.67 ± 0.1, and FR-T 0.67 ± 0.05 mmol/L). FR increased serum urea concentrations on −14 d ($P = 0.05$), but on −10 d it was lower than the control ($P = 0.02$). No differences among treatments were observed for the remaining metabolites. This model of feed restriction to mimic that seen in the week before calving produced substantial fat mobilization but not, based on the present indicators, the hypothesized induction of SI.

Key Words: dairy cow, transition period, metabolic inflammation

M70 Impact of colostrum pasteurization on serum concentration of selected cytokines. M. Terré*[1], A. Bassols[2], M. Vidal[1], J. Galian[3], and A. Bach[4,1], [1]*Institut de Recerca i Tecnologia Agroalimentàries, Caldes de Montbui, Spain,* [2]*Universitat Autònoma de Barcelona, Bellaterra, Spain,* [3]*Granja Murucuc, Gurb, Spain,* [4]*Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.*

Levels of IgG are usually used to evaluate colostrum quality. However, there are other bioactive substances such as cytokines, which pasteurization may also affect. The objective of the study was to evaluate the effects of colostrum pasteurization (60°C during 60 min) on performance and cytokine plasma levels. Sixty dairy Holstein male calves (44.9 ± 0.38 kg BW) from birth to weaning (56 d of age) were enrolled in the study. Calves were born in a commercial dairy farm, and they were offered within the first 2 h after birth 4 L of colostrum either non-pasteurized (NP) or pasteurized (P). All colostrum was previously frozen at −18°C within the following 2 h after being milked. All calves received the same MR feeding program and the same concentrate starter during the preweaning period. Animals were weighed weekly, intakes recorded daily, and blood samples obtained at 24 h after the colostrum was fed, and at 2 and 5 week of age to determine general biochemistry. Fecal and respiratory scores from 1 to 3 were recorded daily. Fifteen calves per treatment that consumed the 4 L of colostrum offered were selected to determine IgG, TNFα, and IL-1β in serum samples. Data were analyzed with a mixed-effects model with repeated measures being pasteurization and time the main effects of the model. There were no differences in growth rate among treatments, neither in the gain to feed ratio nor in the incidence of fecal and respiratory scores. Although colostrum IgG concentration was similar in both NP and P colostrum (2,366 ± 251.5 mg/dL), greater IgG and a tendency in greater TNFα serum concentrations were observed in NP compared with P colostrum fed calves (3,356 vs 3,183 ± 0.053 mg/dL 58.3 vs 28.5 ± 11.38 pg/mL, in NP and P colostrum fed calves, respectively). There were no differ-

ences in general biochemistry at 24-h samples, but P fed calves had greater serum levels of cholesterol, total proteins, and tended to have lower AST serum levels throughout the study. Although no effects on performance were demonstrated when feeding P or NP colostrum to calves, some blood biochemical changes were observed, which might affect calf development.

Key Words: calf, colostrum, cytokine

M71 Genetic diversity and associated enterotoxin production patterns of *Staphylococcus aureus* isolates from cases of bovine mastitis. J. M. Vaughn*, R. D. Abdi, B. E. Gillespie, C. E. Merrill, and O. K. Dego, *The University of Tennessee, Knoxville, TN.*

Staphylococcus aureus is an important zoonotic pathogen which has significant consequences for animal and human health. *S. aureus* is one of the many foodborne pathogens that causes food poisoning through its diverse enterotoxins. It is believed that *S. aureus* strains that infect different host species are genetically distinct although some strains are thought to be infective to wide range of host species. Some reports also showed the presence of distinct strains of *S. aureus* with specific tissue-tropism that are adapted to infect specific tissues such as the mammary gland. However, there are no clearly defined clonal patterns of *S. aureus* isolates that cause infection in any given species of animals or human. The objectives of this study are to (1) evaluate clonal diversity of *S. aureus* isolates from cases of bovine mastitis to determine dominant clones responsible for bovine mastitis; and (2) determine enterotoxin gene patterns of *S. aureus* isolates from cases of bovine mastitis. Milk samples from cases of bovine mastitis collected from various farms in the eastern Tennessee area for diagnosis were evaluated at Tennessee Quality Milk Laboratory (TQML) for causative agents. Overall, 111 *S. aureus* isolates from these cases were evaluated for toxins genes such as toxic shock syndrome toxin gene 1 (*tsst1*) and enterotoxin genes by each gene-specific primer pair using PCR. All 111 *S. aureus* isolates were evaluated for *seA*, *seB*, and *seC, seE, seJ,* and *tsst1* using PCR. Negative and positive controls were used in each PCR reaction. The clonal diversity of *S. aureus* isolates from cases of bovine mastitis has been evaluated by pulsed field gel electrophoresis (PFGE). These data were analyzed using GLIMX model for logistic regression in SAS 9.4. Results showed that few isolates were positive for the enterotoxins tested. The genetic diversity analysis shows 16 PFGE types throughout 11 farms. One type stood out, with half of the population of isolates being present among 9 farms. These results suggest a negative pattern for toxins and indicate that one main type predominates in the areas tested, which could help area specific control of *S. aureus* mastitis.

M72 Galectin expression in blood of cows with high and low milk somatic cell count. B. K. Mulakala*, E. Eluka-Okoludoh, S. Adjei-Fremah, E. Asiamah, K. Ekwemalor, S. Ibrahim, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Galectins (Gal) are β-galactosidase-binding proteins that play a role in inflammatory diseases. Somatic cell counts (SCC) in milk are indicators of inflammation. The objective of this study was to assess the expression of galectin genes (LGALS) in cow blood and its possible relationship to SCC in milk. Blood was collected from the jugular vein of 10 clinically healthy Holstein cows. Based on the average of Dairy Herd index reports, 5 cows each were assigned to the High or low SCC group. Total RNA was isolated from whole blood, pooled and transcribed to cDNA, for Real-time PCR. Cow *LGALS-1*, *-2*, *-3*, *-4*, *-7*, *-8*, *-9*, and *-12* specific primers were designed using the NCBI Primer 3 tool. Housekeeping gene β-actin served as internal control. Fold changes were calculated using the Livak method. Plasma was extracted from whole blood. Total plasma protein concentration was determined using the Bicinchoninic acid assay kit. Cow Gal-1, -2, -4, -8, -9, and -12 specific ELISA kits were used to assess secretion. Data were analyzed using Proc ANOVA procedure in SAS 9.4. *LGALS-1*, *-2*, *-4*, *-12* were expressed in HSSC and LSCC. Transcription of *LGALS*, *-1*, *-2*, *-4*, *-12* was higher in HSCC (1.12-, 6.68-, 5.82-, 9.25-fold, respectively) cows compared with LSCC. *LGALS-7* and *-8* were not observed in both groups. Transcription of *LGALS-3* and *-9* were expressed only in LSCC. Total protein concentration was higher in LSCC ($P = 0.0362$) compared with HSCC. Gal-1, -2, -4, -8, -9, and -12 were secreted in both groups. Concentrations of Gal-4 was 11.4% higher in HSCC compared with LSCC, whereas Gal-2 was 3.5% higher in LSCC. Gal-12 was higher in HSCC ($P = 0.036$) compared with LSCC. Gal-1, -8, and -9 remained unchanged. LGALS transcription and Gal translation patterns were different between HSCC and LSCC cows. The observed difference in HSCC and LSCC cows warrants further study using more animals, this will aid in the better definition of the role of galectins in the inflammatory response.

Key Words: galectin, milk, somatic cells

M73 Protective effects of staphylococcal surface proteins as vaccine antigens to control mastitis in dairy cows. C. E. Merrill*[1], D. B. Ensermu[1], R. D. Abdi[1], B. E. Gillespie[1], J. Vaughn[1], S. I. Headrick[1], K. Hash[2], T. B. Walker[2], R. A. Almeida[1], S. P. Oliver[1], and O. K. Dego[1], [1]*The University of Tennessee, Department of Animal Science, Knoxville, TN,* [2]*The University of Tennessee, East Tennessee Research and Education Center-Little River Animal and Environmental Unit, Walland, TN.*

Staphylococcus aureus mastitis is the major cause of economic losses in dairy production worldwide. Dairy cows are very susceptible to mastitis during periparturient and early dry periods. Currently there is no effective vaccine for *S. aureus* mastitis. Despite adoption of hygienic milking practices and subsequent reduction in *S. aureus* mastitis in most well managed dairy farms, overall *S. aureus* mastitis continues to be the most common disease of dairy cattle. This organism usually causes chronic mastitis and treatment with antibiotics is of limited success. The objective of this study was to evaluate the immune responses and protection in dairy cows vaccinated with *Staphylococcus aureus* surface proteins (SASP) and *Staphylococcus chromogenes* surface proteins (SCSP). A total of 17 Holstein dairy cows were randomly divided into 3 groups. Cows in group 1 (n = 5) and group 2 (n = 6) were vaccinated with 1.2 mg of SASP and SCSP with an Emulsigen-D adjuvant respectively. Cows in a group 3 were injected with PBS mixed with an Emulsigen-D and served as a control. Three consecutive vaccinations were given subcutaneously in the neck area at 28 d before drying off (d −28), 14 d before drying off (d −14) and at drying off (d 0). Milk or mammary secretion and serum antibody titers were evaluated by ELISA. All cows were challenged with *S. aureus* strain UT2 by teat dipping in a bacterial suspension at an average cell density of approximately 10^6 cfu/mL of growth medium. Statistical analyses were performed using GraphPad Prism version 7.03. Differences in antibody titers, number of *S. aureus* counts and somatic cell counts were analyzed using a one-way ANOVA, followed by a Kruskal-Wallis test. Results showed that vaccinated cows had significantly increased serum IgG, IgG1, IgG2 antibody titers compared with the control cows ($P \leq 0.05$). Vaccinated cows shed reduced number of *S. aureus* through milk and SCSP vaccine cross-protected against *S. aureus* clinical mastitis.

Key Words: mastitis, vaccine

M74 Metabolic stress biomarkers in dairy cows during early involution. L. M. Sordillo*, A. Putnam, J. Brown, and J. C. Gandy, *Michigan State University, East Lansing, MI.*

Metabolic stress occurs in dairy cows when physiologic homeostasis is disrupted as a consequence of aberrant nutrient metabolism, chronic inflammation, and oxidative stress. Early lactation cows that suffer from metabolic stress are susceptible to health disorders that cause significant production losses. There is little information, however, regarding the occurrence and impact of metabolic stress during involution. The purpose of this study was to investigate well-known metabolic stress biomarkers at various time points during the early dry period when dairy cows are subjected to dramatic changes in physiologic homeostasis. A descriptive study was performed by collecting blood samples from the coccygeal vein of 27 healthy dairy cows at a commercial dairy herd. Sampling points were d −6, d 0, d +1, d +2, d +6, and d +12 relative to dry-off date. Serum samples were utilized to quantify biomarkers related to metabolic stress, including calcium, cholesterol, nonesterified fatty acids, β-hydroxybutyrate, albumin, haptoglobin, cortisol, reactive oxygen species, antioxidant potential, oxidative stress index, and isoprostanes. Data were checked for normality in SAS and analyzed by one-way ANOVA with Tukey's adjustment for multiple comparisons. Within altered nutrient metabolism biomarkers, calcium and NEFA concentrations changed most from d 0 to d +2 during the sampling period. Haptoglobin is a biomarker of inflammation that gradually increased during involution with highest concentrations observed at d +12. In contrast, concentrations of albumin, a negative acute phase protein, were significantly lower at d +12 compared with other sampling points. All indicators of oxidative stress generally increased throughout most of the sampling period, suggesting altered oxidative stress states throughout early involution. Therefore, many of the biomarkers associated with metabolic stress changed during the transition from late lactation to the early dry period, but not to the same magnitude and duration reported previously in periparturient cows. Future studies should be directed toward assessment of other metabolic stress biomarkers and their effect on dairy cattle health and well-being during involution.

Key Words: metabolic stress, involution, inflammation

M75 Genome-wide variation for visceral fat deposition in Holstein dairy cows. P. Melendez*[1], S. Poock[1], P. Pinedo[2], D. Manriquez[2], S. Moore[3], M. Lucy[3], P. Pithua[1], J. Neal[3], and J. Taylor[3], [1]*College of Veterinary Medicine, University of Missouri, Columbia, MO,* [2]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [3]*Division of Animal Sciences, University of Missouri, Columbia, MO.*

The objective of this study was to assess genetic variation in visceral adiposity in US Holstein cows. The study included adult Holstein cows sampled from a slaughterhouse (Green Bay, WI) during September 2016. Only animals with a body condition score (BCS) between 2.75 and 3.25 were considered. The extent of omental fat at the level of the insertion of the lesser omentum over the pylorus area was assessed. A group of 100 Holstein cows with an omental fold <5 mm in thickness, and a second group of 100 cows with an omental fold ≥20 mm in thickness were sampled. A piece of muscle from the neck was collected from each cow into a sterile container for DNA extraction. Samples were submitted to a commercial laboratory for interrogation of genome-wide genomic variation using the Illumina BovineHD BeadChip. A genome-wide association analysis was performed to test potential associations between fat deposition and genomic variation. A univariate mixed linear model analysis was performed using GEMMA to identify SNP significantly associated with variation in visceral fat deposition. The chip heritability was 0.686 and the estimated additive genetic and residual variance components were 0.427 and 0.074, respectively. Eleven SNP defining 4 QTL regions were found to be significantly associated with visceral fat deposition ($P < 0.00001$). Two of the QTL were detected with 4 and 5 significantly associated SNP, respectively, while the QTL detected on BTA12 and BTA19 were each detected with only 1 significantly associated SNP. No enriched gene ontology terms were found within the gene networks harboring these genes when supplied to DAVID using either the *Bos taurus* or human gene ontology databases. We conclude that excessive omental fat in Holstein cows with similar BCS is not caused by a single Mendelian locus and that the trait appears to be at least moderately heritable; consequently, selection to reduce excessive omental fat is possible but would require the generation of predicted transmitting abilities from larger and random samples of Holstein cattle.

Key Words: visceral fat, subcutaneous fat, genome-wide association study (GWAS)

M76 Acetoacetate induces hepatocytes apoptosis by the reactive oxygen species (ROS)-mediated MAPKs pathway in ketotic cows. X. Du, G. Liu, and X. Li*, *College of Veterinary Medicine, Jilin University, Changchun, Jilin, China.*

The objective of this study was to investigate hepatic oxidative stress and the apoptotic status of ketotic cows, and the mechanism of acetoacetate (AcAc)-induced hepatic oxidative damage in ketotic cows. Liver and blood samples were collected from healthy and clinically ketotic cows. Hepatocytes were isolated from calves and treated with 0.3, 1.2, 2.4 or 4.8 m*M* AcAc in presence or absence of antioxidant *N*-acetylcysteine (NAC), JNK inhibitor SP600125, p38MAPK inhibitor SB203580, and ERK activator SC3527, respectively. Compared with healthy cows, ketotic cows exhibited severe hepatic injury and oxidative stress. The blood concentration of the apoptotic marker CK18 M30 and the number of TUNEL-positive cells in the liver of ketotic cows were 1.19- and 2.61-fold, respectively, higher than the values observed in control cows. Importantly, the levels of phosphorylated c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38MAPK) were significantly increased but the level of phosphorylated extracellular signal-regulated kinase1/2 (ERK1/2) was markedly decreased, which further promoted tumor protein 53 (p53) expression and inhibited nuclear factor E2-related factor 2 (Nrf2) expression. In vitro, AA treatment increased reactive oxygen species (ROS) content and further induced oxidative stress and apoptosis of calf hepatocytes. AA treatment increased the phosphorylation levels of JNK and p38MAPK and decreased the phosphorylation level of ERK, which could increase p53 and inhibit Nrf2 expression, nuclear localization and DNA-binding affinity, thereby inducing the overexpression of pro-apoptotic molecules Bax, Caspase 3, Caspase 9, PARP and inhibition of anti-apoptotic molecule Bcl-2. Antioxidant NAC treatment or interference of MAPKs pathway could attenuate the hepatocytes apoptosis induced by AA. Collectively, ketotic cows display severe hepatic oxidative stress and the hepatic MAPK-p53/Nrf2 apoptotic pathway is over induced and partially mediated apoptotic damage.

Besides, AA triggers hepatocytes apoptosis via the ROS-mediated MAPKs pathway in ketotic cows.

Key Words: ketotic cows, acetoacetate, apoptosis

M77 Enhanced hepatic mitochondrial functional in dairy cows with mild fatty liver. Z. Shi, X. Li, G. Liu, and X. Li*, *College of Veterinary Medicine, Jilin University, Changchun, Jilin, China.*

The inevitable deficiency in nutrients and energy at the onset of lactation requires an optimal adaptation of the hepatic lipid metabolism to overcome metabolic stress. Fatty liver is one of the main health disorders after parturition. The regulation of hepatic lipid metabolism is largely dependent on mitochondria. The aim of this study was to investigate the changes in hepatic mitochondrial function in dairy cows with mild fatty liver. Liver and blood samples were collected from healthy (n = 15) and mild fatty liver (n = 15) cows. The mRNA levels of oxidative phosphorylation complexes (CO I-V) and the protein levels of the mitochondrial function regulators were detected by RT-qPCR and Western blotting, respectively, and analyzed with paired *t* tests. The number and volume of mitochondria in the liver were detected by transmission electron microscopy. The hepatic ATP content and mitochondrial DNA (mtDNA) were measured using biochemical kits and analyzed with Wilcoxon-signed-rank-test. Healthy cows were defined as less than 1% liver TG on wet weight basis and mild fatty liver cows were defined as 1–5% liver TG on wet weight basis. Compared with healthy cows, ultrastructural analysis revealed a significantly increased number and volume of mitochondria in the liver of mild fatty liver cows. Hepatic mtDNA was also markedly increased in mild fatty liver cows. These results indicate that mitochondrial function is enhanced in the liver of mild fatty liver cows. We further explored the molecular mechanisms involved. The protein levels of mitochondrial function regulators, PGC-1α and NRF1, were significantly increased in the liver of mild fatty liver cows. The mRNA levels of CO I-V regulated by PGC-1α were significantly higher in the liver of mild fatty liver cows than healthy cows. The ATP content was increased, which was consistent with the increase of CO I-V. In summary, our data show that the hepatic mitochondrial function of dairy cows with mild fatty liver is enhanced, and enhanced mitochondrial function may be the adaptive mechanism of dairy cows with mild fatty liver.

Key Words: mitochondrial function, adaptation mechanism, dairy cow with mild fatty liver

M78 Yeast culture alters volatile fatty acids production in a ruminal in vitro fermentation system. S. A. Armstrong*, S. S. Bascom, and D. J. McLean, *Phibro Animal Health Corporation, Teaneck, NJ.*

The objective of this study was to evaluate the effect of a commercial yeast culture, [Cellerate Culture Classic (CC), Phibro Animal Health Corp.] on volatile fatty acid (VFA) production and kinetics of gas production using an in vitro rumen fermentation system and lactating cow TMR. Bottle (250 mL) was considered the experimental unit; each bottle contained TMR (1.4 g) and treatment in 100 mL of buffered rumen fluid. Treatments were CNTL (no additive) and CC (0.0567 g/bottle). All treatments were incubated in triplicate for 24 h at 39°C and constant agitation (60 rpm). Bottles were capped with units to capture temperature and pressure every 15 min (RF1, Ankom Technology, Macedon, NY). At the end of incubation, final pH and temperature measurements were taken; a sample of rumen fluid was frozen for analysis of VFA and ammonia nitrogen. Three replicate incubation days were performed and data were analyzed using PROC GLIMMIX of SAS; significance was defined as $P \leq 0.05$. Treatment did not affect gas production kinetics, final pH, final temperature, ammonia nitrogen, isobutyric acid (mmol/L), acetate: propionate, or acetate, propionate, isobutyrate, isovalerate, or valerate proportions (percent of total VFA). CC (vs CNTL) increased total acetate (72.67 vs 68.12 ± 4.69 mmol/L; $P = 0.01$), propionate (24.02 vs 22.21 ± 2.68 mmol/L; $P = 0.003$), butyrate (10.22 vs 9.32 ± 0.68 mmol/ L; $P = 0.001$), isovalerate (0.83 vs 0.76 ± 0.02 mmol/L; $P = 0.01$), valerate (1.51 vs 1.40 ± 0.32 mmol/L; $P = 0.006$) and total VFA concentrations (110.17 vs 102.69 ± 8.22 mmol/L; $P = 0.005$). CC increased butyrate proportions vs CNTL ($P = 0.001$). Our results indicate that adding yeast culture to a lactating cow diet may increase volatile fatty acid production without altering kinetics of gas production, pH, or temperature in an in vitro system which may modulate ruminal fermentation.

Key Words: yeast culture, in vitro fermentation, VFA

M79 Time of rumen fluid collection relative to feeding alters in vitro fermentation outcomes. S. A. Armstrong*[1], L. A. Pless[1,2], A. N. Brewster[1,2], and D. J. McLean[1], [1]*Phibro Animal Health Corporation, Teaneck, NJ,* [2]*Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR.*

The objective of this study was to determine the effect of time of rumen fluid collection relative to feeding on gas production kinetics and volatile fatty acid (VFA) production for in vitro rumen fermentation. Three ruminally cannulated Holstein heifers were rumen fluid donors. Feed was removed from heifers 12 h before feeding, rumen fluid was collected from each heifer before feeding (0 h), and at 2, 4, and 6 h after feeding, repeated on 3 separate incubation days. Buffered rumen fluid (100 mL) was incubated in 250-mL bottles containing 1.4 g of dried TMR, in duplicate for each heifer at each collection time. All bottles were incubated for 24 h at 39°C and constant agitation (60 rpm), and capped with monitors to capture temperature and pressure every 15 min (RF1, Ankom Technology, Macedon, NY). Gas data were fit with nonlinear regression in GraphPad Prism 7 using the formula $y = V_m(1-(e^{(-K_d(x-lag))}))$ where y is gas produced at time X (mmol), V_m is the asymptotic gas production (mmol), K_d indicates the fractional rate of gas production (mmol/h), X is time (h), lag refers to the lag time before the start of fermentation (h). At the end of incubation, final pH and a sample of rumen fluid was collected for VFA and ammonia nitrogen. Data were analyzed using PROC GLIMMIX of SAS, with donor as the experimental unit and day as the random blocking factor; significance defined as $P \leq 0.05$. Time of rumen fluid collection significantly affected gas production kinetics (lag $P = 0.01$, V_m $P = 0.03$, K_d $P < 0.0001$), acetate (mmol/L; $P = 0.0004$), propionate (mmol/L; $P = 0.02$), isobutyrate (mmol/L; $P < 0.0001$), valerate (mmol/L; $P = 0.004$), isovalerate (mmol/L; $P < 0.0001$), and total VFA concentrations (mmol/L; $P = 0.004$). All VFA relative proportions were altered due to time of rumen fluid collection ($P < 0.02$). Gas production was highest in fermentor units fed with rumen fluid collected 2 h post-feeding and VFA production was highest when rumen fluid was collected 4 h post-feeding. Our findings suggest gas and VFA production are maximized when rumen fluid is collected between 2 and 4 h post-feeding.

Key Words: in vitro fermentation, rumen gas production, VFA

M80 Factors affecting prevalence of subclinical hypocalcemia in a 1,724-cow survey. S. A. Armstrong*, S. S. Bascom, G. A. Holub, and D. E. Nuzback, *Phibro Animal Health Corporation, Teaneck, NJ.*

The objective of this study was to analyze factors affecting the prevalence of subclinical hypocalcemia on commercial dairies. Serum samples were collected from fresh dairy cows (n = 1,724) and analyzed for total Ca concentration (mg/dL). Time of sample collection and time of parturition were recorded. Other information collected included parity and postpartum Ca supplementation. A TMR sample was collected to determine prepartum diet mineral concentrations (including DCAD, regardless of source of supplemental anions). Data were analyzed using PROC Glimmix of SAS, using the binomial distribution option to generate LSMeans ± SE output describing percent subclinical hypocalcemia. Cows with serum Ca concentrations ≤8.5 mg/dL were considered subclinically hypocalcemic (SCH) and significance was determined at $P \leq 0.05$. Fixed factors included parity, time of blood collection (0–6, 6–12, 12–36, 36–60, and >60 h post-calving), DCAD (<0, 0–11, 11+ mEq/100 g DM), dietary Ca (<0.75%, 0.75–1.50%, or >1.51% DM) and Ca supplementation (any form or method of Ca administered before blood sample collection). Parity, time of sample collection, DCAD and dietary Ca were analyzed for interactions with Ca supplementation. Parity influenced SCH ($P < 0.0001$), with increased prevalence of SCH occurring in 4+ parity cows. Time of sample collection affected SCH ($P = 0.007$). Cows fed negative DCAD diets prepartum had lower (13.84 percentage units) prevalence of SCH than those fed a prepartum diet with a DCAD greater than 11 mEq/100g DM ($P = 0.008$). Prepartum dietary Ca concentrations affected SCH ($P = 0.04$), with high Ca diets (>1.51%; 54.68 ± 4.41% SCH) tending to have lower prevalence of SCH than medium (0.75–1.5%; 60.37 ± 3.94% SCH) or low Ca (<0.75%; 65.08 ± 5.04% SCH) diets ($P = 0.07$ and $P = 0.09$ respectively). The interaction of prepartum dietary Ca and postpartum Ca supplementation significantly affected SCH ($P = 0.03$). These results indicate that numerous factors and their interactions affect serum Ca status in postpartum dairy cows and the prevalence of SCH.

Key Words: subclinical hypocalcemia, DCAD, calcium

M81 Effects of fully acidified close-up diets and dietary calcium content on blood metabolites and mineral concentrations of transition dairy cows. K. M. Glosson*[1], X. Zhang[2], S. S. Bascom[3], A. D. Rowson[3], and J. K. Drackley[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan, China,* [3]*Phibro Animal Health Corporation, Teaneck, NJ.*

Subclinical hypocalcemia (SCH) has been linked to other fresh cow metabolic disorders and many practices have been used to prevent the postpartum drop in circulating Ca. Both low Ca diets and acidogenic diets with negative dietary cation-anion differences (DCAD) to prime Ca homeostasis are common strategies. The objective of this study was to determine the effects of 3 close-up dry cow dietary strategies on concentrations in blood of Ca and other minerals as well as metabolites associated with energy status in multiparous Holstein dairy cows (n = 81) through the transition period. Cows were assigned to 1 of 3 treatments during the close-up dry period (−28 d to calving): (1) a positive DCAD diet with low dietary Ca (0.4%DM; CON); (2) a fully acidified DCAD diet (urine pH 5.7) with low dietary Ca (0.4%DM; LOW); or (3) a fully acidified DCAD diet (urine pH 5.7) with high dietary Ca (2.0%DM; HIGH). An iSTAT (Abbott, Princeton, NJ) was used to analyze ionized Ca concentrations (iCa) in whole blood within 1 h (±1 h), 24 h, 48 h, and 4 d after calving. The iCa concentration was considered a risk for SCH if <1.0 mmol/L. Plasma BHB and nonesterified fatty acids (NEFA) were determined at calving, 24 h, 48 h, and 4, 7, 14, 21, and 28 d postpartum. If BHB values were >1.0 mmol/L at 24 h after calving the cow was diagnosed with subclinical ketosis (SCK). The MIXED procedure in SAS was used to contrast: (1) CON vs the average of LOW and HIGH; and (2) LOW vs HIGH. Cows fed LOW or HIGH had greater iCa at calving (1.10 and 1.11 mmol/L) and 24 h postpartum (1.11 and 1.05 mmol/L) when compared with cows given CON (0.98 mmol/L; $P < 0.01$). Inversely, postpartum BHB was lower in cows fed LOW or HIGH (0.91 and 0.88 mmol/L) compared with cows given CON (1.47 mmol/L; $P < 0.01$). Postpartum plasma NEFA concentrations did not differ among treatments. Cows receiving CON had greater occurrence of SCH and SCK based on iCa and BHB, which could increase the risk for clinical disorders and decrease production. Dietary Ca content of prepartum acidogenic diets did not affect these variables.

Key Words: fully acidified close-up diet, ionized calcium

M82 Near-infrared spectroscopy for measuring plasma metabolites in dairy cows. M. Premi, G. Ferronato, M. Nembrini, L. Calamari, E. Trevisi*, and P. Bani, *Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Blood metabolites on individual animals give relevant information on energy, protein and other nutrient metabolism in dairy cows and they are widely used to assess their nutritional and health status, but their analysis is expensive and time consuming. Near-infrared (NIR) spectroscopy represents a rapid, non-destructive sample analysis, cost-effective alternative to traditional methods of analysis that has been successfully used in many fields and that requires small amount of sample. The aim of this study was to evaluate the use of NIR spectroscopy for analysis of a set of blood biochemical parameters included in a metabolic profile for dairy cows. The NIR spectra were acquired from 200 μL of plasma samples of 149 pluriparous dairy cows from 7 herds in different physiological phases, from dry period to mid-lactation. A Fourier-transform (FT)-NIR Analyzer (MPA, Bruker Optics, Germany), with a spectral resolution of 4 cm^{-1} over a wavelength range of 12,500–4,000 cm^{-1} with 32 scans per spectrum, was used in transmission mode. Reference values, obtained using accepted reference biochemical methods, were used as calibrating values to develop predictive models using a partial least square method. The validation was carried out using cross validation method (leave one-out sample procedure) and its accuracy was evaluated considering the coefficient of determination (R^2) and residual prediction deviation (RPD). Predictions were obtained for cholesterol, total protein, globulin and albumin (R^2 from 0.95 to 0.99; RPD from 4.29 to 12.4). Nonesterified fatty acids and total bilirubin had an approximate quantitative prediction level ($R^2 = 0.61$ and 0.62; RPD = 1.61 and 1.62), whereas urea, β-OH-butyric acid and glucose had non-usable predictions (R^2 from 0.36 to 0.22; RPD from 1.25 to 1.13). This study supports the use of NIR spectroscopy as a substitute of some biochemical methods and has comparable performance to those previously obtained with FT-MIR (MilkoScan FT 120, Foss, Denmark). As plasma samples were obtained from healthy animals, a quite narrow range of variability were present in the data set for some plasma parameters. This could have limited the predictive potential of models and, for that reason, further studies on a larger data set are in progress.

Key Words: near-infrared spectroscopy, metabolic profile, dairy cow

M83 Comparison of β-hydroxybutyric acid concentration determined via an electronic meter and a laboratory method to diagnose ketosis in dairy cows in a commercial herd in Northern Mexico. G. Perez-Hernandez*[1], A. Ruiz-Flores[1], J. G. Garcia-Muñiz[1], C. S. McCarthy[2], L. H. Baumgard[2], L. L. Timms[2], and H. A.

Ramirez-Ramirez[2], [1]*Universidad Autónoma Chapingo, Chapingo, Mexico,* [2]*Iowa State University, Ames, IA.*

Although precise and accurate laboratory methods are available for quantifying circulating β-hydroxybutyric acid (BHB), dairy producers would benefit from having an accurate cow-side tool to quickly diagnose ketosis. Therefore, the objective of this study was to validate a handheld electronic BHB meter (BHBCheck; PortaCheck, Moorestown, NJ) in the diagnosis of hyperketonemia using a colorimetric laboratory assay as reference (LiquiColor Assay; EKF Diagnostics-Stanbio, Boerne, TX). Blood samples were collected from multiparous Holstein cows (n = 112) in the peripartum period at −7, 7 and 14 d relative to calving date in a commercial herd in Northern Mexico. Whole blood was obtained by venipuncture from the coccygeal vein/artery and BHB concentration was directly measured with the BHBCheck meter; blood samples were collected at the same time in sterile evacuated tubes with no coagulating agent, centrifuged to obtain serum, and frozen −20°C until further laboratory analysis. Data were analyzed using the UNIVARIATE, FREQ, and REG procedures of SAS for descriptive statistics, sensitivity and specificity, and regression analysis, respectively. Concentration of BHB ranged from 0.30 to 5.20 mmol/L and averaged 0.87 ± 0.47 mmol/L (±SD) for the BHBCheck whereas the laboratory assay ranged from 0.21 to 5.24 mmol/L with a mean of 0.79 ± 0.49 mmol/L. The overall correlation coefficient (r) for the concentrations obtained with the BHBCheck and the laboratory assay was 0.86; when correlation was tested by day of sampling, the coefficients were 0.47, 0.80, and 0.92 for d −7 (n = 112), 7 (n = 107), and 14 (n = 106) respectively. The proportion of samples with BHB ≥1.2 mmol/L was 12.0% for the electronic meter and 8.9% for the laboratory assay. The overall sensitivity and specificity obtained were 80.3 and 95.1%, respectively, when BHB values were ≥1.2 mmol/L. Results indicate that the BHBCheck meter can be used as a reliable and rapid postpartum diagnostic test to detect hyperketonemia in field conditions.

Key Words: cow-side diagnostic, ketone bodies, transition cow

M84 Ionized calcium and glucose changes in refrigerated heparinized blood samples from dairy cows. A. Valldecabres*, R. Lopes, and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.*

The aim of the present study was to evaluate the changes of ionized calcium (iCa) and glucose in refrigerated heparinized blood samples from dairy cows. Blood samples were collected from the coccygeal vessels of 16 adult cows into vacuum tubes coated with heparin. Measurements of iCa and glucose were performed using a biochemical analyzer (Stat Profile PRIME CCS Analyzer, Nova Biomedical, Waltham, MA). After collection, samples were immediately refrigerated, stored at 4°C and analyzed at 0, 5, 20 and 35 min, 1, 2, 4, 26, 50 and 74 h. Before each analysis, samples were homogenized for proximately 30 s using a tube rocker. After homogenization a specimen was extracted from the vacuum tube using a syringe to minimize gas exchange. The iCa and glucose changes were evaluated with the MIXED procedure of SAS with cow as a random measure and time as a repeated measure. Initial iCa ranged from 1.27 to 0.92 mmol/L and glucose from 58 to 87 mg/dL. Least squares means of iCa and glucose concentrations over time are shown in Table 1. There was a significant effect of storage time for glucose ($P < 0.001$) and a tendency for iCa concentration ($P < 0.10$). The observed changes in iCa over time were minimal (<1%). Glucose concentration remained stable from 0 to 26 h but decreased 2.37% at 50 h and 5.24% at 74 h. Our results suggest that iCa concentration remains stable for 74 h and glucose concentration for 26 h when heparinized samples are kept refrigerated in vacuum tubes.

Table 1 (Abstr. M84). Variations in the concentrations of iCa and glucose in refrigerated blood samples from 16 dairy cows

Duration of storage	iCa (mmol/L)	Glucose (mg/dL)
0 min	1.146 ± 0.026	64.64 ± 3.30[a]
5 min	1.148 ± 0.026	65.13 ± 3.30[a]
20 min	1.151 ± 0.026	64.13 ± 3.30[a]
35 min	1.154 ± 0.026	64.47 ± 3.30[a]
1h	1.157 ± 0.026	64.48 ± 3.27[a]
2 h	1.158 ± 0.026	64.81 ± 3.24[a]
4 h	1.156 ± 0.026	64.44 ± 3.24[a]
26 h	1.154 ± 0.026	63.38 ± 3.24[a]
50 h	1.152 ± 0.026	61.88 ± 3.24[b]
74 h	1.152 ± 0.026	60.06 ± 3.24[c]

[a–c]Different letters show significant difference within column ($P < 0.05$).

Key Words: calcium, glucose, dairy cow

M85 Association of subclinical hypocalcemia and teat canal diameter after milking in dairy cattle. A. A. Barragan*[1], L. da Costa[2], S. Bas[2], A. Della Libera[3], E. Hovingh[1], S. Rassler[1], M. A. Ostach[1], and F. da Costa[4], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [3]*Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil,* [4]*Olentangy Liberty High School, Powell, OH.*

Hypocalcemia (clinical and subclinical) is a metabolic condition that affects more than 50% of dairy cows within 48 h of parturition. Hypocalcemia has been associated with increased risk of retained placenta, metritis, mastitis, and displaced abomasum. It has also been proposed that this condition negatively affects the closure of the teat canal, thereby increasing the risk of new intramammary infections. The objective of this study was to evaluate the association of subclinical hypocalcemia (HYPO; < 8.0 mg/dL serum Ca) and teat canal diameter after milking in postpartum dairy cows. Twenty-five dairy cows from 2 commercial dairy farms were enrolled in this study. Immediately after the morning milking (T1), and 30 min later (T2), ultrasonography (US) of each teat was performed using a 7.5-MHz linear transducer. Blood samples were collected via coccygeal venipuncture at T2 to assess circulating Ca concentration. US images were analyzed with an image-processing program to determine the diameter in millimeters (mm) of the teat canal at the mid-point between the teat end and Furstenberg's rosette at T1 and T2. The measurements were read blindly 3 times by 3 different individuals. The data were analyzed using ANOVA by the MIXED procedure of SAS. The proportion of cows with HYPO in this study was 20% (5/25). For all cows, the teat canal diameter at T1 tended to be larger compared with T2 (T1 = 3.42 ± 0.12 mm; T2 = 3.19 ± 0.11 mm; $P = 0.09$). There was no difference in teat canal diameter at T1 between normocalcemic (NORMO; ≥8.0 mg/dL serum Ca) and HYPO cows (HYPO = 3.27 ± 0.12 mm; NORMO = 3.58 ± 0.20 mm; $P = 0.17$). However, HYPO cows had larger teat canal diameter at T2 compared with NORMO cows (HYPO = 3.54 ± 0.19 mm; NORMO = 2.84 ± 0.20 mm; $P = 0.002$). The preliminary results from this study suggest that subclinical hypocalcemia may affect the proper closure of the teat canal after milking, an important udder defense mechanism against mastitis

pathogens, highlighting the importance of effective prevention of this metabolic disease in dairy operations.

Key Words: dairy cattle, hypocalcemia, teat canal diameter

M86 Reference blood parameter for Holstein dairy cows diagnosed with different health events. A. A. Barragan*[1], S. Bas[2], K. Heckman[1], and A. Ludwikowski[1], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Department of Veterinary Preventive Medicine, Columbus, OH.*

Dairy cattle veterinarians rely on detailed clinical examination of ill animals to identify specific diseases. However, even the most experienced clinicians occasionally may not arrive at a definitive diagnostic after clinical examination. Assessment of blood parameters (e.g., blood cells, hepatic enzymes) is a common practice performed by veterinarians to aid in disease diagnosis and determine best treatment approaches. The objective of this study was to provide reference blood parameters of dairy cows that experienced common health disorders. A total of 399 health records from a veterinary practice that provided veterinary care to 5 dairy farms were analyzed. Health events were grouped in 9 categories: (1) DIG (digestive diseases; n = 134); (2) UTD (uterine diseases; n = 101); (3) DC (down cow; n = 73), (4) MAST (mastitis; n = 55); 5) PNEU (pneumonia; n = 16); (6) LAME (lameness; n = 15), (7) INJ (injury; n = 13), (8) OTHER (n = 31) and (9) UNKNOWN (no diagnosis; n = 59). The data were analyzed using the MEANS and MIXED procedures of SAS. After clinical examination, 79.20%, 17.04% and 3.76% of ill animals were diagnosed with 1, 2, or 3+ health events, respectively. DIG health events were the most common (24.12%) reason that dairy farmers called their veterinarian, followed by DC (17.14%) and UTD (14.92%). Interestingly, veterinarians did not arrive at a definitive diagnosis in 14.78% of the clinical cases. Regardless, cows diagnosed with only 1 health event had higher ($P = 0.02$) concentration of neutrophils (3.40 ± 1.07 cells $\times 10^6$/mL) compared with cows diagnosed with 3+ health events (1.28 ± 1.42 cells $\times 10^6$/mL). UNKNOWN group cows had increased microhematocrit compared with normal values. DIG cows had increased concentrations of aspartate transaminase (AST), creatinine kinase (CK), and total bilirubin (TB) compared with normal values. Similarly, UTD cows had elevated concentration of AST and CK, while albumin and globulins were decreased when comparing to normal values. Cows diagnosed with DC had increased concentration of AST, CK, TB, and potassium, while albumin and sodium were decreased compared with normal values. The results of this study may help veterinarians to rapidly and accurately diagnose and treat ill animals, therefore improving disease prognosis.

Key Words: dairy cattle, blood parameter, health event

M87 Mineral profile of grazing dairy cows in the northwestern of Argentina. G. M. Martinez*[1], J. F. Micheloud[1], V. H. Suarez[1], G. Matioli[2], and D. Rosa[2], [1]*Instituto Nacional de Tecnología Agropecuaria, Salta, Argentina,* [2]*Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina.*

In the last decade, an intensification process has been developed in the dairy production of northwestern Argentina. In Salta province, the bovine milk basin is located in Lerma Valley. Minerals are essential for dairy cows, and the amounts normally found in most feeds are inadequate for high milk production. If these requirements are scarce or not met, deficiency symptoms can occur. As in other parts of the world, mineral imbalances are one of the most important problem in Salta's basin. To known imbalances prevalence of calcium (Ca), phosphorus (P) and magnesium (Mg), 584 dairy cows of 4 farms of basin Lerma Valley were sampled in 4 opportunities. At each visit were bled from the jugular vein of 9/10 animals in each category: prepartum, fresh cow, 2nd and 3rd third of lactation. Values Ca and Mg serum were obtained by atomic absorption spectrophotometry and P values by UV-visible spectrophotometry. Associations between variables were analyzed using linear correlation and linear regression, in addition to analyzing the probability of occurrence of hypocalcemia by odds ratio. Differences between variables and moment of lactation were examined by ANOVA and Tukey's test and Chi-squared using Fisher's test. The mean serum Ca was 8.85 mg/dL, with no differences found all over the periods. The average prevalence of hypocalcemia was 46.2% in all production periods. The mean serum Mg was 2.26%, with a prevalence of 10.6% hypomagnesemia. Animals with hypomagnesemia had a 3.5 times greater chance of having hypocalcemia compared with those with normal values. The prevalence of animals with abnormal values of P was high (48.3%), without association with hypocalcemia levels. Based on our results, there are potential risks of imbalances Ca, Mg and P in the dairy herds of the Lerma Valley.

Key Words: imbalance, mineral, dairy cow

M88 Relationships between test-day fatty acid concentrations and early lactation survival. I. W. Haagen* and C. D. Dechow, *The Pennsylvania State University, University Park, PA.*

The purpose of this study was to quantify early lactation test-day fatty acid concentrations and their associations with survival during the first 90 DIM. Test-day fatty acid concentrations (g / 100 g milk) were available from 2,059 Holsteins located on 19 Pennsylvania herds during the spring of 2014. Concentrations of test-day BHB and acetone were also available and were natural log-transformed. Each cow was restricted to the earliest test-day fatty acid observation available during the first 90 DIM. Test-day yields and reasons for leaving herd were obtained from Dairy Records Management Systems. Cows were recorded as left herd if culled for involuntary reasons or died on farm within 90 DIM. Cows were stratified into quartiles based on concentrations for each fatty acid category, log BHB, and log acetone. Logistic regression models were used to test the effect of fatty acid, log acetone, or log BHB concentration quartiles on the odds of leaving the herd within 90 DIM. The model included lactation group ($1, \geq 2$), test day yield, and either a single fatty acid, log BHB, or log acetone. The mean concentrations were 3.84, 1.57, 1.39, 0.31, 1.21, and 2.29 for total fat, long-chain fatty acid (LCFA), medium-chain fatty acid (MCFA), short-chain fatty acid (SCFA), unsaturated fatty acid (UFA), and saturated fatty acid (SFA), respectively. Means for log acetone and log BHB were 0.07 and 0.04, respectively. When evaluating odds ratios between trait quartiles, cows with intermediate concentrations had lower odds ($P < 0.05$) of leaving the herd within 90 DIM than cows in the top quartile for total fat, LCFA, MCFA, SCFA, UFA, SFA, and acetone. For BHB, cows in the lowest quartile had reduced odds of leaving the herd compared with cows in the highest quartile. The measure most strongly related to survival was SCFA, with an odds ratio of 0.26:1 for the third to the fourth quartile. These results suggest that cows with intermediate concentrations of fatty acids had greater odds of survival than cows with higher concentrations of fatty acids, and cows with smaller concentrations of BHB were favored over large concentrations.

Key Words: fatty acid, culling

M89 Effect of ketosis on lying time in transition dairy cows. J. M. Piñeiro*[1], B. T. Menichetti[1], A. A. Barragan[1], A. Relling[2], W. P. Weiss[2], S. Bas[1], and G. M. Schuenemann[1], [1]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [2]*Department of Animal Sciences, The Ohio State University, Wooster, OH.*

The objective of this study was to assess the effect of ketosis status on behavioral activity in transition dairy cows. A total of 1,024 Holstein dairy cows (390 primiparous and 634 multiparous) in 3 commercial dairy herds were enrolled at 14 d before calving until 14 d post-calving. Monthly, a cohort of 20 to 36 cows was enrolled at each farm and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual cows to assess their lying time. Holstein animals were screened for NEFA at 7 d prepartum (dpp), calcium at calving (Ca), ketosis (KET) at 7 ± 3 and 14 ± 3 DIM by measuring β-hydroxybutyrate (BHB) in serum samples, and haptoglobin at 7 ± 3. A case of KET was recorded when lactating cows had serum concentration of BHB ≥1.2 mmol/L. Cases of retained placenta, milk fever, metritis, pneumonia, digestive disorders, and mastitis during the study period were recorded. Lactating cows were allocated into 1 of 4 groups: (1) non-disease (ND, n = 613; cows without KET and any other health conditions), (2) cows with only KET (n = 152), (3) sick cows experiencing ≥1 health conditions, but without KET (SICK, n = 198), or (4) cows with KET plus at least 1 health condition (KET+H, n = 61). Data were analyzed using MIXED procedure of SAS. Multiparous cows had greater LT ($P < 0.0001$) compared with primiparous cows, regardless of health status. Cows experiencing KET+H had increased LT (~1 h; $P < 0.05$) compared with ND cows for the first 6 DIM after parturition. Cows experiencing KET, KET+H or SICK had increased NEFA prepartum ($P < 0.0001$) with greater BCS loss early in lactation ($P < 0.0001$), reduced Ca ($P = 0.004$), and increased HAPTO ($P = 0.0002$) compared with ND cows. These results suggest that LT along with energy and Ca balance are critical for transition cow health.

Key Words: lying time, health, dairy cattle

M90 Using once per day milking as an adjunct treatment of hyperketonemia. M. E. Williamson*, T. F. Duffield, S. Leblanc, T. DeVries, and B. W. McBride, *University of Guelph, Guelph, ON, Canada.*

Subclinical ketosis affects approximately 40% of dairy cows in North America. Current recommended treatments for ketosis focus on providing cows with more energy. However, the most effective treatments, such as oral propylene glycol, only resolve ketosis approximately 50% of the time. The objective of this research was to evaluate the effect of decreasing milking frequency from 2 milkings/day, to one milking/day for 2 wk; in conjunction with a standard treatment of 5 d of oral propylene glycol in cows diagnosed with ketosis. Ketosis was defined as a blood β-hydroxybutyrate concentration of ≥1.2mmol/L. From November 2016 to September 2017, 104 ketotic cows from the University of Guelph Livestock Research Innovation Centre were studied. Fifty-five cows were randomly allocated to the once/day milking group, and 49 were enrolled in the twice/day milking group. All cows inhabited the same pen during their time on trial, and all cows were milked in a DeLaval VMS robot equipped with Herd Navigator. Blood, milk, and urine samples were collected in a 21-d period to analyze ketones over time in the cows. Disease occurrence was recorded up to 60 DIM and reproductive performance, examining days to first breeding, and pregnancy on the first breeding were explored. CanWest DHI herd recording data were collected on a weekly basis, for a period of 15 weeks, examining milk production, milk component data, and SCC. Results indicate that once per day milking reduced the risk of subsequent ketosis, this was determined through the use of a logistic regression model in SAS. For cows in lactation 1, the odds of ketosis for once/day milking treatment was reduced by 99% compared with those in the twice/day milking treatment group on d 18 of trial ($P = 0.0005$, CI $3.38 \times 10^{-6} – 0.0258$). For cows in lactation 2+, the odds of ketosis for once/day milking treatment group is 0.18 times the odds of ketosis in the twice/day milking treatment group on d 18 of trial ($P = 0.0002$, CI 0.0729 – 0.426).

Key Words: ketosis, milking frequency, dairy cow health

M91 Epidemiology of subclinical hypocalcemia in early-lactation Holstein cows. R. Neves[2], B. Leno[1], K. Bach[1], and J. McArt*[1], [1]*Cornell University, Ithaca, NY,* [2]*Texas Tech University, Lubbock, TX.*

Our objective was to characterize the epidemiology of subclinical hypocalcemia (SCH) in Holstein cows by assessing the temporal associations of plasma Ca concentrations in the first 4 d in milk (DIM) with the risk of cows being diagnosed with metritis/and or displaced abomasum in the first 60 DIM and milk production across the first 15 wk of lactation. We conducted a prospective cohort study in 2 dairy herds in New York State in which cows had blood samples collected daily for the first 4 DIM; a total of 389 cows (186 primiparous and 253 multiparous) were enrolled. Multivariable Poisson regression models were built to evaluate the disease outcomes, and generalized linear mixed models were built to evaluate the milk production outcome. Plasma Ca concentration was assessed in the continuous scale in all models; dichotomization and SCH classification only occurred if the Ca concentration variable was meaningful by creating an optimized threshold based on receiver operating characteristic curve analyses. Plasma Ca concentration at 1 DIM was not associated with the risk of metritis in primiparous cows ($P = 0.22$), but we observed an association at 2, 3, and 4 DIM (critical thresholds were plasma Ca concentration ≤2.15, 2.10, and 2.15 mmol/L, respectively; all $P \leq 0.001$). Plasma Ca concentration was associated with the risk of metritis and/or displaced abomasum diagnosis for 2nd parity cows at 2 DIM (threshold ≤1.97 mmol/L; $P = 0.03$) and at 4 DIM for parity ≥3 cows (threshold ≤ 2.20 mmol/L; $P = 0.03$). Reduced plasma Ca concentration was associated with higher milk production when assessed at 1 DIM in primiparous and multiparous cows (2.9 ± 0.8 kg/d, $P \leq 0.01$) and lower milk production when assessed at 4 DIM in multiparous cows only (−1.8 ± 0.8 kg/d, $P = 0.02$). For primiparous cows, plasma Ca concentration was not associated with lower milk production at any of the DIM assessed ($P > 0.30$). We conclude that assessment of SCH at the individual cow level must take into account the DIM of Ca concentration measurement and parity of the cow, as the epidemiology of SCH is highly dependent on these variables.

Key Words: calcium, subclinical hypocalcemia, epidemiology

M92 Using milk fatty acid profile to identify early ketosis in dairy cows. J. K. Poncheki[1], P. M. Souza[1], R. Locatelli-Dittrich[1], G. T. Santos[2], D. P. D. Lanna[3], and R. de Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Universidade Estadual de Maringá, Maringá, PR, Brazil,* [3]*Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, SP, Brazil.*

The aim of this study was to identify milk fatty acids that could indicate ketosis in early lactation cows. A total of 694 Holstein cows (314 primiparous and 380 multiparous), with average body condition score (BCS) 3.29 ± 0.62, calving from September, 2015 to August, 2016 were evaluated from a commercial dairy farm located in Palmeira county, Paraná State, Southern Brazil. A milk sample was collected from each

cow between d 5 and 15 after calving, and they were frozen and stored in bottles at −20°C. Later, these milk samples, 90 from primiparous and 92 from multiparous, and one-third from healthy and two-thirds from ketotic cows, were analyzed by gas chromatography to determine 52 milk fatty acids (Finnigan Focus CG, Thermo Fisher Scientific). On d 5 and 10 after calving, BHB was also measured using a blood drop in ketone test strips (FreeStyle Optium Ketone Monitoring System, Abbott). Each association between milk fatty acid and ketosis was analyzed to obtain AUC, using MedCalc software. Averages for BHB were 1.03 ± 0.75 and 1.14 ± 0.90 mmol/L on d 5 and 10, respectively. Cows categorized as subclinical ketotic (BHB ≥1.2 mmol/L) were 26% (d 5) and 32% (d 10). Averages for milk fat and milk protein contents from the 182 samples were 4.73 ± 1.03% and 3.50 ± 0.39%, respectively, with 8.5 DIM. Milk fatty acids with AUC ≥0.90 were C12:0, C14:0, C18:1 *cis* 9, C17:0/C15:0, C18:1 *cis* 9/C14:0 ratio, and C18:1 *cis* 9/C15:0 ratio. The sensitivity and specificity of these variables were 90.5 and 79.2, 95.2 and 71.4, 85.7 and 84.4, 85.7 and 81.8, 91.4 and 83.1, 94.3 and 72.7, respectively. The cut-point for these fatty acids and their ratios associated with subclinical ketosis were ≤1.481, ≤6.597, >27.423, >1.359, >4.305, and >43.330 g/100 g, respectively. These results show that many fatty acids and their ratios could be used to identify ketosis in early lactation. *Acknowledgment: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.*

Key Words: fresh cow, negative energy balance, transition period

M93 Detection of health problems by changes in milk estimated blood nonesterified fatty acids (NEFA) and milk fat, protein, and fatty acids. A. Pape*[1], H. M. Dann[1], D. M. Barbano[2], and R. J. Grant[1], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*Department of Food Science, Northeast Dairy Food Research Center, Cornell University, Ithaca, NY.*

The objective was to examine the relationship between changes in milk composition and the onset of ketosis or displaced abomasum (DA). The approach taken was to test the extent to which machine learning models could differentiate between milk samples from cows that went on to experience either ketosis or a DA and milk samples from cows that did not. Milk samples were analyzed with mid-infrared methodology. Milk-estimated blood NEFA (881.6 ± 304.6 μEq/L), de novo fatty acid (FA; 18.7 ± 3.7 g/100 g FA), preformed FA (50.6 ± 7.0 g/100 g FA), fat (5.1 ± 0.9%), the ratio of fat to protein (FTP; 1.4 ± 0.3), and the ratio of preformed FA to de novo FA (1.0 ± 2.9) were the specific milk composition features examined. Each milk sample from a cow with ketosis or DA was matched with ~10 milk samples from healthy cows. Each sample was from the same DIM and within 30 calendar days as the corresponding sample from a sick cow. Samples were drawn from a pool of 46,860 samples from 764 cows that were ≤21 DIM. A total of 1,436 samples were selected for the ketosis data set and 1,240 for the DA data set. Logistic regression and random forests models were used and evaluated with area under the ROC curve (AUC) from 10 replicates of 10-fold cross-validation. For ketosis and DA, milk-predicted blood NEFA, de novo FA, and preformed FA yielded AUC of ~0.8 from 5 to 0 d in advance of the event, with predictive power generally increasing up to an AUC of ~0.89 on the day of the event for logistic regression. This was found to be true for these 3 predictors both when considered individually and when combined. Fat, the FTP ratio, and the ratio of preformed FA to de novo FA yielded similar results with logistic regression, especially within 2 d of the event. Random forest results were more erratic but the overall predictive power found was not substantially different, indicating the selected variables are linearly related to the onset of health events. Overall, these results indicate that certain health events can be predicted with at least moderate accuracy several days in advance using changes in milk composition.

Key Words: transition cow, NEFA, milk fatty acid

M327 The effects of fatty acid supplementation and provision of a dry teat on disease in veal calves. L. L. Deikun*[1,2], G. Habing[1], J. D. Quigley[2], and K. L. Proudfoot[1], [1]*The Ohio State University, Columbus, OH,* [2]*Provimi, Brookville, OH.*

Veal calves are at a high risk of disease early in life. Research is needed to determine interventions that reduce disease. The aim of this study was to determine the effects of fatty acid supplementation (NeoTec5g, Provimi) and the provision of a dry teat on bovine respiratory disease (BRD), navel inflammation, and diarrhea. A total of 240 Holstein bull calves from 2 cohorts were randomly assigned to 4 treatments using a 2 × 2 factorial design (n = 60/treatment): control, NeoTec5g, NeoTec5g+Teat, and Teat upon arrival to a commercial veal facility (d 0). Calves were housed in individual pens from 0 to 9 wk and then paired by treatment at wk 9. Milk replacer (MR) was fed twice daily using a proprietary step-up program. NeoTec5g was added to MR at a feeding rate of 0.5g/kg of BW/hd/day for NeoTec5g groups. Calf starter was offered to all calves from d 1. Serum IgG was determined using radial immunodiffusion assays on d 1; 33% of the calves had failure of passive transfer (<10 mg of IgG/mL.) Health exams were conducted twice weekly for 6 wk to diagnose BRD (UC Davis scoring system: 0 = total score for all clinical signs <5, 1 = total score ≥5), navel inflammation (0 = no or mild inflammation, 1 = moderate or severe inflammation) and diarrhea (0 = normal feces, 1 = loose or watery feces). Health data were analyzed using logistic regression (PROC GENMOD in SAS) using calf as the experimental unit; the model included IgG, disease at arrival, cohort, NeoTec5g, Teat, and NeoTec5g × Teat. Data are reported as odds ratios (OR). There was no effect of NeoTec5g (OR = 1.0; $P = 0.86$), Teat (OR = 1.1; $P = 0.57$) nor their interaction ($P = 0.48$) on the odds of BRD. There was no effect of NeoTec5g (OR = 1.3; $P = 0.18$), Teat (OR = 1.2; $P = 0.33$) nor their interaction ($P = 0.64$) on the odds of diarrhea. There was no effect of NeoTec5g (OR = 0.4; $P = 0.14$) or Teat (OR = 1.2; $P = 0.63$) on the odds of navel inflammation. There was a tendency for a NeoTec5g × Teat interaction for navel inflammation ($P = 0.09$). We saw no effect of our interventions on calf health. More research is needed to determine the effect of other factors, such as milk allowance and ventilation, on disease in veal calves.

Key Words: bovine respiratory disease (BRD), diarrhea, inflammation

M94 Genetic analysis of daily milk yield variability. V. S. Moncur*, L. C. Hardie, and C. D. Dechow, *Penn State University, University Park, PA.*

With the increased availability of daily milk yield measurements, data exists for analyses of additional traits that could be related to health and fertility. Selection for milk yield variability would be feasible if cows with more consistent milk yield exhibit greater levels of fertility or health. The objective of this study was to determine if day-to-day variation in milk yield is heritable and how it is correlated with daily milk yield (dMY). We retrieved 789,266 dMY records from 5 to 250 d in milk for 2366 lactations of 1184 Holsteins; of these, 470 had 42K genotypes. We merged dMY with the previous day's milk yield (pMY) to derive the absolute value of dMY − pMY (ΔABS), and relative change in milk yield (ΔREL), which was ΔABS/pMY. Only lactations with at least 200 observations were retained, and the average ΔREL, ΔABS, and pMY for the first 250 d of lactation were calculated and included in a 3-trait single-step genomic evaluation in ASReml. The fixed effects included lactation group (lactations 1, 2, ≥3), age at calving, and year-season of calving, and the random effects encompassed the genomic relationship effect, permanent environment, and residual error. Pedigree and genotypic data were blended to create the genomic relationship matrix with PREGSF90. All traits were heritable with estimates of 0.23 ± 0.04, 0.15 ± 0.04, and 0.26 ± 0.05 for ΔABS, ΔREL, and pMY, respectively. The genetic correlation estimate between ΔABS and pMY was 0.76 ± 0.07, whereas the genetic correlation estimate between ΔREL, and pMY was −0.15 ± 0.16. The phenotypic correlation estimates of pMY with ΔABS and ΔREL were 0.60 ± 0.02 and −0.32 ± 0.02, respectively. The 2 measures of variability were moderately correlated with a genetic correlation estimate of 0.52 ± 0.12 and phenotypic correlation estimate of 0.52 ± 0.02. Last, repeatabilities ranged from 0.32 ± 0.03 (ΔREL) to 0.41 ± 0.03 (pMY). In conclusion, daily milk yield variability was heritable and ΔREL was more independent of milk yield than ΔABS.

Key Words: milk yield variation, heritability, daily milk yield

M95 A resolution to breed identification of Pakistani Sahiwal cattle. M. Moaeen-ud Din*, G. Bilal, R. D. Muner, and N. Wahid, *Laboratories of Animal Breeding and Genetics, Faculty of Veterinary and Animal Sciences, PMAS Arid Agriculture University, Rawalpindi, Rawalpindi, Punjab, Pakistan.*

Molecular identification of animals is becoming increasingly important to preserve and maintain pure breeds worldwide. The issue is aggravated with rise in import of foreign animals and germplasm in Pakistan. It is becoming difficult to find pure males of Sahiwal breed for breeding purpose in public as well as private semen production units. The present study was designed to develop standard molecular markers for Sahiwal to ascertain their purity for breeding purpose. In this study, 50 and 48 unrelated males were sampled for each Sahiwal and Crossbred cattle respectively. Candidate molecular markers present in Sahiwal but absent in Crossbred and vice versa were detected using amplified fragment length polymorphism (AFLP) method. Eleven markers were developed that were converted to SNP markers for genotyping. The allele frequencies in both breeds were determined for discrimination ability using AFLP. Data were analyzed using Arlequin 3.5. The probability of identifying Sahiwal breed was 0.86 and probability of misjudgment was 0.021 using single selected markers. However, probabilities for judgment and misjudgment with 2 markers and combined with 3 markers were 0.745, 0.367 and 0.964, 0.376 respectively. Sahiwal breed and crossbred could be tested using the given markers and can be verified for purity before entering into breeding program.

Key Words: molecular marker, breed identification, Sahiwal

M96 Causes of inflation in genomic evaluations for young genotyped dairy bulls. S. Tsuruta*[1], D. A. L. Lourenco[1], I. Misztal[1], and T. J. Lawlor[2], [1]*University of Georgia, Athens, GA,* [2]*Holstein USA Inc., Brattleboro, VT.*

The objective of this study was to investigate causes of inflation in genomic predictions for dairy cattle. The simulated data included phenotypes, pedigrees, and genotypes, mimicking a dairy cattle population, which was selected by breeding values or not selected. With the simulated data, genomic (G)EBV were calculated with a single-step genomic BLUP and compared with true breeding values (TBV). Phenotypes and genotypes were simulated for 10 generations and the last 4 generations, respectively. Phenotypes in the last 2 generations were removed to predict breeding values using only genomic and pedigree information. For comparison, (G)EBV were also calculated using all phenotypes and genotypes in 10 generations. Pedigrees with and without inbreeding and pedigrees with unknown dams were used to construct the pedigree-based relationship matrix (**A**). Regression coefficients (b_1) of TBV on (G)EBV were calculated to investigate inflation in GEBV. In addition to the simulation study, inflation in GEBV for 18 linear type traits of US Holsteins were examined as well. Regression coefficients of daughter yield deviations on GEBV for young genotyped bulls were calculated. The results from the simulation indicated that GEBV for bulls were inflated regardless of selection whereas EBV were not inflated with no selection. The inflation was greater with no inbreeding or with no dams in **A**. On the other hand, inflation in GEBV for cows were minimal with no selection or with genotypes in all generations. For linear type traits, GEBV (and parent averages) were always inflated ($b_1 < 1.0$). To minimize the inflation, **A** and the genomic relationship matrix (**G**) should be consistent; including exact inbreeding in **A** is one way and using a weight (ω) < 1.0 on the inverse of **A** for genotyped animals ($\mathbf{A}_{22}^{-1}$), which increases the pedigree contribution ($\mathbf{A}^{-1} - \omega\mathbf{A}_{22}^{-1}$) for genotyped animals, is another way. Smaller or current additive genetic variances could be useful. In dairy cattle, known (accurate) pedigree information and consistency between **G** and **A** (and $\mathbf{A}_{22}$) could be essential to reduce the inflation in genomic predictions for young genotyped bulls.

Key Words: genomic prediction, linear type trait, US Holstein

M97 Genetic analysis of subclinical mastitis resistance in early lactation. S. G. Narayana*[1,2], F. Miglior[2,3], S. A. Naqvi[1], F. Malchiodi[2], P. Martin[2], and H. W. Barkema[1], [1]*Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada,* [2]*CGIL, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Canadian Dairy Network, Guelph, ON, Canada.*

Subclinical mastitis (SCM) causes economic losses for producers by affecting milk production and leading to higher incidence of clinical mastitis and premature culling. The incidence of SCM in first lactating cows is usually higher during early lactation. The somatic cell count (SCC) can be used for the diagnosis of subclinical mastitis. The objective of this study was to estimate genetic parameters for SCM in early lacta-

tion in first parity Holsteins. Test-day records for SCC were collected monthly between 2005 and 2009 in 90 Canadian herds participating in the national cohort of dairy farms of the Canadian Bovine Mastitis Research Network. Only the first test-day record available between 5 to 30 DIM was considered for the analysis. The final data set contained 8,518 records from first lactating Holstein heifers. Six alternative traits were defined as indicators of subclinical mastitis using different cut-off values of SCC (between 150,000 to 400,000 cells/mL). Linear and threshold animal models were used for the analysis. The prevalence of subclinical mastitis ranged from 15 to 24%. Estimated heritabilities from linear and threshold model ranged from 0.037 to 0.057 and 0.040 to 0.050, respectively. Strong genetic correlations were found among alternative SCC traits (from 0.90 to 0.99), indicating that these 6 traits were genetically similar. Despite a low heritability, estimated breeding values (EBV) predicted from both models showed a large genetic variation among sires. Higher EBV of SCM resistance corresponded to sires with higher percentage of healthy daughters. The percentage of diseased daughters varied between 5 to 13% and 19 to 33% among sires with best and worst EBV. The Spearman's rank correlations between EBVs of sires predicted from linear (0.76 to 0.95) and threshold (0.74 to 0.95) models were moderate to high.

Key Words: mastitis, heritability, somatic cell count

M98 Genetics of functional traits related to resistance of diseases and milk yield in Friesian × Bunaji crosses. I. Adedibu[1], A. Mshelia[1], A. Iyiola-Tunji[2], P. Barje[3], C. Lakpini[3], and T. O. Ososanya*[4], [1]*Ahmadu Bello University, Zaria, Kaduna State, Nigeria,* [2]*National Agricultural Extension and Rural Liaison Services, Zaria, Kaduna State, Nigeria,* [3]*National Animal Production Research Institute, Shika, Kaduna State, Nigeria,* [4]*University of Ibadan, Ibadan, Oyo State, Nigeria.*

This study estimated the relationship between milk yield and health trait indicators in F_1 (50:50) and F_2 (75:25) Friesian × Bunaji crosses (F×B) of cows. The data were collected from 500 F_1 and F_2 F×B cows on which routine standard measures: vaccinations, control of ecto- and endo- parasites were carried out. The data were collected between 2000 and 2017. The F_1 showed significantly ($P < 0.01$) positive genetic correlation (r_g) between milk yield (MY) and lameness (LM) and between MY, foot and mouth disease (FM). The LM was significant ($P < 0.01$) and moderately positive r_g with skin ulcer (SU); helminths (HM); FM and skin rashes (SR). Mastitis had significantly ($P < 0.05$) low positive r_g with HM and SR. There was only significant ($P < 0.05$) phenotypic relationship (r_p) between MY and FM but negative. The r_p between SU and mastitis, SU and LM, SU and HM were highly significant ($P < 0.01$) and highly positive. The F_2 showed significant ($P < 0.01$) moderate positive r_g between MY and LM as well as between MY with FM. LM had significant ($P < 0.01$) moderate positive r_g with HM, SU, SR and FM. There were moderate significant ($P < 0.05$) r_p between MY and LM, MY and FM. The SR and HM had highly significant ($P < 0.01$) positive r_p. Selection of F_1 F×B cows for improved MY can also benefit from genetic selection against FM and LM due to antagonistic pleiotropic effect. Selection against incidence mastitis may lead to selection of cows with low resistance to ecto- and endo-parasitic incidences. The F_1 F×B cows that were high milk producers in this study were likely to have lower incidences of FM. Selection of the F_2 F×B cows for improved MY can be undertaken and would aid selection for population with lower incidences of LM and FM. Pleotropic gene effect could have influenced LM, HM, SU, SR and FM in the F_2 indicating that selection can be effectively utilized to improve these health traits. In the F_2, the quantity of MY may not be affected by the traits LM and FM. In the F_1 and F_2 F×B cows, genetic relationships exist between MY and common dairy disease traits like LM and FM thus can be selected against at the onset of any breeding program.

Key Words: functional trait, milk yield, selection

M99 Development and application of the GENEX Jersey Ideal Commercial Cow Index (ICC$). H. Adams*[1], G. Abdel-Azim[1], L. James[2], J. Hanson[2], N. Hemauer[2], S. Carson[2], and R. Fourdraine[1], [1]*CRI International Center for Biotechnology, Mount Horeb, WI,* [2]*GENEX Cooperative Inc., Shawano, WI.*

Selection indexes are a valuable way for producers to simultaneously select several significant traits in a well-adjusted formula, allowing emphasis in multiple areas such as production, health and fertility. The GENEX (Shawano, WI) Holstein Ideal Commercial Cow Index (ICC$) has already proven itself to be a well-designed and useful economic-based selection index, allowing breeders to make selection decisions based on a single ICC$ value, or focus on a more specific area of improvement using sub-indexes that make up ICC$. As the GENEX ICC$ was originally designed for Holstein dairy producers, surveys were conducted on some of the largest and most progressive US Jersey herds with the aim to understand the projected future direction for the Jersey breed, and identify the traits and selection areas of most importance to Jersey producers. The gathered information was used to formulate an ICC$ specific to the Jersey breed. Data used in the model formulation was pulled from the CRI internal dairy research database (54 million health records on 12 million cows) utilizing 128K lactation records on 60K Jersey cows. Three sub-index categories were established: Cheese Maximizer (ChMAX$; component traits), Sustainability (SUST$; health traits), and Fertility (FERT$; cow and heifer fertility). Based on the producer survey responses, several indexes were tested using various trait combinations and corresponding economic weights that directly tie to on-farm profitability, to identify an ideal model. The final index was broken down into 43% ChMAX$, 35% SUST$ and 23% FERT$. Validation was done using active GENEX Jersey bulls measuring individual trait responses and compared with selection on JPI and NM$ based on the ranking of bulls within each index. The Jersey ICC$ is unique from currently available Jersey indexes in allowing individual producers to focus their selection on an area specific to their own farm's needs using the available sub-index values. The index also places more weight on fertility and health and emphasizes milk components while staying neutral to milk yield, which sets the index apart from currently available indexes.

Key Words: Jersey, ICC$, selection index

M100 Allele frequency of β-casein gene in local dairy animals of Pakistan. G. Bilal* and M. Moaeen-ud-Din, *Laboratories of Animal Breeding and Genetics, Faculty of Veterinary and Animal Sciences, PMAS Arid Agriculture University, Rawalpindi, Rawalpindi, Punjab, Pakistan.*

The objective of the present study was to identify the genotypes of our leading dairy cattle and buffalo breeds concerning A1 and A2 β-casein bovine milk protein. Blood samples were collected from local dairy cattle [Sahiwal (n = 13), Cholistani (n = 12), Holstein (n = 42), Crossbred (n = 18)] and buffalo (Nili Ravi; n = 15) bulls. DNA was extracted through GeneJet Genomic DNA Purification Mini kit (Thermoscientific, EU, Lithuania). Allele-specific primers for A1 and A2 alleles of β casein gene were designed using Primer Premiere (Ver. 6.0). Primers sequences used for A1 allele were (forward) CCCTTCCCTGGGCCCATCCC and

(reverse) TCAGTGAGAGTCAGGCTCTGG and for A2 allele were (forward) TCCCTTCCCTGGGCCCATCCC and (reverse) TCAGTGAGAGTCAGGCTCTGG. PCR (Proflex, Singapore) was performed at annealing temperature of 57°C for all primers and all samples. Briefly, the PCR mix had total volume of reaction mixture of 20 µL, including 10 µL of (2×) master mix, 7 µL of PCR water, 2 µL of primer (both forward and reverse) and 1 µL of extracted DNA template. After completing PCR, the PCR products were subjected to Gel Electrophoresis (Cleaver Scientific, UK). Briefly, 3 µL of PCR product was run on agarose gel (2%) at 90 V for 90 min and visualized in UV light in Gel Doc system (Syngene, UK). Bands with the specific band size were seen under UV light and confirmed for A1 and A2 by sequencing from Macrogen (Korea). The preliminary results suggest that our major dairy cattle and buffalo breeds (i.e., Sahiwal, Cholistani, and Nili Ravi) have desirable (A2A2) genotype and consumption of milk from our local breeds is healthier and safe. Moreover, crossbred and Holstein dairy animal present in the country have considerable presence of undesirable A1A1 or A1A2 genotypes (i.e., 0.17 and 0.74, respectively), indicating that import of exotic animals and crossing/inseminating local cows with exotic semen could result in the introduction of the undesirable and unhealthy β-casein A1 allele in the local dairy populations.

Table 1 (Abstr. M100). Frequency of A1A1, A1A2, and A2A2 genotypes among major local and exotic dairy animals in Pakistan

Species and breed	Sample size (N)	Genotype frequency		
		A1A1	A1A2	A2A2
Dairy cattle				
Sahiwal	13	0.00	0.00	1.00
Cholistani	12	0.08	0.00	0.92
Cross-bred	18	0.00	0.17	0.83
Holstein	42	0.07	0.67	0.26
Buffalo				
Nili-Ravi	15	0.00	0.00	1.00

Key Words: dairy animal, β-casein, Pakistan

M101 Comparative transcriptomic and iTRAQ proteomic analyses of bovine mammary glands in response to *Streptococcus agalactiae*-induced mastitis. H. Zhang*, H. Jiang, Z. Chen, Y. Mao, and Z.P. Yang, *College of Animal Science and Technology, Yangzhou University, Yangzhou, China.*

Mastitis is a prevalent disease in dairy cattle that causes large economic loss, mostly through its effects on milk production. *Streptococcus agalactiae* is a common and major cause of bovine mastitis, but the immune response related to *S. agalactiae*-induced mastitis is a very complex biological process. To understanding the pathophysiological process and the host immune response to *S. agalactiae*-induced mastitis, we used nipple tube perfusion of *S. agalactiae* to establish a Chinese Holstein cattle clinical mastitis model. Visual inspection, somatic cells count analysis, histopathology and transmission electron microscopy examination have confirmed the successful establishment of the *S. agalactiae*-induced mastitis model. Then, we used Bovine Gene Expression microarray and isobaric tags for relative and absolute quantitation (iTRAQ) to screen potential genes and proteins associated with mastitis. Compared with healthy mammary glands tissue, 129 differentially expressed genes (fold change ≥2, $P \leq 0.05$) and 144 differentially expressed proteins (>1.2-fold) were identified. Most significantly, differentially expressed genes were involved in immune and inflammatory reactions, such as complement and coagulation cascades, inflammatory mediator regulation of TRP channels, and bacterial invasion of epithelial cells. iTRAQ analysis also showed that the immune and inflammatory related proteins were significantly upregulated in the mastitis group, such as fibronectin 1, complement factor H, and von Willebrand. In conclusion, this joint analysis of transcriptomic and proteomic profiles provides an enhanced understanding of immune response to *S. agalactiae*-induced mastitis in bovine mammary glands.

Key Words: *Streptococcus agalactiae*-induced mastitis, transcriptomic analyses, proteomic analyses

M103 Genome-wide association study (GWAS) for bovine respiratory disease in pre-weaned Holstein calves. A. E. Quick*, T. L. Ollivett, B. W. Kirkpatrick, and K. A. Weigel, *University of Wisconsin, Madison, WI.*

Bovine respiratory disease (BRD) is one of the leading causes of morbidity and mortality in dairy calves. NAHMS (2011) reported that BRD affected 18.1% of pre-weaned heifer calves, with a mortality loss of 2.3%. The objective of this study is to establish a protocol for objective and efficient assessment of bovine respiratory disease (BRD) phenotypes in dairy calves and identify markers associated with BRD in a genome-wide association study (GWAS). 1,107 calves from 6 dairy farms in southern Wisconsin were measured at 3 and 6 weeks of age. Each calf was given a clinical score based on visual appraisal of eyes, nose, ears, attitude, cough, and temperature, as well as a subclinical score based on thoracic ultrasonography. The interaction of clinical and subclinical phenotype was represented on a 1 to 6 scale as overall BRD score. 1016 calves were genotyped with a commercially available single nucleotide polymorphism (SNP) array, and upon completion of quality control and imputation, 28,696 SNP and 1,014 individuals remained. A preliminary GWAS analysis was performed using a linear mixed model with SNP genotype as a fixed effect and with background polygenic effect as a random effect. Three- and 6-wk phenotypes were analyzed separately, and BRD scores were considered as binary (healthy or affected) or ordinal (6 levels reflecting increasing severity). At 3 wk of age, 8 and 6 significant SNP (P-value $<5 \times 10^{-5}$) were detected in the binary and ordinal analyses, respectively, with common SNP on chromosomes 1, 7, 17, and 18. At 6 wk of age, 3 significant SNP were found in each of the binary and ordinal analyses, with common SNP on chromosomes 8 and 9. Combining the clinical and subclinical scoring systems allows objective and efficient assessment of BRD for detection of important SNP using GWAS or whole-genomic selection against BRD. Further analysis is needed to identify putative genes affecting BRD and to assess the reliability of whole-genome predictions.

Key Words: genome-wide association study (GWAS), bovine respiratory disease

M104 Effect of feed selenium supplementation on dairy cattle Se transformation and cheese antioxidant activity. Z. Liu[1], Y. Xiao[2], C. Wang[3,1], J. Liu[1], and D. Ren*[1], [1]*Institute of Dairy Science, College of Animal Science, Zhejiang University, Hangzhou, Zhejiang, China,* [2]*Institute of Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China,* [3]*College of Animal Science and Technology, Zhejiang A&F University, Hangzhou, Zhejiang, China.*

Selenium (Se) is an essential micronutrient important to human and animal health. China is one of the countries experiencing serious Se deficiency. Our previous study found feed Se supply could improve Se content in milk. In the present study, the effects of milk Se additives; that is, Se-Met and Se-Yeast, on raw milk and cheese Se content, Se transformation in milk, blood, urine and feces, and antioxidant activity of cheese after 2 mo storage were compared. Three groups of 24 multiparous Jersey cows in mid-lactation were selected in local dairy farm. The first group as control received the basal diet; the second group received the basal diet with Se-Met (0.3 mg Se/kg DM), and the third group received the basal diet with Se-Yeast (0.3 mg Se/kg DM). After 2 mo feeding, milk Se content in the Se-Met group (76.5 μg/L) was higher than in the Se-Yeast group (59.5 μg/L). Se content in milk, blood, urine and feces were also compared, both Se-Met and Se-Yeast group was significantly higher than control. For the milk Se transformation, the Se-Met group (9.58%) is higher than Se-Yeast (8.45%) and control (7.91%). Using this Se-enrich milk make mozzarella cheese, a relatively high Se content was found in the Se-Met Mozzarella cheese (488 μg/kg) and Se-Yeast (415 μg/kg) than control (272 μg/kg). Antioxidant activity of cheese was evaluated by DPPH and reducing powder. For both index, Se-enriched cheeses showed higher values than control, whereas a similar result was found between Se-Met and Se-Yeast. Results of this study showed that both Se-Met and Se-Yeast supplementations could improve Se content in Jersey milk and cheese. The antioxidant activity of the Se-enriched cheese also improved, which may be of importance for human health.

Key Words: Se-Yeast, Se-Met, antioxidant activity

M105 Impact of inulin on the quality parameters of low-fat Cheddar cheese. M. S. Murtaza*[1], A. Sameen[2], M. A. Murtaza[3], and U. Farooq[1], [1]*Department of Food Science and Technology, Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan,* [2]*National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Punjab, Pakistan,* [3]*Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Punjab, Pakistan.*

Low-fat cheese is the demand of this era due to overconsumption and sedentary life style and increase in diseases such as hypertension, obesity, and cardiovascular diseases. Despite its fat content, cheese is a good source of valuable protein and peptides and a good source for lactose intolerant people. Removal of fat causes various quality problems so fat must be replaced with a fat-replacing agent and inulin is a good choice. Inulin is a food ingredient that belongs a class of carbohydrates known as fructans with degree of polymerization range from 2 to 70. Chemical structure is linear fructan with (2–1)-linked β-D-fructosyl units. Fructan with degree of polymerization <10 are called fructo-oligosaccharides. They have functional and health-promoting properties as they reduce caloric value, add dietary fiber, and endorse prebiotic effects. Inulin is frequently used in industrially processed dairy as a bulking agent for fat replacement, textural modifications, and organoleptic improvements. Hence, inulin can be used in manufacturing different kind of cheeses to have reduced or low fat, texturized or symbiotic product. The present study was designed to evaluate the effect of different levels of inulin in low-fat Cheddar cheese to improve its quality. Different levels of inulin have significant effects on the physico-chemical (moisture, fat, protein, ash) characters. Meltability and flowability showed inverse relationship with levels of inulin. Meltability and flowability decreased with increasing inulin and increasing hardness. Maximum meltability and flowability was noted in I_1 as 54.00 mm and 18.70% respectively. Yield calculation showed nonsignificant effect within levels but significant effect as compared with control. The fat substituting property is based on its ability to stabilize the structure of aqueous phase, which improves creaminess. The addition of inulin as fat replacement improved the sensory characteristics of low fat cheese samples when added up to the level of 0.5% inulin. Increase in inulin levels in cheese samples decreases the scores awarded for various parameters.

Key Words: meltability, flowability, hardness

M106 Development of a rapid method using near-infrared spectroscopy to quantify starch in shredded mozzarella cheese. L. Vázquez-Portalatín* and T. C. Schoenfuss, *University of Minnesota, Saint Paul, MN.*

Shredded mozzarella cheese poses challenges for manufacturers and consumers due to stickiness during shredding, caking of shreds, or growth of mold after packing. One solution is to add starch or cellulose-based flow aids, often with oxygen scavengers or antimycotics. Companies need to be able to quantify the flow-aid for quality assurance reasons, and to protect themselves from fraud when purchasing shredded cheese from others. Starch and cellulose wet chemistry methods are time consuming and require highly trained analysts. We developed a rapid method to quantify starch in shredded mozzarella using Fourier transform near-infrared spectroscopy (FT-NIR). Samples of low-moisture part-skim mozzarella loaves were shredded, and 0 to 10% of a starch/cellulose flow-aid was added. Treatments were ground, weighed, formed into a ball and pressed in the middle of the glass Petri dish with a force of 300 N for 1 min to create a homogeneous scanning surface. Samples were scanned using a Buchi NIRFlex N-500 FT-NIR spectrometer (Buchi Labortechnik AG, CH). NIRCal 5.2 Chemometric Software (Buchi Labortechnik) was used to analyze the spectra after first dividing the spectra of the 154 samples into 102 calibration sets and 52 validation sets. Reducing wavelength regions, adjusting the number of principal components, and pretreatment of spectra using Standard Normal Variate to minimize sample presentation variations, optimized the calibration. The resulting calibration was accurate with an R^2 of 0.9872, and a SD of 0.3321 when full-fat cheese >4 wk post-purchase, and smoked mozzarella were removed as outliers. Future research will determine if cellulose and starch can be identified and quantified separately in the same sample, and the effect of different starch and cellulose types on quantification. It is likely that different calibrations will be necessary to quantify smoked, aged, and full-fat mozzarella.

Key Words: mozzarella, flow-aid

M107 β-Lactam antibiotics in goat's milk affecting the characteristics of mature cheeses. P. Quintanilla[1], M. C. Beltrán[1], A. Molina[2], I. Escriche[1], and M. P. Molina*[1], [1]*Universitat Politècnica de València, Valencia, Spain,* [2]*Universidad de Castilla-La Mancha, Albacete, Spain.*

Antibiotic residues in milk may pose a risk for public health and for the dairy industry, especially in fermented products such as cheeses. The objective of this study was to analyze the effect of β-lactams (amoxicillin, benzylpenicillin, and cloxacillin) in goat's milk on the biochemical and texture characteristics of semi-hard Tronchón cheese. Milk from Murciano-Granadina goats with a good health status and not having received any veterinary drugs was used. The milk was spiked with β-lactam concentrations equivalent to their maximum residue limit (MRL) and ripened cheeses were made. One batch of cheeses from raw antibiotic-free milk was used as control. The proteolysis level was determined by free amino acids content (FAA, mg of leucine/g of cheese), the lipolysis level measured as the free fatty acids concentration (FFA, mEq KOH/100 g of fat) and texture parameters assessed by texture profile analysis (TPA: hardnesss, adhesiveness, springiness, cohesiveness, chewiness). Also, the residual concentration of antibiotics by HPLC-MS/MS in cheeses ripened for different periods (1, 30, and 60 d) was studied. The presence of amoxicillin in milk at MRL affected the FFA concentration in cheeses, being lower in antibiotic-spiked cheeses compared with control cheeses (2.21 vs. 2.67 mEq KOH/100 g of fat). However, the presence of benzylpenicillin in milk did not have any negative effects on the cheese properties. While, in cheeses made from cloxacillin spiked milk FFA concentration being lower than in control cheeses (2.40 vs. 2.67 mEq KOH/100 g of fat), also springiness was affected likewise (0.60 vs. 0.63). Amoxicillin (<2 μg/kg), benzylpenicillin (4.8 ± 1.8 μg/kg) and cloxacillin (28.8 ± 1.7 μg/kg) residual amounts were quantified at beginning of ripening (1 d), while antibiotic residues were not detected in the cheese at the end of ripening (60 d). It can be concluded that the β-lactam residue concentrations in matured goat's cheeses do not represent a potential public health risk. Nevertheless, similar studies with other antibiotic groups and using different cheesemaking processes are recommended.

Key Words: β-lactam antibiotic, goat milk, ripened goat cheese

M108 Influence of increasing milk protein concentration from 4 to 9% using ultrafiltration on Cheddar cheese pH and moisture. M. M. Motawee*[2] and D. J. McMahon[1], [1]*Western Dairy Center, Utah State University, Logan, UT,* [2]*Department of Nutritional Evaluation and Food Sciences, National Organization for Drug Control and Research, Giza, Egypt.*

Increasing protein levels of milk influences rate of whey expulsion during cheesemaking and buffering capacity of curd, which in turn influences moisture and pH of cheese. Our objective was to develop manufacturing procedures for making Cheddar cheese using milk containing 4 to 9% protein. Pasteurized milk was ultrafiltered to ~3.5× concentration then diluted with permeate to required protein levels. A time-standardized make procedure was prepared based on ~4 to 12 kg of milk such that each mini-vat contained ~400 g of casein. Acidification was by adding ~0.5% of a pH-controlled bulk starter. The milk was renneted and before cutting, 1.5 kg of permeate was overlaid upon the curd to minimize curd breakage upon stirring and facilitate curd syneresis. The pH and moisture of curd was monitored during cheesemaking and in the final cheese. Whey expulsion decreased as protein concentration increased such that curd made from 9% protein milk was lower ($P < 0.05$) in moisture than curd made from 4% protein milk after cooking and before draining. After cheddaring, milling and salting there was slight but not statistically significant differences in curd moisture. The final cheeses made using higher protein concentrations were about 1% lower in moisture than those made at the lower concentrations ($R^2 = 0.55$). Even though culture was standardized to protein concentration, this did not completely compensate for increased buffering capacity of the curd and there was less pH drop during cheesemaking as protein level increased: with differences of 0.1 units before draining and 0.2 units before pressing, such that the pH after 1 d increased from pH 4.9 to pH 5.1 for cheese made from 4% and 9% protein milk, respectively. During 30 d of storage, the pH of the cheese increased ~0.2 units. In conclusion, provided the starter activity added is increased to match the protein concentration, and permeate is added to help float the curd upon cutting, Cheddar cheese can be manufactured within moisture and pH targets with milk containing up to 9% protein.

Key Words: concentrated milk, ultrafiltration, starter culture

M109 Method development to quantify paste stability for surface mold-ripened cheeses. D. Batty*, J. Waite-Cusic, and L. Meunier-Goddik, *Oregon State University, Corvallis, OR.*

Surface mold-ripened cheeses undergo biochemical reactions during ripening that transform the curd from firm and chalky to soft and flowable over a short ripening period (3–12 wk). The degree and rate of softening varies depending on the procedure used for manufacture (lactic curd vs stabilized curd). The objective of this study was to objectively quantify the degree of paste stability for surface mold-ripened cheeses using a method that accounts for their heterogeneous consistency during maturation. In this study, 5 varieties of Camembert type cheese were manufactured using different cheese making procedures in triplicate and measured for composition. The paste displacement was quantitatively measured 35 and 50 d post manufacture. Cheese wheels (7 cm in diameter) were separated by a vertical cross sectional cut in the middle of the wheel. The initial temperature was 7°C at the start of the test. The paste displacement (flow) distance was measured at the furthest point it traveled away from the cheeses after 15, 30, 45, and 60 min. There were significant differences ($P < 0.05$) between the varieties, most notably between the lactic curd (furthest displacement), washed curd (middle displacement), and stabilized curd (least displacement) varieties. After 35 d of maturation and 60 min in to the test the lactic curd, washed curd, and stabilized curd traveled average distanced of 24 ± 3.4 mm, 7 ± 1 mm, and 3 ± 0.7 mm and after 50 d of maturation the distance traveled was 27 ± 1.9 mm, 19 ± 2.1 mm, and 4 ± 0.3 mm. An objective method for quantifying paste stability of non-homogeneous cheeses can help determine the level of paste stability in soft ripened cheeses, maturity of the cheese, as well as help cheese makers to optimize cheese recipes for customer requirements. Further investigation using various cheese wheel sizes will be necessary to verify this method is applicable to all cheese sizes.

Key Words: soft cheese, rheology, paste stability

M110 The effect of high hydrostatic pressure on the microbiological quality and shelf life of Camembert-type cheese. D. Batty*, A. Emch, L. Meunier-Goddik, and J. Waite-Cusic, *Oregon State University, Corvallis, OR.*

Surface-ripened cheeses, such as Camembert, typically have a short shelf life and are susceptible to contamination by environmental pathogens. High hydrostatic pressure processing (HPP) has been shown to be effective at extending shelf life and reducing pathogens post-manufacture in various food products. The objective of this study was to determine the

effect of HPP on the microbiological quality of a surface mold-ripened cheese. Three varieties of Camembert type cheese (traditional, stabilized curd, and a hybrid of the 2) were manufactured. The cheese was HPP treated (550 MPa for 10 min at 25°C) in duplicate treatments at either 3 (treatment A) or 10 d (treatment B) post manufacture. Treatment was applied either before (treatment A) or after (treatment B) surface yeast/mold growth. They were analyzed and monitored through shelf life for pH of the rind, pH of the paste, and microbiological quality (yeasts and molds, total plate count, and lactic acid bacteria). There were significant differences ($P < 0.05$) across both treatment time and types of cheese. *Penicillium candidum* had the largest decrease post-HPP with an average reduction of 2.3 ± 0.1 log cfu/g across all varieties 7 d after manufacture. At 21 d post manufacture there was an average reduction of 1.0 ± 0.3 log cfu/g for treatment A and 3.8 ± 0.8 log cfu/g for treatment B. The pH of the rind, another critical parameter for Camembert maturation and surface mold growth, was significantly lower after 14 d with treatment A having an average decrease of 1.69 ± 0.3 units (from 7.52 to 5.83) and treatment B decreasing by 2.36 ± 0.1 units (from 7.52 to 5.16) compared with the non-treated cheeses. Our study demonstrates that HPP of Camembert type cheese has a negative effect on the surface mold. However, HPP of cheeses before mold growth has a less negative effect on mold compared with pressure treating following surface mold development, indicating that HPP treatment time will influence the microbiological quality of surface-ripened cheeses.

Key Words: *Penicillium candidum*, pH, high pressure processing treatment time

M111 Selective growth using MRS broth for raw milk microbiome of naturalized Brazilian breeds Curraleiro Pé-Duro and Pantaneiro. N. R. Soares[1], M. C. Sola[2], C. Gebara*[1], G. V. Barancelli[3], O. F. Zaccaroni[4], M. C. S. Fioravanti[4], E. S. Nicolau[1], and C. S. Minafra-Rezende[1], [1]*Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil,* [2]*Unified Higher Education Institute, Objetivo Faculty, Goiânia, Goiás, Brazil,* [3]*Department of Agro-Industry, Food and Nutrition, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil,* [4]*School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil.*

The genus *Enterococcus* involves bacteria that are part of the natural microbiota of the gastrointestinal tract of animals and humans and is also found in water, soil and food. In the food industry, is used as starter or probiotic cultures, developing sensory characteristics and contributing to the balance of the intestinal microbiota. It should be noted that *E. faecium* can be considered probiotic or pathogenic, depending on the strain. The aim of this study was to select and identify potentially bacteriocin-producing lactic acid bacteria in raw milk from naturalized Brazilian breeds Curraleiro Pé-Duro and Pantaneiro after a selective growth on MRS medium. Raw milk samples were aseptically collected from Brazilian breeds Curraleiro and Pantaneiro (10 animals each) and aliquots were submitted to a selective growth using MRS broth at 37°C/16 h. The broth was centrifuged twice to obtain a cellular concentrate for performing molecular analysis. LAB was determined through DNA extraction from milk, the 16S rRNA gene was amplified by PCR and optimized by Illumina TruSeq platform and analyzed with QIIME. A total of 42,899 sequences was formed. The only genus identified was *Enterococcus*, with higher frequency of *E. faecium* (86.18% of sequences) on 90% of the samples. Others species identified were *E. mundtii* (90% of samples and 9.34% of sequences), *E. faecalis* (10% of samples, 3.47% of sequences), *E. lactis* (90% of samples, 0.84% of sequences), *E. durans* (45% of samples, 0.11% of sequences), and *E. hirae* (15% of samples, 0.05% of sequences). Our hypothesis is that lactic acid bacteria naturally present in milk of these breeds have the ability to produce inhibitory compounds against pathogens, because these animals present low mastitis incidence and produce milk with low SCC, despite the absence of management to control mastitis. These compounds could be organic acids or bacteriocins and some strains of the identified species, such as *E. faecium*, are bacteriocins producers. Therefore, these microorganisms have technological potential for isolation and using in dairy products and affect animal health and food safety.

Key Words: *Enterococcus,* molecular analysis, milk

M112 Effect of farm interventions on sporeformers and milk quality. R. A. Crespo*, B. A. Martínez, J. Stratton, and A. Bianchini, *University of Nebraska-Lincoln, Lincoln, NE.*

Bacillus and *Paenibacillus* spp. are spore-forming bacteria with the ability to survive pasteurization due to its spore structure. The powdered-milk industry may need spore counts as low as 50 spores/g to achieve the high quality standards required by foreign customers; hence, a microbial load of <5 spores/mL in raw milk would be required. Control of sporeformers below this limit is crucial to benefit the dairy industry. Interventions at the farm level are key because raw milk has been identified as a main entry point of these organisms into the milk chain. Previous research indicates that teat cleaning, changes in bedding material, and CIP procedures could be potential interventions to decrease sporeformer bacterial populations in milk. Therefore, the objective of this research was to implement these previously mentioned interventions in 2 farms and analyze their microbial effect. Samples were collected for 7 d to establish a microbiological baseline. After the interventions were applied and an acclimatization period of 15 d, samples were collected for 8 d to compare with baseline data. Environmental and raw milk samples were analyzed for mesophilic and thermophilic sporeformers. Additionally, microbial quality and prevalence of psychrotrophic sporeformers (7°C) for raw milk samples were performed. Raw milk quality analysis after interventions showed that total plate counts were around 3.0 log cfu/mL, whereas *Enterobacteriaceae* and coliforms were around 1.0 log cfu/mL. *E. coli* counts ranged from 0.25 to 0.45 log cfu/mL. Mesophilic spore counts ranged from 0.81 to 1.0 log cfu/mL, and thermophilic spore counts varied between 0.76 and 0.98 log cfu/mL in raw milk samples. Results suggest that a change in the sanitation (i.e., CIP) and bedding protocols (i.e., new bedding material) leads to a statistically significant reduction in sporeformers in raw milk. Changes in sanitizing teat dips showed different results among brands. The prevalence of psychrotrophic sporeformers did not seem to be affected by the interventions. This research showed that farm practices appear to exert an important effect on the levels of some sporeformers in raw milk.

M113 Increasing producer profitability through farm-level interventions designed for optimization of spore counts in raw milk. R. L. Evanowski*, D. J. Kent, N. H. Martin, K. J. Boor, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Spore-forming bacteria, such as *Paenibacillus* sp. and *Clostridium* sp., can survive pasteurization and affect the quality of dairy products (e.g., spoilage in fluid milk and late blowing in certain cheeses). With the demand for higher quality finished products that can be distributed further and to new markets, dairy processors are becoming more concerned with low spore counts in raw milk. Some processors have begun to offer premiums to producers who can supply low spore count raw milk for certain applications. The present study used results from a previous data collection to develop and test intervention strategies aimed at reducing transmission of spore-forming bacteria from environmental sources into bulk tank raw milk. These strategies involved (1) training milking staff to focus on teat end cleaning during milking preparation, and (2) implementing changes in towel treatment (i.e., use of detergent, chlorine bleach, and drying). Study design included collecting bulk tank raw milk samples for a week before and a week after initiating the intervention strategies (e.g., milker training on the importance of teat end cleaning and towel treatment). The interventions were conducted 3 times over the course of 15 mo at 5 New York farms, each of which had varying management practices. Teat end condition and udder hygiene scores were also collected. The 288 raw milk samples to date were analyzed for mesophilic and thermophilic spore counts. Results showed bulk tank milk mean spore counts of 1.5 and 1.3 cfu/mL for MSC and TSC respectively before the intervention, and bulk tank milk mean spore counts of 0.9 cfu/mL after the intervention for both MSC and TSC for a spore reduction of 41% and 42% in bulk tank raw milk for MSC and TSC respectively. This was found to be significant using a mixed effects linear regression model. The intervention strategies tested provide an easy to execute milking hygiene enhancement (e.g., focusing on teat end hygiene and towel washing procedures) that can reduce bulk tank

raw milk spore levels. Future studies on additional farms in a range of regions will be needed to further test these interventions.

Key Words: dairy, spore-forming bacteria, milk

M114 Sporulating behavior influences the population dynamics of sporeformers during raw milk holding. N. Awasti*[1], S. Anand[1], and G. Djira[2], [1]*Midwest Dairy Food Research Center, Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Department of Mathematics and Statistics, South Dakota State University, Brookings, SD.*

Thermoduric sporeformers are predominant in raw milk and form thermoduric endospores. Our previous research showed these sporeformers to cause biofouling of dairy contact surfaces and membranes, leading to cross contamination of final products. A critical factor influencing thermal inactivation is their form as vegetative cells or endospores. It would thus be of interest to understand the population dynamics of sporeformers in raw milk during storage at low temperatures. In our previous study, a low sporulating strain of *Bacillus licheniformis* showed an increasing trend in vegetative cell population during 72 h of storage at 10°C or higher, while maintaining spore population relatively static. In continuation, this study investigates population dynamics of a high sporulating strain of *B. licheniformis* (ATCC 14580). Raw milk samples were separately spiked with an average 4.0 log vegetative cells and 2.0 log spores/mL, and stored at 4°, 6°, 8°, 10°, and 12°C for 0, 24, 48, and 72 h. Standard protocols were followed for enumerating vegetative cells and spores. Three trials were conducted, in replicates of 3, and means were compared using ANOVA. Contour plots were developed using quadratic regression models to predict the population of vegetative cells and spores. In the vegetative cell spiking study, cell population remained mainly unchanged for 72 h up to 10°C, with more than 1.0 log change observed only at 12°C. As it was a sporulating strain, the spore spiking study validated a shift toward spores during storage at 4° to 8°C, with evidence of some parallel germination at 10°C or higher. The regression models helped us to develop contour plots across the holding temperature and duration. Based on the initial cell population of the spore former, such contour plots would help predict the presence of vegetative cells and spore populations in raw milk at a given time and temperature. This information will prove useful in optimizing raw milk holding conditions to keep the sporeformer population toward vegetative cells, which can subsequently be inactivated easily with thermal treatments such as pasteurization.

Key Words: sporulation, spore, *Bacillus*

M115 Feasibility of hydrodynamic cavitation, in line with HTST pasteurization, for inactivating sporeformers and spores in skim milk. P. Chaudhary*, S. Anand, and S. M. Monteagudo, *Midwest Dairy Foods Research Center, Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Sporeformers and their endospores are of concern for the dairy industry due to their thermal resistance. A previous study conducted in our laboratory revealed the effectiveness of a 6- to 8-pass cavitation effect (CE), in combination with batch pasteurization, in reducing vegetative cells and endospores of some common dairy sporeformers. In continuation, the current study evaluates the feasibility of reduced pass hydrodynamic cavitation, combined with HTST pasteurization for the reduction of sporeformers. The equipment set up was assembled in Davis Dairy plant to conduct a continuous cavitation and HTST pasteurization process. The spiking studies were conducted by inoculating sterilized skim milk with either vegetative cells (at 4.76 ± 0.03 log/mL) or spores (2.65 ± 0.09 log/mL) of a thermoduric strain of *Bacillus coagulans* (ATCC 12245). The flow of the spiked skim milk samples varied from 50 to 200 L/h, with a cavitation frequency of 60 Hz, using an APV cavitator. A small plate heat exchanger and a holding tube set up to achieve 73°C ± 1.0°C for 15 s effected the pasteurization. The spiked milk samples were processed through the above set-up for a single pass CE, followed by pasteurization to establish a baseline. The study was expanded up to 4-pass CE, combined with pasteurization, to observe their feasibility in enhancing spore inactivation. All trials were conducted thrice, with samples drawn in triplicates, and data were analyzed statistically using one-way ANOVA Tukey's test. The initial load of 4.76 ± 0.03 log of vegetative cells was reduced to 0.91 ± 0.04 log by a single pass CE at a flow rate of 200L/h, followed by pasteurization, as compared with 1.19 logs by pasteurization alone. In the case of spores, a 4-pass CE at 100L/h flow rate was found necessary to reduce the mean spore counts from 2.65 ± 0.09 log to 2.06 ± 0.02 log. The single pass CE combined with HTST pasteurization appeared to be feasible for reducing thermoduric sporeformers; for spore reduction, multiple CEs were necessary. Further evaluation studies are in progress.

Key Words: hydrodynamic cavitation, sporeformer, spore

M116 Efficacy of sub-pasteurization thermal treatments to inactivate *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* in fluid milk. A. Emch*, L. Goddik, J. Kovacevic, and J. Waite-Cusic, *Oregon State University, Corvallis, OR.*

Cheeses made with milk treated at temperatures below time/temperature requirements for pasteurization are subject to the 60-d aging rule as is required by the FDA for raw milk cheeses. Recent reports from the FDA have indicated their lack of confidence in the 60-d aging rule. It is likely that the FDA will be increasing their scrutiny of a variety of dairy products produced with non-pasteurized milk. Several commercial dairy companies produce cheeses made from milk that has been treated at these "sub-pasteurization" temperatures, these treatments are commonly referred to as "heat-shocked." Currently, there is very little research on the efficacy for heat shock treatment to reduce pertinent pathogens in fluid milk. The purpose of this study is to determine the efficacy of sub-pasteurization treatments to reduce pertinent foodborne pathogens (*Salmonella*, Shiga toxin-producing *E. coli*, and *L. monocytogenes*) in fluid milk. Raw milk (1 mL) samples were individually inoculated with a cocktail of *Salmonella, E. coli* O157:H7, or *Listeria monocytogenes* to reach a final concentration of approximately 8 log cfu/mL. Milk samples were heated in at 63°C in a recirculating water bath for 0–6 min. Survivors were enumerated using standard serial dilution and spread plating techniques using suitable selective-differential media: Hektoen enteric agar (*Salmonella*), Eosin Methylene Blue agar (*E. coli*), or CHROMagar Listeria spp. (*Listeria*). Plates were incubated at 37°C for 48 h before enumeration. A thermal treatment of 63°C for 6 min resulted in a 2.85 ± 0.95 log reduction of the *Salmonella* cocktail. These conditions were more effective at reducing *E. coli* O157:H7 and *L. monocytogenes* achieving 5.52 ± 0.98 log reduction and 5.12 ± 0.44 log reduction, respectively. *E. coli* and *Listeria* did not have significantly different reductions for the full treatment. The reduction of *Salmonella* was significantly less than both *E. coli* O157:H7 and *L. monocytogenes* (Tukey HSD $P < 0.05$). This study demonstrates that *Salmonella* is more resistant to heat-shock treatment in raw fluid milk under bench-scale conditions. Therefore, *Salmonella* would be an appropriate target organism for future thermal validation studies in fluid milk.

Key Words: fluid milk, heat-shock

M117 Effect of freezing and hardening on injured versus intact cells of *Listeria* in ice cream mix. N. Neha*[1], S. Anand[1], B. Kraus[2], and S. Sutariya[2], [1]*South Dakota State University, Brookings, SD,* [2]*Wells Enterprises Inc., Le Mars, IA.*

Ice cream manufacturing relies on pasteurization to eliminate any *Listeria* contamination. However, factors such as cross-contamination levels, entrapment in polymorpholeukocytes, and product matrices may influence cellular injury. Our previous studies demonstrated a dose-dependent, random presence of heat-injured cells of *Listeria* in pasteurized ice cream mix. Such cells did not show any recovery within the mix itself under normal handling conditions. The present study evaluates the effect of freezing and hardening of ice cream mix on heat-injured and intact cells of *Listeria*. Raw ice cream mix (42% TS) samples were spiked with 4.36 ± 0.13 logs per gram of *Listeria innocua* (a surrogate) and subjected to pasteurization (69°C for 30min), which resulted in the random presence of heat-injured cells. To simulate the post-pasteurization contamination of mix with intact cells, 2.65 ± 0.07 logs per gram of *L. innocua* were spiked in the pasteurized mix samples. The mixes containing injured and intact cells were followed through aging (72h at 7°C), freezing (−4.4°C), and hardening (12 h at −40°C) steps. Direct plating on *Listeria* selective agars enumerated intact cells, while *Listeria* enrichment broth (BLEB) was used to recover heat-injured cells before plating. All trials were conducted in triplicates and data were statistically analyzed. Although no post-pasteurization survivors were observed on direct plating, the enrichment protocol revealed heat-injured cells at all of the post-pasteurization stages of processing, tested. Freezing and hardening steps thus did not appear to have any detrimental effects on heat-injured cells. Injured cells have not been associated with any outbreaks; however, it would be interesting to study their ability to recover during any handling-abuse at retail or consumer end. In case of spiked intact cells, no detrimental effect of freezing and hardening was observed. This implies that post-pasteurization contamination of mix might pose a greater risk. Results from this study emphasize a need to design stage-specific critical control points to prevent any potential *Listeria* outbreaks.

Key Words: injured, *Listeria*, ice cream

M118 Enhanced efficacy of nisin loaded zein microcapsules against *Listeria monocytogenes* in Queso Fresco. L. A. Ibarra-Sanchez*, Y. Feng, Y. Lee, and M. J. Miller, *University of Illinois at Urbana-Champaign, Champaign, IL.*

Queso Fresco (QF), a widely produced Hispanic-style fresh cheese, is known to support the growth of *Listeria monocytogenes*. Nisin is a biopreservative with antimicrobial activity against *L. monocytogenes*; however, it has limited stability in near neutral pH foods, such as QF. The objective of this study was to evaluate the efficacy of nisin microencapsulated in zein against *L. monocytogenes* in QF. Zein microcapsules with low (LNM) and high (HNM) nisin loading (3–5 and 12–13 µg nisin/mg microcapsules, respectively) were prepared with a microfluidic device and nisin concentration was quantified via HPLC. Microencapsulated and free nisin were added to QF at a final concentration of 250 µg of nisin/g of cheese, and untreated cheeses were included as a control. The antilisterial activity of microencapsulated and free nisin was tested by inoculating cheese curds with approximately 4 Log cfu/g of *L. monocytogenes* cocktail of 5 different foodborne outbreak-associated strains, and *L. monocytogenes* cells were enumerated by spread plating on PALCAM agar supplemented with ceftazidime, across 7 d of storage at 4°C. All experiments were repeated 3 times with samples prepared in duplicate. Free nisin reduced the viable cell count of *L. monocytogenes* by approximately 0.8 Log cfu/g after 3 d, but subsequent regrowth led to a final population comparable to untreated QF. All treatments with nisin-loaded zein microcapsules achieved overall lower viable cell counts relative to free nisin, notably at early cheese storage. HNM reduced the initial viable population of *L. monocytogenes* by up to 1.5 Log cfu/g after 3 d. LNM showed higher viable pathogen reduction relative to HNM, reducing up to approximately 2 Log cfu/g from the initial inoculum after 7 d of cold storage. In conclusion, our results support the use of encapsulation technology to improve nisin's effectiveness to control *Listeria* in QF.

Key Words: Queso Fresco, *Listeria monocytogenes*, microencapsulation

M119 High-voltage atmospheric cold plasma on inactivation of *Listeria innocua* on Queso Fresco cheese. Z. Wan*, S. K. Pankaj, G. Li, and K. Keener, *Iowa State Universit, Ames, IA.*

Queso Fresco cheese (QFC), a type of Hispanic-style soft and fresh cheese, is a popular food in Latin-American diet. Due to its high moisture content, near neutral pH and moderate salt content, QFC provides an optimal environment for the growth of spoilage and pathogenic microorganisms. Currently, there are no effective commercial methods or technologies to reduce microorganisms in soft cheeses such as QFC. High-voltage atmospheric cold plasma (HVACP) is a novel, non-thermal technology that can be used to treat packaged food products and achieve significant reduction of pathogenic and spoilage microorganisms without compromising products' qualities. In this study, 2 types of gases, dry air and MA50 (50% CO_2, 50% N_2), were evaluated for inactivation of *Listeria innocua* (LI), a non-pathogenic surrogate for *Listeria monocytogenes*, in QFC by HVACP treatment. Survival of LI after HVACP treatments was evaluated by enumeration on *Listeria* selective agar. Quality effects after HVACP treatments were analyzed for lipid oxidation, pH, and moisture content. Plasma characterization was done using optical emission spectrometry. ANOVA was used for statistical analysis of microbial and quality data. The results have shown that HVACP treatment was able to achieve a maximal of 4.9 Log_{10} cfu/g LI reduction after dry air treatment and 1.7 Log_{10} cfu/g reduction after MA50 treatment. Higher lipid oxidation was found in samples after dry air treatment than MA50 treatment due to the presence of O_2 in dry air. HVACP treatments have resulted an average of 2.14 mg/kg malondialdehyde (MDA) formed in dry air treated samples, and 1.07 mg/kg MDA in MA50 treated samples. Negligible changes were observed in moisture content and pH after HVACP treatments. HVACP treated samples had an average pH and moisture content of 5.5 and 48.8%, respectively, versus 5.7 and 49.4% in control samples. LI reductions were found to be dependent on the gas composition and treatment time. The results demonstrate the efficacy of HVACP treatment for LI inactivation in QFC. This study shows the potential of HVACP technology for non-thermal processing of delicate dairy products.

Key Words: atmospheric cold plasma, *Listeria innocua*, Queso Fresco

M120 Screening of lipolytic, proteolytic, and antibacterial activities of lactic acid bacteria with biotechnological significance isolated from dairy products. I. García-Cano*, D. Rocha-Mendoza, J. Ortega-Anaya, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Fermented dairy products such as yogurt and cheese are an important source of high quality nutrients for consumers. The wide variety of fermented products, in terms of chemical composition, are the result

of the metabolism of lactic acid bacteria (LAB). The predominant enzymes that contribute to the diversity of fermented dairy products include lipases, proteases, and antibacterial proteins such as bacteriocins and peptidoglycan hydrolases, which are synthesized directly by LAB having a high biotechnological importance. Microbiome studies based in metagenomics indicate that there are specific enzymes contributing to the fermentation process and bioavailability. The objective of this work was to identify and characterize strains of LAB that possess high enzymatic expression of lipolytic, proteolytic, or antibacterial activity. Additionally, we aimed to identify and localize the proteins involved in these processes; weather it takes place in the intra or extra cellular milieu to assess the potential application in the dairy industry. The LAB (131 strains) were identified by 16S DNA sequencing. The bacteria were grown in CGB medium and harvested by centrifugation at the end of the log phase. Both the pellet and the supernatant of each strain were tested for lipolytic, proteolytic, and antibacterial activity by agar diffusion and zymograms. Five genera of LAB were identified as most active; 2 of them showed lipolytic activity in the cell fraction, hydrolyzing tributyrin agar and a-naphthyl-acetate in zymography. The active enzyme was identified by LC/MS-MS as a phospholipase of 40 kDa. Other strains in the supernatant fraction showed 3 proteolytic enzymes (45, 60, and 75 kDa) hydrolyzing casein. The antibacterial activity by zymogram against *M. lysodeikticus* was found in the supernatant fraction. *P. acidilactici* was unique in possessing 1 bacteriocin and 2 peptidoglycan hydrolases with putative *N*-acetylmuramidase and *N*-acetylmuramoyl-L-alanine amidase activities. So far, 40, 56, and 65% of total strains showed proteolytic, lipolytic, and antibacterial activity, respectively. A significant value of this report is the characterization of these enzymatic activities and their relevance in their potential biological activity in dairy foods

Key Words: dairy product, bioactive compound, lactic acid bacteria

M121 Addition of *Lactobacillus paracasei* and *Lactobacillus rhamnosus* bacteria to yogurts for inhibition of yeast growth and improvement of their quality. C.-H. Kim[1], M. S. Nam[2], and Y. W. Park*[3], [1]*Binggrae Company, Kyuunki-Do, South Korea,* [2]*Chungnam National University, Deajeon, South Korea,* [3]*Fort Valley State University, Fort Valley, GA.*

Yogurt is manufactured with 1.25% of active *Lactobacillus delbrueckii* ssp. *bulgaricus* and 1.25% *Streptococcus thermophilus* in milk by weight. Yeast contamination can occur during and after manufacture of yogurt products through unsanitary ingredients, equipment and processing personnel. *Lactobacillus paracasei* (Lp) and *Lactobacillus rhamnosus* (Lr) have been known to possess antimicrobial and heat resistant activities, respectively, against the growth of yeasts in cultured dairy products. The objectives of this study were to determine effects of the addition of Lp and Lr on inhibition of yeast cell growth in yogurts, and evaluate the improvement of organoleptic quality and storage stability of the fermented products. Two separate experiments were conducted by additions of Lp and Lr strains at 3 different inoculation rates of 3×10^1, 3×10^2 and 3×10^3 cfu yeast/2L yogurt and control group without bacterial addition. All control and experimental yogurt samples were stored at 2 temperature treatments (10°C and 25°C). The number of yeast cells in all experimental yogurt samples were counted weekly, and the swellings occurred due to gas production by contaminated yeast cells in all yogurt samples were evaluated. The results showed that the Lp and Lr bacteria added yogurt groups showed much slower rates of yeast cell growth and lower frequency of swelling in comparison with those of the control yogurts, suggesting that the growth of yeast was inhibited by the additions of Lp and Lr strains to the experimental yogurts. With respect to the effect of storage temperature, the yogurts stored at 10°C had little post-acidification in the Lp and Lr added groups compared with control samples, and also showed lower post-acidification than samples stored at 25°C. These outcomes suggest that the addition of Lp and Lr bacteria to the yogurt products could improve their organoleptic quality. It was concluded that the fortification of *L. paracasei* and *L. rhamnosus* bacteria improved storage stability and organoleptic properties of the yogurts, attributable to the delayed or inhibited yeast cell growth and post-acidification in the products.

Key Words: *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, yeast inhibition

M122 Growth of lactic acid bacteria in milk phospholipids enhances lipolysis and increases the possible absorption in Caco-2 cell line. D. Rocha-Mendoza*, I. García-Cano, J. Ortega-Anaya, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Fermented milk products like yogurt have been recognized for their beneficial effects on health. This is largely due to the presence of lactic acid bacteria (LAB) who are responsible for the synthesis of bioactive compounds and other molecules that positively affect human health and also modify the physicochemical and sensory characteristics. Owing to the complexity in nutrients in milk, LAB are known to "turn on" a standard set of genes that generate enzymatic activities well identified in fermented products so far. However, little is known about the metabolic potential of LAB when grown in minimal medium or the effect on lipolytic activity specifically toward milk phospholipids (PL). In this work, we aimed to evaluate the growth of different LAB strains from our culture collection (OSU library) in a minimal medium (glucose and tryptone modified) added with different concentrations of PL (0, 0.5 and 1% wt/vol) to determine lipolysis and its sub-products. The biological effect of the resulting metabolites on intestinal cells was further evaluated measuring absorption to Caco-2 cell. After screening 95 different strains of LAB we have found that *L. casei, L. pentosus, L. plantarum, P. acidilactici* and *P. lolli* are able to grow in a medium enriched with an optimized concentration of milk PL (0.5% wt/vol) producing an interesting mixture of metabolites (not identified peaks by HPLC-MS). Even though the growth rate decreased with the higher concentration of milk PL, as well as biomass production, the lipolytic activity was greatly increased as determined in vitro using 4-nitrophenyl acetate and 4-nitrophenyl octanoate, and α-naphtyl-acetate as substrate in zymogram experiments. We also isolated a protein with an approximate mass of 40 kDa, evaluated by SDS-PAGE, which is responsible for the predominant lipolysis observed by zymography. This enzyme product on PL modification or fragmentation represents a key factor that affects cell culture response as shown by our preliminary Caco-2 cell trials.

Key Words: milk phospholipid, lipolysis, lactic acid bacteria

M123 Rapid method for measuring the effect of prebiotics on probiotic bacterial growth. D. Hoffman*, C. Oberg, and M. Domek, *Weber State University, Ogden, UT.*

Prebiotics are used to stimulate probiotic bacterial growth in the gut to optimize their health benefits. A rapid method was developed to evaluate growth enhancement by prebiotics on probiotic bacteria using a programmable spectrophotometer, microtiter plates, and commercial media, with results ready in 12 h. *Lactobacillus* strains were grown in MRS broth while *Bifidobacterium* strains were grown in MRS broth with L-cysteine. Cultures were back diluted to an OD_{600} of 0.1 and then inoculated into wells (48-well plate) containing individual prebiotics. Plates were placed in a Tecan Infinite M200 spectrophotometer and

incubated at 37°C with A_{600} readings taken for 12 h. Growth curves were done in triplicate with results compared with controls to determine extent of prebiotic growth enhancement. To optimize the method, MRS concentrations of 20, 35, 50, and 100% were tested at selected pH values (7.0, 5.5, 5.0, 4.5, and 4.0) using 5 probiotic cultures. Addition of the bio-catalytic oxygen-reducing reagent, oxyrase, to the test wells significantly enhanced *Bifidobacterium* species and *Lb. acidophilus* growth. Results indicated a 25% MRS broth at pH 5.0 with 2% oxyrase optimized prebiotic growth enhancement comparisons. Using this method, the stimulatory effect of prebiotics (2% vol/vol) FOS, GOS, and XOS were determined for *B. infantis* M-63, *B. longum* BB536, and *B. lactis* BL-04, *Lb. rhamnosus* LR-32, and *Lb. acidophilus* NCFM. A one-sided *t*-test was used to determine significance ($P < 0.05$) between treatments and the control (no added prebiotic). All 3 significantly improved growth of M-63 (12 h OD_{600} for GOS-.85, FOS-.68, XOS-.64, and control-.60), but only FOS significantly increased growth of BL-04 (12 h OD_{600} for FOS-.67, GOS-.60, XOS-.57, and control-.60). For BB536, just GOS (12 h OD_{600} for GOS-.75, FOS-.70, XOS-.68, and control-.70) significantly enhanced growth. GOS and FOS slightly improved growth of NCFM whereas no oligosaccharides enhanced growth of LR-32. This method allows rapid testing of inoculum levels, prebiotic concentrations, media pHs, and prebiotic combinations for any probiotic strain including *Bifidobacterium.* With multiple samples run concurrently, comparisons can readily be made to determine optimum enhancement by individual prebiotics or prebiotic combinations for any probiotic strain.

Key Words: prebiotic, probiotic bacteria

M124 Flax seed enhances acid tolerance of *Streptococcus thermophilus* ST-M5. I. Moppert*[1] and K. Aryana[2,1], [1]*Louisiana State University, Baton Rouge, LA,* [2]*Louisiana State University Agricultural Center, Baton Rouge, LA.*

Flax seed (*Linum usitatissimum* L.) has been reported to provide several health benefits which include, reduction of cholesterol, protection against several cancers and improvement in blood sugar. For this benefit, the consumption of 62 g of flax seed per day is recommended. Acid tolerance is an important probiotic characteristic. The objective was to determine the effect of various amounts of flax seed powder on the acid tolerance of *Streptococcus thermophilus*. M17 broth of several pH values (1, 1.5, and 2) were prepared without and with flax seed powder incorporated at 62 g/L. Flax seed powder was dispersed using a magnetic stirrer. After sterilizing and cooling the M17 broths, freshly thawed *Streptococcus thermophilus* ST-M5 was incorporated and incubated at 42°C for 4 h. Samples were drawn at 2, 2.5, 3, 3.5, and 4 h, serially diluted and pour plated on M17 agar. Pour plates were aerobically incubated at 42°C for 48 h and colonies were counted. Samples were plated in duplicate. Entire experiment was replicated 3 times. Data were analyzed using PROC ANOVA of SAS, with means being separated using Fisher Least Significant Difference test. Mean counts of control pH 2, 1.5 and 1.00 and flax seed pH 2, 1.5 and 1.00 were 9.23 ± 0.25; 8.50 ± 0.43; 4.01 ± 0.54; 9.28 ± 0.26; 8.82 ± 0.37 and 4.39 ± 0.41 log cfu/mL. Flax seed counts at pH 1.5 and 1 were significantly ($P < 0.05$) higher than control. Flax seed enhanced the acid tolerance of *Streptococcus thermophilus* ST-M5.

Key Words: flax seed, yogurt, *Streptococcus thermophilus*

M125 Isolation of protein fractions of serum of milk by preparative disc-electrophoresis. V. Yukalo*[1], O. Tsisaryk[2], and K. Datsyshyn[1], [1]*Ternopil Ivan Pul'uj National Technical University, Ternopil, Ukraine,* [2]*Lviv National University of Veterinary Medicine and Biotechnology, Lviv, Ukraine.*

More than hundreds of different bioactive peptides are located in the primary structure of milk serum proteins. β-Lactoglobulin (β-LG), α-lactalbumin (α-LA), serum albumin (SA), lactoferrin and immunoglobulins belong to the bioactive peptides precursors. For the study and use of bioactive peptides, it is necessary at the first stage to select their homogeneous precursors. The purpose of the study was to isolate homogeneous precursors of bioactive peptides from serum proteins by preparative electrophoresis in a polyacrylamide gel. Homogeneity of protein fractions at different stages of their selection was analyzed by analytical disc-electrophoresis (Davis system) for acidic neutral proteins. The protein concentration was determined by spectrophotometer (SF-46, λ = 280 nm). In this case, previously established absorption coefficients ($D^{1\%}_{1cm}$) were used: 12.3 for total serum protein, 9.6 for β-LG, 20.9 for α-LA, 6.9 for SA, 9.91 for lactoferrin and 13.6 for immunoglobulins. Homogeneous fractions for identification of milk serum proteins were isolated by double gel-filtration on Sephadex G-100 (fine). Preparative electrophoresis was performed on a modified apparatus of Stadier type. Milk serum was isolated after precipitation of caseins at the isoelectric point. From the 3 variants of the anode electrophoretic systems (disc-electrophoresis with sodium dodecyl sulfate, disc-electrophoresis in the native conditions, electrophoresis in the presence of uric acid), disc-electrophoresis in the native conditions was selected due to its efficiency and accessibility. The isolation of homogeneous fractions included preparative disc-electrophoresis, identification of fractions, their extraction and drying. Based on the results of 5 preparative electrophoresis, it was shown that the total protein yield was 67.9% ($P < 0.05$). Among fractions, the immunoglobulins had the lowest yield (<60%). The β-LG, α-LA, and SA fractions had a yield close to the total protein one.

Key Words: milk serum protein, fraction, electrophoresis

M126 Combined effects of bovine casein or caprine casein and resveratrol on the chemical stability of α-tocopherol in sunflower oil-in-water emulsions. A. Mora-Gutierrez*, R. Attaie, M. T. Nuñez de González, Y. Jung, J. M. Kirven, and S. Woldesenbet, *Prairie View A&M University, Prairie View, TX.*

The objective of this study was to determine the combined effects of bovine casein or caprine casein and resveratrol on the chemical stability of α-tocopherol in sunflower oil-in-water emulsions. The highly hydrophobic nature of types I and II caprine caseins may improve the stability of resveratrol in dairy food systems. Previous studies have reported an increase in the chemical stability of α-tocopherol in the oil phase of sunflower oil-in-water emulsions stabilized by whey proteins when the *cis*-isomer of resveratrol partitioned into the oil-in-water interface. The whey proteins were also useful to entrap resveratrol, which is a hydrophobic bioactive compound. Sunflower oil-in-water emulsions (5% wt/wt) stabilized with bovine casein or caprine caseins type I and type II (0.5% wt/wt), α-tocopherol (0.015% wt/wt), and resveratrol (0.024% wt/wt) were prepared using a high-pressure homogenizer. The pH of the emulsions were adjusted to 7.0. The chemical stability of α-tocopherol was assessed by measuring the changes in its concentration in the emulsions during incubation at 21°C for 28 d using a HPLC method. The chemical stability of α-tocopherol increased significantly ($P < 0.05$) in emulsions with types I and II caprine caseins compared with bovine casein. Microencapsulation of α-tocopherol with either caprine casein-resveratrol or bovine casein-resveratrol protected α-tocopherol in sunflower oil-in-water emulsions and significantly reduced ($P < 0.05$) the chemical degradation of α-tocopherol during 28 d of storage at 21°C compared with the treatments without resveratrol. This information will be useful for understanding the behavior of α-tocopherol in dairy foods.

Key Words: casein, α-tocopherol, resveratrol

M127 Interactions of micellar casein and β-glucan as a functional ingredient in a model food system. S. Sarantis*, F. Maleky, R. Jimenez-Flores, and V. Alvarez, *Department of Food Science and Technology, The Ohio State University, Columbus, OH.*

Consumers' demand for "clean label" foods requires replacing commonly used additives with natural ingredient alternatives such as oat β-glucan, a soluble dietary fiber. Its consumption is associated with the reduction of coronary heart disease risk and its use improves the texture and mouthfeel of food products. However, adding β-glucan in dairy formulations often causes phase separation. This phenomenon is attributed to the thermodynamic incompatibility of micellar casein and β-glucan. The objective of this study was to investigate the casein - β-glucan interaction mechanism. Casein and β-glucan were mixed at 9 concentrations with ranges of 1.2 to 2% β-glucan and 4 to 6% casein and processed under 3 thermal conditions, namely pasteurization at 73°C/15 s, retort sterilization at 121°C/5 min and control (not treated). A phase behavior diagram was constructed to determine the critical biopolymer concentrations for phase separation. The stability of the samples was calculated as the percentage of the total volume occupied by the upper phase. Dynamic light scattering (DLS) was applied to investigate the biopolymer interactions through particle size distribution. The experimental design is a full factorial of 2 factors with multiple levels each. The phase behavior diagram showed that the heat treatment intensity increased significantly ($P < 0.05$) the critical total biopolymer (casein and β-glucan) concentration from 6.75% to 7.8%. Increasing the biopolymer concentration reduced the stability to 28%, while increasing the thermal treatment intensity increased the stability at levels higher than 90%. DLS results showed that increasing β-glucan concentration increased significantly the mean particle size from 300 nm to 1.8 μm ($P < 0.05$) revealing aggregate formation. The results indicate that segregative phase separation occurred due to limited biopolymer compatibility. Increasing the biopolymer concentration decreased the formulations' stability, while increasing the thermal treatment intensity resulted to more stable samples. This was confirmed by DLS, which showed smaller particle size for high heat treatment and low concentrations.

Key Words: casein, β-glucan, interaction

M128 Inclusion of ground coffee to dairy cattle rations and its impact on the milk proteome. M. C. Honan*[1], S. L. Zeger[1], D. B. Ebenstein[1], Y.-W. Lam[2,3], J. Kraft[1], R. A. Scuderi[1], and S. L. Greenwood[1], [1]*Department of Animal and Veterinary Sciences, The University of Vermont, Burlington, VT,* [2]*Vermont Genetics Network Proteomics Facility, The University of Vermont, Burlington, VT,* [3]*Department of Biology, The University of Vermont, Burlington, VT.*

Byproducts, co-products, and waste products from other industries are commonly utilized in dairy rations. Coffee is a popular phytochemical-rich commodity in the human food chain and processing of such yields numerous waste products, many of which have the potential to be included in livestock feed. Given the known effect of coffee and coffee residues on the rumen microbiota and metabolizable protein profile, it was hypothesized that inclusion of ground coffee in dairy cattle diets would affect the milk proteome and healthfulness of the milk produced. The objective of this study was to use proteomic analyses to identify and characterize the milk proteome produced by cows fed ground coffee. Ten mid lactation (151 ± 25 DIM) Holstein cows were fed the same base diet ad libitum over a 28-d period in a complete randomized block design, which included 2 supplementation treatments of either (1) 140 g/d (DM basis) of molasses (CON), or (2) 140 g (DM basis) of molasses + 70g/d of ground coffee (GC). Milk samples collected in the last week of the trial were stored for milk proteomic analysis. Milk samples were analyzed by HPLC for analysis of high abundance protein concentrations, including casein isoforms, α-lactalbumin, and β-lactoglobulin. For analysis of the low abundance protein-enriched milk fraction, LC-MS/MS techniques were utilized. All parameters were statistically analyzed using the PROC MIXED of SAS. While no differences in DMI, milk yield, or high abundance milk protein concentrations were observed across treatments, 102 proteins were identified using LC-MS/MS techniques. Abundances of 7 proteins decreased due to dietary treatment, including ENO2 protein, antithrombin-III, glycosylation-dependent cell adhesion molecule, junction plakoglobin, and several immunoglobulin-related proteins. Bioactive proteins including osteopontin, lactoferrin, zinc-2-α-glycoprotein, and lactoperoxidase were not affected by dietary treatment. The changes in the milk proteome are likely due to shifts in post-absorptive metabolism and confirm the potential use of secondary compounds in feeds as a mechanism to alter the milk protein profile.

Key Words: polyphenols, LC-MS/MS

M129 Simultaneous texturization and extraction of phospholipids (STEP) from a dairy by-product (whey protein phospholipid concentrate) using ethanol. N. R. Price*, T. Fei, S. Clark, and T. Wang, *Iowa State University, Ames, IA.*

There has been great interest in phospholipids (PL) found in dairy products due to their health and functional properties. Concentrating these polar lipids with microfiltration has been successful; however, this technology is significantly affected by protein interference which limits the achievable PL concentration. In this study, we demonstrated a method that successfully removed the protein while extracting the lipid and PL. The technology that was originally developed for egg yolk PL extraction, simultaneous texturization and extraction of PL (STEP), has been successfully applied to whey protein phospholipid concentrate (WPPC). This method successfully precipitated the protein present in WPPC and extracted the lipid and PL with a green alcoholic solvent ethanol. We also evaluated the effect of extraction temperature and ethanol concentration on the recovery of lipid and PL. A dairy lipid fraction with 65.62% PL was obtained under optimal conditions, and that is approximately 3 times higher than PL concentration reported in the literature.

Key Words: milk phospholipid, whey protein phospholipid concentrate (WPPC), texturization

M130 Effect of different phosphate mixtures and homogenization pressure on the particle size distribution of whole milk. I. Lima de Paula[2], E. F. Maurício[1], Í. T. Perrone[1,2], A. Fernandes de Carvalho*[1], L. F. Cappa de Oliveira[2], and R. Stephani[2], [1]*Universidade Federal de Vicosa, Vicosa, MG Brazil,* [2]*Universidade Federal de Juiz de Fora, Juiz de Fora, MG Brazil.*

The application of different phosphate combinations is a usual industrial practice in Brazil, aiming to increase protein stability during UHT processing. Milk homogenization is an essential step whose aims are to reduce the fat globule size and avoid the physical separation of the lipid fraction due to the formation of a new fat globule membrane composition with direct participation of the proteins. The objective of this study was to evaluate the influence on the nanostructure and microstructure of whole milk when 4 concentration levels of 3 different commercial phosphate mixtures were added, subjected to 200, 5000, or 800 bar of homogenization pressure, after 3 storage times at 5°C. Particle size distribution was analyzed by laser refraction on LS 13.320 Beckman Coulter particle analyzer (Brea, CA). From the 81 experiments, 80% of the treatments presented d_{90} values higher than their corresponding values without addition of the phosphate mixtures, indicating an increase in the fat globules diameters, due to the participation of the caseins after the influence of the salts used.

Key Words: protein stability, nanostructure, microstructure

M131 Efficacy of local vitamin D-fortified dairy products versus oral vitamin D supplementation in Saudi adolescents. N. Al-Daghri*[1], M. G. A. Ansari[1], S. Sabico[1], Y. Al-Saleh[2], N. Aljohani[2], H. Alfawaz[1], M. Alharbi[3], A. Al-Othman[4], M. Alokail[1], and S. Wimalawansa[5], [1]*King Saud University, Riyadh, Saudi Arabia,* [2]*King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia,* [3]*Ministry of Health, Riyadh, Saudi Arabia,* [4]*Sehhati National Medical Company, Riyadh, Saudi Arabia,* [5]*Cardio Metabolic Institute, New Jersey, NJ.*

Vitamin D deficiency is rampant in the Middle East, even in children and adolescents. This study was designed to investigate the effects of claimed vitamin D-fortified dairy products versus oral vitamin D supplementation on serum vitamin D levels of Saudi adolescents. A 6-mo multi-center, controlled clinical study, involving 34 schools in the central region of Riyadh, Saudi Arabia, was conducted. Four locally available milk products having "vitamin D-fortified" labels [200 mL fortified milk of different brands] and vitamin D tablet (1,000 IU) were tested. Anthropometrics were taken and fasting blood samples withdrawn at baseline and after intervention for the quantification of serum glucose, lipid profile, and 25(OH) vitamin D. A significant increase in 25(OH)D level was observed in subjects supplemented with vitamin D tablet, milk brands 2 and 4, whereas subjects supplied with fortified milk brands 1 and 3, respectively, exhibited a significant decrease in 25(OH)D levels. Analysis of covariance showed that after adjusting for baseline 25(OH)D, age, gender, and BMI, the mean 25(OH)D levels of children who were taking vitamin D tablet (9.1 ± 0.8 nmol/L) and milk brand 4 were significantly higher (7.3 ± 1.1 nmol/L) than children taking milk brand 2 (1.6 ± 1.0 nmol/L). Subjects supplied with milk brands 1 and 2 exhibited a significant increase in total cholesterol level, while it dropped significantly in subjects taking milk brand 3. No changes were observed in other groups. Although the superiority of oral vitamin D supplementation versus fortified milk is unquestionable, different dairy products used in this clinical study elicited varying degrees of improvement in serum 25(OH)D level. The observed outcomes were dependent on the strategy and gender in the Saudi adolescent population, with oral tablet supplementation being favored in boys.

Key Words: vitamin D deficiency, fortified milk, school children

M132 Influence of milk pH on the manufacture of Licor de Oro, a beverage produced in Chiloé island, Chile. R. A. Ibáñez*[1], M. F. Muñoz[1], N. Brossard[1], S. Wyhmeister[1], F. Osorio[2], and E. Vargas-Bello-Pérez[3], [1]*Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Universidad de Santiago de Chile, Santiago, Chile,* [3]*University of Copenhagen, Copenhagen, Denmark.*

Li-Cor de Oro (or Gold Liqueur; LO) is a traditional alcoholic beverage produced in Chiloé island, Chile. LO is made by mixing milk, alcohol (usually *aguardiente*, a distilled alcohol from wine) and sugar at a ratio of 1.0:1.0:0.7, along with lemon juice and other spices (such as vanilla, cloves and saffron), leading to a phase separation. The mixture is stored up to 2 wk and then filtered to obtain a product with a yellowish-transparent appearance, sweetness and acidic taste, milky and alcoholic notes and slightly viscous. The principle used to make LO is based on the destabilization of caseins (CN) due to the presence of alcohol in acidified milks. The lack of information regarding LO processing, mainly in the amount of acid added to the mixture, leads to products with high variability on their quality. The objective of this study was to evaluate the effect of milk acidification on the physicochemical and sensory properties of LO. Independent batches of raw milk (1 L) were acidified using 25% (vol/vol) citric acid to 6 different pH values: 6.7 (control), 6.0, 5.3, 4.6, 3.9 and 3.2. Each milk treatment was then mixed with 1 L of *aguardiente* (50% vol/vol ethanol), 700 g of sucrose and other minor ingredients (vanilla and almond extracts, cloves, and saffron). The mixtures were then filtered using filter paper after 2 wk of storage and the filtrates (LO) were further analyzed for composition, viscosity, turbidity and descriptive sensory analysis (12 trained panelists). As expected, a decrease of milk pH led to LO with higher levels of acidity ($P < 0.05$). LO obtained at pH 6.7 and 6.0 exhibited higher ($P < 0.05$) levels of total protein than other treatments, leading to a higher turbidity and viscosity ($P < 0.05$) when determined by instrumental and sensory analysis, which could be attributed due to the presence of CN as confirmed by protein analysis using HPLC. In contrast, treatments made at pH ≤5.3 had a typical transparent appearance of LO, because CN were not detected. These results suggest that a combined ethanol and acid concentration in milk is necessary to destabilize CN and obtain a desired appearance and composition on LO.

Key Words: milk-based beverage, alcohol destabilization of milk proteins

M133 Use of the simplex-centroid mixture design to development of whey fermented beverages with buttermilk and Brazilian Cerrado fruit. R. T. Pfrimer*[1], L. Damasceno[1], C. F. Cardoso[2], F. A. Freitas[1], E. R. B. de Souza[2], E. Arnhold[3], E. S. Nicolau[1], and C. Gebara[1], [1]*Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil,* [2]*School of Agronomy, Federal University of Goiás, Goiânia, Goiás, Brazil,* [3]*School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil.*

The simplex-centroid mixture design is used for the development of new food products. This design saves time and money by generating mathematical models that predict the centesimal composition of new formulations without the need to repeat experiments. Thus, it is an important innovation tool for the food industry. The aim of this work was to produce different formulations of whey fermented beverages with buttermilk and cagaita pulp (*Eugenia dysenterica*) using the simplex-centroid design. Therefore, 7 formulations with different concentrations of independent variables were prepared: whey/buttermilk mix (x_1), milk (x_2), and cagaita pulp (x_3) and its centesimal composition (dependent variables) was determined. Using design, it was possible to determine the best regression model and to generate the equations that predict the behavior of each dependent variable by changing the relation between the concentrations of the independent constituents. The following equations were obtained, where y = response estimate. For moisture: $y = 78.12x_1 + 84.33x_2 + 83.41x_3 + 8.25x_1x_3 – 13.38x_2x_3$; lipids: $y = 0.50x_1 + 3.40x_2 + 2.73x_3 – 1.76x_1x_3 – 5.83x_2x_3$; proteins: $y = 0.50x_1 + 1.55x_2 + 2.58x_3 + 3.49x_1x_2 + 0.02x_1x_3 – 0.20x_2x_3$; and total energy value: $y = 88.50x_1 + 75.89x_2 + 79.49x_3 – 45.53x_1x_3 + 24.00x_2x_3$. The interaction x_1x_3 shows synergism with moisture and antagonism with lipid and total energy value content. The interaction x_2x_3 shows antagonism with the moisture, lipids, and protein. A positive correlation was observed by interaction x_1x_2 only with the protein content of formulations. By using the models generated through the simplex-centroid design, it is possible to predict components like moisture (which influences the shelf life of the product), lipids and proteins (which influence the viability of microorganisms and the sensorial characteristics of the product), as well as total energy value and other constituents of the food. Therefore, the composition can be predicted and modified without having to do new experiments.

Key Words: innovative product, dairy coproduct, cagaita pulp

M134 Effects of polymerized whey protein on goaty flavor and texture properties of goat milk yogurt in comparison with β-cyclodextrin. C. Wang*[1], C. Wang[1], F. Gao[1], Y. Xu[1], and M. Guo[2,1], [1]*Jilin University, Changchun, Jilin, China,* [2]*University of Vermont, Burlington, VT,* [3]*Northeast Agriculture University, Harbin, Heilongjiang, China.*

Goaty flavor and poor consistency may affect consumer acceptance of goat milk yogurt. The special flavor and poor consistency may be mainly attributed to the presence of short- to medium-chain free fatty acid (SM-FFA) especially C_6–C_{10} fatty acids and low casein content in goat milk. This study aimed to investigate the effects of polymerized whey protein (PWP) on goaty flavor as well as texture of goat milk yogurt compared with β-cyclodextrin (β-CD). Samples were evaluated on sensory properties, SM-FFA contents, texture, and viscosity. Compared with control, the contents of fatty acids (C_6, C_8, C_{10}) of the yogurt samples significantly decreased approximately by 22, 71, and 54% in goat milk yogurt with 0.5% β-CD while they decreased approximately by 45, 58, and 71%, respectively, with 0.7% PWP. There was a synergistic effect of 0.3% β-CD and 0.6% PWP by decreasing the contents of SM-FFA (C_6, C_8, C_{10}) sharply by 89, 90, and 79%, respectively. Under the same percentage of addition in the yogurts with β-CD showed a higher ($P < 0.05$) viscosity than those with PWP. However, addition of PWP increased the texture parameters of goat milk yogurt ($P < 0.05$). Combination of PWP and β-CD presented a more desirable texture and consistency in goat milk yogurt. Results indicated that polymerized whey protein could be used to reduce the goaty flavor and improve the texture of goat milk yogurt.

Key Words: goat milk yogurt, goaty flavor, polymerized whey protein

M135 The role of hydrocolloids and saliva in rheological behavior and texture perception of yogurt. M. Baniasadidehkordi and H. S. Joyner (Melito)*, *University of Idaho, Moscow, ID.*

Texture plays a key role in consumer acceptance of semisolid foods such as yogurt. Hydrocolloids can significantly alter semisolid food structure, which affects their texture. Additionally, semisolid foods show different oral behaviors as a result of interactions with saliva. A better understanding of how different hydrocolloids and saliva interactions

affect semisolid food textures could help in the development of low-fat or fat-free products with textures similar to their full-fat counterparts. Thus, the objective of this study was to determine the effect of saliva and hydrocolloids on rheological and tribological behaviors of yogurts. Twelve different yogurt formulations were prepared with skim milk, skim milk powder (0–2.8% wt/wt), cream (0–3.5% wt/wt), whey protein isolate (0–2.8% wt/wt), and hydrocolloids (corn starch, potato starch, locust bean gum and cellulose gum; 0–1.55% wt/wt). Yogurt mixtures were pasteurized at 85°C for 30 min, homogenized at 5,000 rpm for 1 min, and cooled to 42.2°C for the addition of starter cultures. The mix was incubated for 5–6 h to reach a pH of 4.55–4.6, and then the gel was broken and refrigerated overnight at 4°C. Tribometry was performed at 25°C and rheometry (shear rate, strain, and frequency sweeps) was performed at 8°C and 25°C. Both rheometry and tribometry were conducted with and without human whole saliva. Two-way ANOVA was used to analyze rheological data; one-way ANOVA was used for tribological data. Addition of saliva caused significant differences in viscosity at 1 s^{-1} and friction coefficient at 0.1 mm s^{-1}. Critical strains with and without saliva were not significantly different. Most samples showed solid-like behavior; the highest storage moduli were for the sample containing WPI alone. Control samples had the lowest viscosity; the addition of hydrocolloids and WPI significantly increased viscosity. In general, friction coefficient decreased with added saliva. Confocal images showed a distinct, heterogeneous protein network with longer clusters and fat coalescence for samples with added saliva and hydrocolloids. These results can help manufacturers design low-fat semisolid foods with similar textures to their full-fat counterparts.

Key Words: rheology, texture, yogurt

M137 Storage stability of commercial powder goat milk in relation to changes in physico-chemical properties under different temperature and time treatments. B. I. Davis, R. Paswan*, A. Siddique, and Y. W. Park, *Fort Valley State University, Fort Valley, GA.*

Shelf-life of dehydrated foods can be predicted by several factors including water activity (a_w), moisture content, types of lipids present in the products and environmental conditions. Studies have shown that reduced overall acceptability and spoilage of foods are highly related to a_w and stability of lipid moiety in food products. Storage stability of dehydrated bovine milk with respect to a_w and lipid oxidation have been reported extensively, while few studies have been conducted on powdered goat milk (PGM) products. The objectives of this study were to determine changes in a_w, pH, lipid oxidation of the PGM during storage, and evaluate the stability of PGM products in relation to a_w, moisture content, peroxide value (POV) stored under different temperature and time treatments. Three batches of commercial whole PGM were purchased at a local retail outlet at Warner Robins, Georgia. Each batch of the experimental PGM samples were placed in 120 mL size light-proof amber glass bottles in triplicates, and stored at 4°C and 22°C for 0, 2 and 4 mo. At each storage period, samples were tested for differences in basic nutrients, moisture content, water activity, pH, peroxide value (POV) and their correlations between these parameters at different storage time and temperature treatments. Results showed that there were no differences in levels of moisture (%) and a_w between the 2 temperature treatment groups. The respective mean a_w for 0, 2, and 4 mo at 4°C and 22°C were 0.266, 0.251, 0.243; 0.291, 0.266, 0.219, suggesting that a_w and moisture contents were significantly ($P < 0.05$) reduced during the 4 mo storage. No differences were found between temperature × storage interaction effects. Significant ($P < 0.05$) differences were found in POV among different storage time and temperature treatments. We concluded that the a_w and moisture content of the commercial PGM were decreased by storage period, but not affected by temperature, while lipid oxidation of the PGM was significantly affected as the storage time advanced.

Key Words: storage stability, powder goat milk, water activity

M138 Effect of relative humidity and storage temperature on the physical, chemical, and thermal properties of deproteinized whey powders. P. Thorakkattu* and J. K. Amamcharla, *Kansas State University, Manhattan, KS.*

Deproteinized whey (DPW) is obtained during ultrafiltration of whey and it is processed into a non-hygroscopic and free-flowing DPW powder. DPW powders may undergo changes such as caking and Maillard browning during storage due to its high lactose content. This study investigated the effect of temperature and relative humidity (RH) on the physical, chemical, and thermal properties of DPW powders. Six DPW powders were obtained from a commercial manufacturer with varying protein contents ranging from 2.47 to 3.42%. The water activity (a_w), color, pH, titratable acidity, and fluorescence of advanced Maillard products and soluble tryptophan (FAST index) were determined for the fresh powders. The a_w of the fresh powders was in the range 0.222 to 0.285. It was observed that as the protein content increased the titratable acidity increased in the fresh powders. The lightness (L*) values ranged from 86.7 to 88.8 and the FAST index values ranged from 60.83 to 153.92 for the fresh powders. The DPW powders were stored over saturated salt solutions with RH (11.2, 22.6, 43.8 and 52.9%) and temperature (25 and 40°C) for 1 mo. The color and FAST index were determined for the powders after storage. It was observed that temperature, RH, and their interactions significantly influenced ($P < 0.05$) color and FAST index. We found that the L* values decreased on storage and ranged from 51.76 to 80.81. Also, FAST index values increased with storage ranging from 67.23 to 879.43. The Maillard browning was studied using front face fluorescence spectroscopy (excitation: 360 nm and emission: 380 to 480 nm) and the emission spectra showed a broad peak with a maximum around 437 nm for the fresh and stored powders. The thermal properties of DPW powders were determined with differential scanning calorimetry and a dehydration endothermic peak at around 142°C was observed. Overall, the DPW powders stored at RH 43.8 and 52.9% at 25 and 40°C showed poor quality characteristics as indicated by FAST index and color.

Key Words: lactose-rich coproduct, storage changes, fluorescence spectroscopy

M139 Impact of supercritical fluid extraction with CO_2 on milk powders. S. Sreenivasaraghavan* and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

Supercritical fluid extraction has been demonstrated as a reliable technique to extract triglycerides and volatiles from food. The aim of this work was to study the effect of temperature and pressure of supercritical fluid extraction using CO_2 on the physicochemical properties of buttermilk powder (BP) and whey protein isolate (WPI). The properties studied included triglyceride reduction, solubility, lactose crystallization, heat stability, particle size, particle morphology, foaming, and water activity. Triplicate treatments were applied to Land O'Lakes BP and Hilmar WPI using our supercritical fluid extractor (Waters Inc., Milford, MA). All results were statistically analyzed by ANOVA. Temperature ranges tested were 65 to 80°C, and pressure ranges from 250 to 350 Bar. Our resulting surface-response graphs indicate a much larger triglyceride extraction at 80°C and 350 bar. However, there is not a clear preference

on these parameters when measuring solubility indices in either BP of WPI. The increase of solubility of treated BP was significantly higher than that of WPI as compared with untreated samples. This correlated with the lower concentration of triglycerides in WPI initial sample. Foam volume and stability was significantly reduced in BP but not in WPI. One particularly noticeable change was the particle size reduction of both treated powders when reconstituted and analyzed by static laser diffraction. However, this difference was only significant when compared with untreated samples. Other parameters measured were not significantly modified by the treatments tested.

Key Words: dairy processing, supercritical fluid extraction

M140 Effect of storage temperature and protein content on the flowability and morphological characteristics of milk protein concentrate powders. K. S. Babu*[1], K. Siliveru[1], J. K. Amamcharla[1], P. V. Vadlani[1], and K. Ambrose[2], [1]*Kansas State University, Manhattan, KS,* [2]*Purdue University, West Lafayette, IN.*

Milk protein concentrate (MPC) powders are widely used dairy ingredients in a variety of food product formulations to improve the nutritional, functional, and sensory properties. Flowability and morphological characteristics are affected by processing, storage, particle size, and composition of the powder. This study investigated the flowability and morphological characteristics of commercial MPC powders with protein concentrations of 70–90% (wt/wt) after storage for 12 wk at 25 and 40°C. Powder flow properties [basic flowability energy (BFE), flow rate index (FRI), and permeability] and shear properties (cohesion and flow function) were measured using the Freeman Technology FT4 rheometer (FT4, Freeman Technologies, UK). After 12 wk of storage at 40°C, the BFE values significantly increased ($P < 0.05$) from 512 to 722 mJ with the increase in protein content from 70 to 90% (wt/wt). FRI was significantly higher ($P < 0.05$) for samples with high protein content (90%). Dynamic flow tests indicated that MPC powders with high protein content displayed higher permeability. Shear tests confirmed that the samples stored at 25°C were relatively more flowable than samples stored at 40°C. Also, the higher protein content samples showed poor shear flow behavior compared with low protein samples. The results indicated that MPC powders stored at 25°C had lesser cohesiveness and better flow characteristics than MPC powders stored at 40°C. The MPC powders showed distinctly different flow properties due to their difference in intrinsic physical and chemical composition. The circle equivalent diameter and elongation of MPC powders increased as protein content and storage temperature increased, whereas convexity decreased as protein content and storage temperature increased. The flow, shear, and morphological characterization can help us understand the behavior of MPC powders with different protein contents during storage and will be useful in the process design of handling, processing, and storage.

Key Words: high-protein dairy powder, FT4 rheometer, storage

M141 Freezing point determination of raw milk using Fourier-transform infrared spectroscopy (FTIR). E. L. Duarte, J. S. Lima, W. L. F. T. Vicentini, J. P. M. Heringer, D. C. S. Z. Ribeiro, R. Rodrigues, M. O. Leite, M. M. O. P. Cerqueira, and L. M. Fonseca*, *Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.*

The milk freezing point is correlated with the soluble components and can be an indicator of fraudulent addition of water. The objective of this study was to investigate the interference of milk fat content upon the freezing point obtained by Fourier-transform infrared (FTIR), and to evaluate the relationship among freezing point, milk components, and somatic cell count (SCC). A fraction of raw milk was skimmed, obtaining cream and skim milk. Whole and skim milk were added with water (0, 2, 5, 10, 15, and 20%; experiment 1). The remaining skim milk was added with cream (5, 10, 15, 20%) and water (0, 2, 5, 10, 15, and 20%; experiment 2). Samples were analyzed for freezing point (thermistor cryoscope) and freezing point and composition (CombiScope FTIR, Delta Instruments). Additionally, 4,000 bulk tank raw milk samples with abnormal characteristics (freezing point, composition, and SCC) were screened and analyzed for adulteration (sugar, starch, salt, formaldehyde, neutralizers and hydrogen peroxide). Freezing point measured by FTIR was highly correlated with thermistor cryoscope method (above 98.5%) and, as expected, water addition was significant ($P \leq 0.001$) upon increasing milk freezing point in both methods. However, high fat content in raw milk resulted in decreasing freezing point when using FTIR ($P \leq 0.001$). One hundred and ninety-six samples (4.9%) were screened as suspect from a total of 4,000 bulk tank raw milk samples routinely analyzed in the laboratory. From these, 74.5% presented freezing point above −0.512°C, indicating potential fraudulent addition of water. Although sugar, starch, neutralizers or inhibitors were not found, high chloride concentrations were found in 12.2% of the samples. However, high values of SCC and freezing point were found in 7% of the samples. SCC is correlated with high chloride concentration. Most of the suspected samples presented fraudulent addition of water, which reinforces the importance of constant monitoring of the milk freezing point. Because water addition caused the inverse effect of high fat on freezing point measured by FTIR, it is important to consider the fat content of milk to avoid misinterpretation of freezing point when using FTIR method.

Key Words: freezing point, Fourier transform infrared (FTIR), composition

M142 Management tools for monitoring milk quality in dairy industries. V. M. Araújo[1], A. H. N. Rangel*[2], S. B. P. Barbosa[1], A. M. V. Batista[1], and J. G. B. Galvão Jr.[3], [1]*Universidade Federal Rural de Pernambuco, Recife, PE, Brazil,* [2]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [3]*Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil.*

This study aimed to evaluate the capability index (C*pk*) and the charts of the statistical process control (SPC) as quality management tools for somatic cell counts (SCC) and total bacterial count (TBC) of raw refrigerated milk supplied to industries. The data were obtained from milk samples collected monthly from tanks for 36 mo from 5 dairy industries. Data transformations were performed for logarithmic scale, somatic cell score (SCS) and transformed bacterial count (tTBC). The capability index was obtained by equation C*pk* = MIN (LSE – μ/3σ; μ – LIE/3σ), and the center line (CL) and the upper (UCL) and lower (LCL) control limits, both distant to 3σ standard deviations from the center line, were calculated from the data of the variables from each industry over time to investigate the potential of control charts in detecting special causes of variation for monitoring the SCS and the tTBC. The maximum and minimum limits considered were 500,000 and 400,000 cells/mL and 300,000 and 100,000 cfu/mL for SCC and TBC, respectively. Only one industry presented an acceptable process or was able to meet the established specifications for SCS and tTBC ($1 \leq C_{pk} \leq 1.33$). The control charts for the 5 industries indicated the presence of special causes (intermittent) in the variation of the processes for both variables (SCS and tTBC). The results highlight how industries need to improve their processes in order for their milk suppliers to meet quality standards. Therefore, the C*pk* index and the SPC charts are viable tools

for detecting violations in quality standards for the SCC and TBC of refrigerated raw milk supplied to industries.

Key Words: process capability, control chart, statistical process control

M143 Refrigerated raw milk quality among dairy processors in northeastern Brazil. V. M. Araújo[3], A. H. N. Rangel*[1], S. B. P. Barbosa[3], J. G. B. Galvão Jr.[2], and A. M. V. Batista[3], [1]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [2]*Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil,* [3]*Universidade Federal Rural de Pernambuco, Recife, PE, Brazil.*

The study objective was to evaluate seasonal influence on the chemical composition, somatic cell count (SCC) and total bacterial count (TBC) of refrigerated raw milk supplied to milk processors situated in northeast region of Brazil. Data were obtained from milk samples collected monthly from tanks in 41 industries registered in the federal inspection service (S.I.F.), distributed across 9 states in northeastern Brazil. A total of 76,230 samples were collected regarding fat, protein, somatic cell count (SCC) and total bacterial count (TBC) over a period of 36 mo. Data transformations to logarithmic scale were performed for SCC and TBC data. An ANOVA and the Tukey test were performed to verify whether there was interference of the month factor in the variables. The months of the year had a significant influence ($P < 0.05$) on the means of chemical composition, SCC and TBC. For chemical composition, the highest averages were recorded between May and July. The average fat content varied from 3.51 to 3.69% and the protein content ranged from 3.07 to 3.17%. The averages for somatic cell score ranged from 4.66 to 4.90 cells/mL, in which the highest was registered in the month of July, while the transformed bacterial count ranged from 2.34 to 2.53 cfu/mL, with the highest in March. 44.25% of the samples for SCC did not comply with the limit of 400,000 cells/mL, and 71.63% of the samples were in non-compliance for TBC with a limit of 100,000 cfu/mL. On a scale of priorities, the TBC is an indicator that deserves stronger action in corrective measures, since the means found for the milk from the cooling tanks in the states of the northeast Brazil are far above that recommended for good quality milk.

Key Words: milk composition, total bacterial count, somatic cell count

M144 Transcriptome analysis revealed that aflatoxin M_1 causes cell cycle arrest in differentiated Caco-2 cells. X. Y. Bao[1,2], Y. N. Gao[1], J. Wang*[1], and N. Zheng[1], [1]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China.*

Aflatoxin M_1 (AFM_1) is known as one of the hydroxylated metabolites of aflatoxin B_1 (AFB_1) and the most threatening aspect of AFB_1 contamination. It could lead to hepatotoxicity and hepato-carcinogenicity, and possess intestinal cytotoxicity. However, little was known about the potential mechanisms of the intestinal cytotoxic effect. The aim of this study was to investigate the intestinal barrier damage induced by AFM1 via transcriptome analysis. Gene expression profiling was analyzed to comparatively characterize the differentially expressed genes (DEG) after differentiated Caco-2 cells were exposed to different concentrations of AFM_1 for 48 h. A total of 165 DEGs were significantly clustered into 2 downregulated patterns. The Search Tool for Retrieval of Interacting Genes (STRING) based protein-protein interaction (PPI) network analysis suggested 23 key enzymes mainly participate in regulation of cell cycle. Q-PCR analysis was performed to validate that the 12 key genes *BUB1*, *BUB1B*, *MAD2L1*, *CCNA2*, *RB1*, *CDK1*, *ANAPC4*, *ATM*, *KITLG*, *PRKAA2*, *SIRT1* and *SOS1* were involved. This study is the first to our knowledge to reveal that the toxicity of AFM1 against intestinal function might be at least partly due to the occurrence of cell cycle arrest.

Key Words: aflatoxin M_1, cell cycle, intestinal barrier

M145 Combined intestine toxicity effects of aflatoxin (AF)B_1 and AFM_1 in mice. M. Zhang[1,2], N. Zheng[1,2], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Milk Product Risk Assessment Laboratory of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Food crops are easily to be contaminated by aflatoxin B_1 (AFB_1), which is more commonly encountered and considered to have most toxicity among the types of aflatoxins. When AFB_1 is ingested by dairy cows in contaminated feed, undergoes liver biotransformation and is converted into aflatoxin M_1 (AFM_1), which is excreted in milk. Because of this, when contaminated cereal and dairy products are ingested together, people will face the simultaneous exposure risk of AFB_1 and AFM_1. Studies confirmed that there is an increasing awareness of the deleterious effects of aflatoxins during their fate within the gastrointestinal tract (GIT). This study employed ICR (CD-1) mice (aged 4 wk) to investigate the combined intestine toxicity effects of AFB_1 and AFM_1. After acclimation, the mice were randomized into 4 groups of 10 each and received an oral administration of 0.5 mg/kg of BW AFB_1, 3.5 mg/kg of BW AFM_1, mixture of AFB_1 and AFM_1 (0.5 mg/kg of BW AFB_1, 3.5 mg/kg of BW AFM_1) and vehicle for 4 weeks (once a day), then blood and intestine were collected. The concentration of diamine oxidase (DAO), citrulline (Cit), intestinal fatty acid binding protein (I-FABP), D-lactate in serum and histomorphology, apoptosis analysis of duodenum, jejunum and ileum were measured. Statistical analysis was carried out by SAS 9.4 software. The results showed individual or combination of AFB_1 and AFM_1 did not affect body weight of mice significantly. And individual of AFB_1 or AFM_1 did not affect the concentration of Cit, I-FABP, and D-lactate in serum, but combined of AFB_1 and AFM_1 increased the DAO, I-FABP, and D-lactate in serum significantly. Individual of AFB_1 or AFM_1 increased the apoptosis ratio of cells in duodenum, jejunum, and ileum slightly, but combined of AFB_1 and AFM_1 increased it significantly. These results demonstrated that compared with individual of AFB_1 or AFM_1, combined of AFB_1 and AFM_1 could increase intestinal mucosa permeability and induce apoptosis of intestinal cells, which could provide basic information for further study of the combined intestinal toxicity effects of AFB_1 and AFM_1.

Key Words: aflatoxin B_1, aflatoxin M_1, toxicity

M146 Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from different raw milks in China. H. Liu[1,2], L. Meng[1,2], L. Dong[1,2], N. Zheng[1,2], and J. Wang*[1,2], [1]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Milk Product Risk Assessment Laboratory of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Staphylococcus aureus is one of the main pathogens involved in dairy cow mastitis. Monitoring antimicrobial resistance in *S. aureus* from raw milk is very important to make decisions regarding antibiotic treatments of animals from a food safety standpoint. The objective of this work was to investigate the prevalence of *S. aureus* strains isolated from different raw milk in China, and to characterize antimicrobial susceptibility of these strains and their key virulence genes. Raw milk samples of cow (n = 195), goat (n = 50), buffalo (n = 25), camel (n = 25), and yak (n = 25) were collected from 8 provinces in China. *S. aureus* was identified by biochemical identification and the *nuc* gene. Antimicrobial susceptibility patterns for recovered *S. aureus* isolates were determined by the agar disc diffusion method according to the guideline of the Clinical Laboratory Standard Institute. Antimicrobial resistance genes and virulence genes were tested by PCR. A total of 90 strains of *S. aureus* were isolated, included 54 strains from cow milks, 16 strains from goat milks, 9 from buffalo milks, 6 isolates from camel milks, and 5 isolates from yak milks. Among the 90 strains, antimicrobial resistance was most frequently observed topenicillin G (71.1%), followed by to ampicillin (61.1%), erythromycin (37.8%). The antimicrobial resistance *S. aureus* isolates were the most frequently observed in cow milk (79.6%), followed by goat milk (31.8%), camel (18%), buffalo (16.2%), and yak (7.69%). The most predominant resistance genes were *blaZ* (52.2%), *mecA* (24.4%), and *cfxA* (21.1%). The antimicrobial resistance genes were most frequently detected in *S. aureus* strains from cow milk (31.2%), followed by goat milk (26.9%), camel milk (14.1%), buffalo milk (10.24%) and yak milk (7.69%). The most predominant toxin genes were *sec* (22.2%), followed by *sea* (12.2%), and *tst* (12.2%). The majority of *S. aureus* strains were multidrug resistant and carried multiple virulence genes, which may pose a potential risk to public health. Our data indicated that the prevalence and antimicrobial resistance of *S. aureus* was a serious concern in different raw milks in China, which may pose potential risk to public health, especially cow milk and goat
milk.

Key Words: antimicrobial resistance, China, *Staphylococcus aureus*

M147 The effect of furosine on gut microflora in ICR mice model. N. Zhao[1,2], H. Li[1,2], N. Zheng[1,2], J. Wang*[1,2], and J. Cheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Furosine, which is produced when milk and milk products are heated, is bad for people's health. Furthermore, its content in milk and milk products will increase sharply as the temperature rises. The intestines harbor a highly diverse microbial community, which is critical to maintain health. To investigate furosine's effect on structure and abundance of gut microflora in ICR mice, a 35-d chronic toxicity model was constructed. Fresh feces sample was collected and detected by Illumina-MiSeq 16s rRNA sequencing technology. We found that the amount of *Actinobacteria* and *Firmicutes* in furosine treatment groups increased at the phylum level ($P < 0.05$); the abundance of *Alloprevotella* and *Bacteroides* in furosine treatment groups increased at the genus level ($P < 0.05$), while the level of *Lactobacillus* in furosine treatment groups decreased significantly ($P < 0.05$). In conclusion, furosine increased the complexity and uncertainty of microflora in mice intestine, embodying the downregulation of probiotics and upregulation of maleficent bacteria, indicating that microflora changes might be related to immune injury and intestinal damage caused by long-term intervention of furosine.

Key Words: milk, furosine, microflora

M148 An UPLC method for the quantitation of furosine in liquid milk. F. Wang[1,5], Y. Zhang[1,2], N. Zheng[1,3], F. Wen[1,4], P. Li[1,4], Q. Wang[1,2], B. Du[1,2], G. Huang[1,2], W. Du[1,2], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*Milk Product Risk Assessment Laboratory of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [4]*Milk and milk product Inspection Center of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [5]*Institute of Quality Standard and Testing Technology for Agro-Products, Shandong Academy of Agricultural Sciences, Jinan, Shandong, China.*

The concentration of furosine in liquid milk, as an indicator to assess the heat load on dairy products, can be used to distinguish different processed milk. The most established detection methods, based on the international standard (ISO 18329:2004/IDF 193:2004), includes acid hydrolysis of milk, solid-phase extraction, HPLC separation and PDA detection at 280 nm. However, the pretreatment is tedious and time-consuming. In this work, we developed a simple and time-saving UPLC method for furosine detection. Water film is used, instead of solid-phase extraction, which lead to easier operation, time and cost savings, and 10% recovery increased. The total runtime, instead of 32 min in the international standard method, is only 8 min. The limits of detection and quantification are 3 and 10 μg/L, respectively. The linearity range, calibrated by diluted furosine standards in matrix solvent, are 0.2 to 5.0 mg/L, with determination coefficient $R^2 > 0.9999$. The recoveries of various concentration of furosine in different liquid milk samples ranged from 78.4 to 112.6%, with relative standard deviations of 0.1 to 6.7%. Moreover, 34 liquid milk samples were measured for their furosine concentrations. The results (furosine content of 4.0–6.8, 9.1–14.2, and 148.3–197.6 mg/100g protein for raw milk, pasteurized milk, and UHT milk samples, respectively) indicated the furosine amounts increased with increasing heat load applied. Therefore, this simple and quick UPLC method can be used to test furosine concentration in liquid milk.

Key Words: furosine, UPLC, liquid milk

M149 Intestinal cells exposed to different thermo treated bovine milk exhibited diverse gene expressive pattern. H. Yang[1,2], N. Zheng[1,2], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Consumption of raw milk may pose a health threat due to a possible contamination with certain pathogens. Thermal processing can guarantee microbial safety, but this procedure meanwhile alters the physicochemical property of milk and denatures original bioactive components. To assess whether these changes could lead to diverse functional consequences, 4 types of bovine milk (raw, pasteurized, UHT sterilized, and in-can sterilized) were digested in vitro and then applied to culture human intestinal Caco-2 cells. Subsequent transcriptomic analysis demonstrated that the genome expressive patterns of cells under the condition of raw milk and pasteurised milk were quite similar, definitely distinct from UHT and in-can sterilized group. Compared with raw milk group, 3, 662, and 1,904 differentially expressed genes (DEG, absolute fold change >1.5 and false discovery rate <0.05) were identified in pasteurized, UHT, and in-can milk culturing cells, respectively. Gene ontology

analysis using overlapping DEG in UHT and in-can group showed that the most significantly enriched biological processes were allocated to cell cycle classes. A key gene *CDK1*, which controls the transition of G_2/M phase, was downregulated in both sterilized milk culturing cells. Moreover, some important genes (*NRAS*, *KRAS*, *PI3KR*, *PIK3C3*, *RHEB*, *EIF4EBP1*, *EIF4E*) involved in PI3K-AKT-mTOR-EIF4EBP1 pathway were deregulated, which together declined protein translation and may eventually cause cell cycle arrest. As many previous studies reported that the gross nutritional value of milk can be reduced by heat treatment, its underlying mechanisms were further illustrated by the transcriptomic profiling in this work.

Key Words: milk, thermal processing, cell cycle

M150 Bringing research to the farm: How producers adopted new practices following an on-farm calf management workshop. D. E. Santschi*, S. Adam, and D. M. Lefebvre, *Valacta, Ste-Anne-de-Bellevue, QC, Canada.*

Finding effective ways to translate knowledge from the latest research into applicable concepts on farm is a continuous challenge for the dairy industry. Valacta held a series of workshops on young calf feeding and management practices in Quebec, Canada, from November 2016 to March 2017. A total of 1,398 producers attended one of the 127 sessions offered. Topics addressed covered mainly the pre-weaning period and focused on colostrum (importance in terms of nutrients, IgG content, management, timing of first feeding), milk feeding (type and quality of milk replacers, feeding levels, proper mixing of milk replacer), solid feeds (forages, concentrates, types and amounts), weaning (protocol and timing), and growth monitoring. In a discussion format, producers were invited to compare and challenge current feeding and management practices for young calves. Producers were also asked to bring a colostrum sample as well as a sample of reconstituted milk replacer to learn how these can be assessed quickly on farm and to generate discussion around the results. Between 2 and 3 mo after their participation in the workshop, producers were sent a survey to evaluate adoption of best practices covered in the workshop. Producers were asked if they used the suggested management practice already before the workshop (Before), since the workshop (After), or not at all (No). Table 1 presents results of the 199 workshop participants who answered the survey. Testing colostrum for quality and daily milk feeding practices were the ones where the workshop had the greatest impact. The importance of growth monitoring still needs to be reinforced.

Table 1 (Abstr. M150). Survey responses

	Yes (%)		
Management practice	Before	After	No (%)
Test colostrum with refractometer	6	72	28
Feed colostrum within 1h of birth	66	92	8
Feed a second meal of colostrum	23	56	42
Keep a bank of frozen colostrum	37	63	35
Thaw colostrum at 40°C	16	50	25
Ensure adequate quality of milk replacer	63	78	9
Weigh amount of milk replacer	26	54	23
Verify mixing temperature	21	50	43
Reconstitute milk replacer at 150 g/L	37	31	9
Verify milk final temperature	21	48	43
Rapidly increase to at least 8 L/d milk	56	86	11
Wean gradually when calf eats 2 kg/d	62	79	18
Monitor growth at 2, 6 and 13 mo	21	33	64
Breed at target BW	69	78	17

Key Words: calf management, producer workshop, knowledge transfer

M151 Factors associated with variation in dry period length. P. Pattamanont* and A. De Vries, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Dry period lengths (DPL) are known to be around 60 d but may systematically vary between and within herds. These systematic variations are not well known. The objective of this study was to describe the distribution of DPL among Holstein herds across the United States and to investigate the association between parity, days to conception, and seasons with DPL. After editing, DHIA lactation records from cows in their first and second parity (n = 438,087) were available for 1,167 herds. Cows calved from January 2012 to 2016. To be included in the analysis, herd size was at least 50 cows, cows completed their first lactation and had a subsequent calving date, and length from conception to drying off was at least 180 d. Daily milk yield was individually quantified by fitting Wood's lactation curve to milk test records. The variable of interest was days from conception to dry off (CtoD) because this measure was less affected by irregular gestations lengths, and hence a better indication of the intended DPL. Four seasons were defined based on date of dry off. The association between season and CtoD was calculated as the largest difference in CtoD among the 4 seasons. Factors explaining within herd variations in CtoD were investigated with simple linear regressions of CtoD with days to conception and parity, by herd. Overall, cows in the first parity had longer CtoD but less variation (224.4 ± 14.31 d) than cows in the second parity (220.3 ± 17.10 d). Compared with second parity CtoD, 2.5% of farms had ≥ 5-d shorter CtoD for first parity cows, whereas 43% of herds had ≥ 5-d longer CtoD for first parity cows. In 15% of the herds, the mean difference between CtoD in first and second parity cows was at least 1 d. In 34.1% of herds, the regression of days to conception on CtoD was significant ($P < 0.01$). In 4.3% of the herds, each later 10 d of conception increased CtoD by at least 1 d. In 18.5% of herds, the regression of season on CtoD was significant ($P < 0.01$). In conclusion, CtoD was typically greater for first parity cows than second parity cows, indicating likely shorter DPL in first parity cows. Days to conception and season all were associated with CtoD in a large number of farms, which indicates that many dairy farmers vary cows' DPL on purpose.

Key Words: dry period length, parity, season

M152 On-farm bacteriologic milk culturing: Producer perception and decision impact. B. L. Bowman, M. D. Denny, and A. E. Stone*, *Mississippi State University, Starkville, MS.*

The objective of this study was to evaluate producer perception and implementation of on-farm bacteriologic culturing (OFBC). Nine Mississippi dairy producers volunteered to participate after attending a local workshop in exchange for a month of free OFBC supplies. Initial farm visits were conducted between June 28 and 31, 2017, and ended 30 d later. Producers were asked to culture samples from cows with clinical mastitis and record the pathogen identified and resulting treatment or management decisions. Four weekly surveys were administered, intended to evaluate change and producer-perceived challenges and benefits. A $\chi 2$ analysis was conducted using the FREQ procedure of SAS for all questions asked during both wk 1 and 4. No significant changes occurred in the producers' confidence in the accuracy of the test, confidence in their ability to run the test, amount of time the test added to their routine, the percent of cows with clinical mastitis they chose to sample, or the amount of antibiotics used on-farm ($P > 0.05$), all of which remained high throughout the study. No significant differences were observed in management decisions between wk 1 and 4 or producer-reported bulk tank SCC ($P > 0.05$). Throughout the study period, many producers treated cows regardless of OFBC results (n = 27; 28%), used a quarter milker (n = 23; 24%), or did nothing (n = 14; 14%) after reading the results. Most culture results were no growth (n

= 41; 31%), followed by staphylococcus species (n = 25; 19%), streptococcus species (n = 24; 18%), *Staphylococcus aureus* (n = 16; 12%), and gram-negative (n = 12; 9%). Most producers reported that OFBC was inexpensive to perform, improved cow health, reduced antibiotic treatment expense, reduced contamination risk to other cows (all n = 6; 75%), reduced endotoxin release risk, and was faster than a laboratory (both n = 5; 63%). At the end of the study, 8 producers chose to purchase the incubator and extra plates to continue OFBC. Although producers did not use the tool for its intended purpose, they still wanted to continue its use. Reasons for lack of management change are not yet fully understood. Producer behavior is difficult to understand or predict.

Key Words: on-farm culture, mastitis, extension

M153 Semillas program: Engaging dairy farm workers' youth to the dairy industry. M. Rovai*[1], D. Bittiker[2], and A. Garcia[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*4-H Program, South Dakota State University, Brookings, SD.*

Dairy expansion in the United States resulted in greater dependence on Latino immigrant labor. The majority of workers' children, although born in the United States still identify with Spanish-speaking backgrounds. No data are available on how they perceive the important role their parents play in this industry. A program titled "Semillas" (Spanish for seeds; SEED) was created by South Dakota State University (SDSU) Dairy Extension with support of 4-H and the South Dakota Department of Education. It reached Latino dairy workers' youth to help embrace their heritage, gain a sense of community, and appreciate their parents' jobs. SEED provided a hands-on learning experience in a culturally rich environment while covering the opportunities in higher education and potential occupations within the dairy industry. SEED consisted of 2 sessions within 6 mo held at SDSU. Youth (n = 50; session 1, 23; session 2, 27) ranged from 6 to 16 year olds in both sessions. Session 1 included making piñata, ice cream, chocolate truffles; touring the SDSU dairy manufacturing plant and campus; and learning the importance of the dairy industry. Guest speakers talked about citizenship, cultural differences, and about sense of community and belonging. During the sessions, questions about their knowledge of ethnicities and stereotypes were discussed and uncovered an alarmingly negative preconception of their own Latino heritage and US nationality. Session 2 continued to focus on embracing culture differences, and although based on dairy, it concentrated on nutritional choices and a cheese-topped pizza cooking class. Pizza is a popular food item across ethnicities and is readily adopted by diverse groups. SEED welcomed the new generation of youth, developed their cultural identity, and helped them learn the benefits of diversity. Goals included changes in cultural misconceptions through interaction, increased participation of local community activities, and gain respect for their parents' role in the dairy industry. Feedback data showed that when youth develop respect for dairy workers they become increasingly visible and active in their communities, while increasing the job satisfaction of their parents.

Key Words: dairy worker, youth, Latino employees

M154 Needs assessment for cooperative extension dairy programs in California. J. P. N. Martins*[1], B. Karle[2], and J. Heguy[3], [1]*University of California Agriculture & Natural Resources, Tulare, CA,* [2]*University of California Agriculture & Natural Resources, Glenn, CA,* [3]*University of California Agriculture & Natural Resources, Modesto, CA.*

The aim of the present study was to better understand dairy producer needs and how to best direct and deliver cooperative extension (CE) programming. In March 2017, a needs assessment survey was mailed to grade A dairy producers in California (n = 1,080). Response rate was 15.4% (n = 166) and herd size averaged 1,405 milking cows (range 83–5,500). The geographic distribution of survey responses was representative of the distribution of dairies throughout the state. Producers were asked to indicate the level of concern for a predetermined list of issues. Rank of concern had 3 numeric levels: (1) very concerned, (2) somewhat concerned, or (3) not concerned. Mean and percentage of responses in each rank for each issue topic was calculated. The top 5 concerns/obstacles indicated were (1) milk price, (2) labor availability/quality, (3) environmental issues/regulations, (4) labor costs, and (5) water quality/availability. Surveyed respondents were also asked to determine the level of priority of a predetermined list of CE research and educational opportunities. Producers ranked topics as (1) high priority, (2) medium priority, or (3) low priority. The 5 highest priority research topics were (1) herd health, (2) environmental issues, (3) reproduction, (4) milk quality, and (5) water quality. The 5 highest priority educational topics were (1) herd health, (2) milk quality, (3) reproduction, (4) environmental issues, and (5) calf and heifer management. Producers were then asked to identify the target audience for CE information delivery and preferred information delivery method. Most respondents indicated that the target audience should be dairy owners (93%) or managers (66%). Fewer producers indicated a target audience of dairy employees (27%) or allied industry (23%). Preferable information delivery methods were newsletter/magazine articles (81%), half-day/short meetings (47%), on-farm training/meetings (39%). Webinars and 2- or 3-d destination meetings were the least preferable methods (27 and 9%, respectively). Survey results will serve to develop future dairy cooperative extension programs in California.

Key Words: dairy, needs assessment, cooperative extension

M155 Silage safety: Preventing serious injury and fatality accidents involving machinery. K. K. Bolsen*[1] and R. R. Bolsen[2], [1]*Kansas State University, Manhattan, KS,* [2]*Keith Bolsen Silage Safety Foundation, Austin, TX.*

Safety is the control of recognized hazards to reach an acceptable level of risk. Farm accidents are caused by unsafe behavior or conditions due to the actions of people. A split second of inattention can cause a fatal accident and nothing will ever be the same again. Few farming operations invite as many opportunities for a serious injury or fatality a silage program. Three hazards in managing bunker silos and silage piles are truck or tractor rollover, entanglement in machinery, and run-over by machinery. Detailed case studies of the hazards and ways to avoid each one are presented. Only the case studies are discussed here. In case study 1, a 21-year-old truck driver was killed when a dump-bed rolled over in a bunker silo at a cattle feedlot in Texas. According to the county sheriff's office, the circumstances of the accident are not uncommon, and silage trucks are known to rollover if not operated correctly. In case study 2, an 18-year-old employee on a dairy farm in New York was fatally injured when he became entangled in a silage defacer. The youth was idling the tractor to allow it to warm up on a cold morning. The defacer was attached on the front of the tractor and was 1 m above ground level. The energized defacer caught the sleeve of the victim's coat as he walked in front of the unit. The farm owner heard an unusual sound and went to investigate. He immediately shut off the tractor's engine and disentangled the victim from the defacer. The youth died at the scene. In case study 3, the adult son of a Nebraska silage contractor was talking with an employee who was in the cab of

a large forage harvester near the edge of a field. After the conversation, the employee started the machine and drove away in the harvester. A few minutes later, another employee found the contractor's son lying unconscious in the field, and he died 2 h later. Although details of the accident were unclear, the son was apparently run over by a rear tire of the forage harvester. Every beef and dairy cattle producer should have written safety guidelines for their silage program and schedule regular meetings with all employees to discuss safety.

Key Words: silage, accident, entanglement

M156 Employee training and updated chemical sanitation may be insufficient for resolution of post-pasteurization contamination in HTST fluid milk plants. S. J. Reichler*[1], S. I. Murphy[1], T. Erickson[2], N. H. Martin[1], K. J. Boor[1], and M. Wiedmann[1], [1]*Cornell University, Ithaca, NY,* [2]*Ecolab Inc., Eagan, MN.*

Among 280 samples of HTST pasteurized fluid milk collected from 10 dairy plants in the northeastern United States from 2015 to 2016, 132 (47%) showed evidence for spoilage due to post-pasteurization contamination (PPC) with gram-negative bacteria, some of which is known to survive normal cleaning and sanitation due to the formation of tough biofilms. PPC negatively affects the quality and shelf life of milk through the development of flavor, odor, texture, and visual defects. To address this high level of PPC, we examined the efficacy of 2 strategies aimed toward reducing the rate of PPC in fluid milk: (1) employee training both alone and (2) with concurrent implementation of updated sanitation protocols intended to remove persistent biofilm within equipment. Two large New York State fluid milk plants received employee training only, and 2 received both employee training and concurrent sanitation changes provided courtesy of Ecolab. Efficacy of the training was evaluated using surveys administered immediately before and after the training exercise, as well as approximately 8 mo after the training. Training and sanitation efficacy were evaluated through collection of finished product and subsequent refrigerated shelf life testing. Isolates collected from the milk over shelf life believed to represent PPC were collected and sequenced to provide identification and subtyping for determination of recurrent isolation. Though plant employees generally indicated that the training altered their perceptions of the causes of PPC, no significant changes in the occurrence of PPC were observed associated with either the training alone or with the training combined with changes in sanitation. Furthermore, multilocus sequence typing revealed a general lack of persistence in bacterial strains responsible for PPC at a given plant over the course of weeks and months. These results suggest that a more holistic and intensive approach may be necessary to reduce the occurrence of PPC in dairy plants, such as redesign or replacement of equipment that cannot be effectively cleaned and sanitized, or workplace modifications to enforce desired employee behaviors.

Key Words: post-pasteurization contamination, sanitation, training

M157 Survey of top-producing Jersey herds in the United States. S. Y. Morrison*[1], K. M. Glosson[1], J. H. Baltz[1], M. F. Hutjens[1], and C. W. Wolfe[2], [1]*University of Illinois, Urbana, IL,* [2]*American Jersey Cattle Association, Reynoldsburg, OH.*

With the Jersey breed increasing in numbers, limited research data and practical information are available to feed high-producing Jersey cows. A survey of current practices of feeding and management on high-producing Jersey farms in the United States was conducted to gather Jersey-specific information that could be applied to future research and extension applications. The top producing herds were identified through the American Jersey Cattle Association. The survey was administered electronically and included herd parameters and management questions. Herd owners were also requested to provide access to their dairy records processing center, ration summaries for lactating and dry cow groups, and forage test results. A total of 38 herds completed the survey to be included in the final analysis. Several questions related to Jersey herd management were addressed, including effects of (1) level of production; (2) recombinant (r)bST use; (3) herd size; and (4) mixed herds. Data were analyzed in SAS. A tendency for the higher producing herds (RHA milk >8981 kg) to milk more than 2×/d ($P = 0.11$) and have lower incidence of metritis ($P = 0.10$) was observed. rbST use did not affect production measurements, but herds that used rbST (n = 25) were more likely to milk more than 2×/d ($P = 0.02$) and push up feed more times per day ($P = 0.03$). No differences in health were observed. Larger farms (>200 cows) had no difference in production parameters, but tended to milk more and push up feed more frequently. Herds that were mixed (% Jersey <100) tended to have lower SCC ($P = 0.08$), more frequent milkings per day ($P = 0.09$), higher percentage of weigh backs ($P = 0.06$), and significantly less ketosis ($P = 0.04$). These comparisons can assist Jersey producers in understanding management and production differences between farms while providing benchmarks and areas of opportunity for Jersey farms across the United States.

Key Words: Jersey, survey, management

M158 Effects of floury and brown midrib corn varieties treated with foliar fungicide on whole-plant corn silage quality fed to lactating Holstein cows. L. J. Wente*[1], M. E. Weatherly[1], R. T. Pate[1], M. S. Akins[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*University of Wisconsin-Madison, Marshfield, WI.*

Producers utilize foliar fungicide on whole-plant corn silage (WPCS) to minimize disease prevalence and improve forage quality. However, limited research is available on the performance effects in dairy cattle fed fungicide-treated WPCS. Therefore, the objective of this study was to determine the effects of foliar fungicide applied at vegetative tassel stage on 2 corn varieties (floury, FLY; brown midrib, BMR) on performance in primiparous and multiparous Holstein cows. Thirty-two lactating Holstein cows [BW = 649 ± 74 kg; DIM = 189 ± 49 d] were assigned to 1 of 4 treatments in a 4 × 4 Latin square design: FLY and fungicide (FF), FLY and no fungicide (control; FC), BMR and fungicide (BF), and BMR and no fungicide (BC). Silage was inoculated (Silo-King; Agri-King, Fulton, IL) and ensiled for 295 d in an Ag-Bag (Ag-Bag; St. Nazianz, WI). Statistical analysis was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). There were no differences in dry matter intake between the 4 treatments ($P > 0.10$). Milk yield was greater for cows in non-fungicide treatments (33.61 ± 0.63 kg/d) compared with cows in fungicide treatments (31.46 ± 0.63 kg/d; $P = 0.01$). Milk fat percentage was greater for cows in BF (3.85 ± 0.11%) compared with cows in BC (3.70 ± 0.11%; $P = 0.009$). Protein yield was greater for cows in FC (0.98 ± 0.02 kg/d) compared with cows in FF (0.92 ± 0.02 kg/d, $P = 0.001$). Protein percentage was greater for cows in non-fungicide treatments (2.97 ± 0.05%) compared with cows in fungicide treatments (2.92 ± 0.05%; $P < 0.001$). Milk urea nitrogen was greater for cows in non-fungicide treatments (14.45 ± 0.23 mg/dL) compared with cows in fungicide treatments (14.03 ± 0.23 mg/dL; $P < 0.001$). In conclusion, foliar fungicide application neither hindered nor improved DMI. However, fungicide application may lead to a decreased milk yield and milk components, as well as an alteration in nitrogen utilization.

Key Words: foliar fungicide, whole-plant corn silage, dairy cow

M159 Foliar fungicide application effects on fiber composition of whole plant BMR and floury corn varieties and corn silage ensiled for 0, 30, 90, or 150 days. M. E. Weatherly*[1], R. T. Pate[1], L. Hedges[2], S. Mideros[2], G. M. Fellows[3], M. Akins[4], M. R. Murphy[1], and F. C. Cardoso[1], [1]*University of Illinois, Department of Animal Sciences, Urbana,IL,* [2]*University of Illinois, Department of Crop Sciences, Urbana,IL,* [3]*B.A.S.F. Corp., Research Triangle Park, NC,* [4]*University of Wisconsin-Madison, Marshfield, WI.*

The objective of this study was to determine the effects of foliar fungicide (FUNG) and ensiling time (0, 30, 90, 150 d) on fiber composition of 2 corn silage (CS) varieties (brown midrib; BMR and floury; FLY). Treatments were assigned to 16 plots in a completely randomized block split-plot design. Treatments were BMR without FUNG, FLY without FUNG, BMR with FUNG, and FLY with FUNG (Headline AMP; BASF Corp., applied at vegetative tassel). Samples of whole plants were collected and separated into leaves, stalks, flag leaf (FL), and cobs. Fresh-cut silage samples were collected at harvest and sealed inside mini silos for the duration of their respective ensiling times. Statistical analysis was performed using the MIXED procedure in SAS (v9.4). No 2- (variety × day, treatment × day, variety × treatment) or 3-way interactions (variety × treatment × day) were observed for stalk, cob, or FL ADF or lignin, or CS NDF, ADF, or lignin ($P \geq 0.11$). No effect of treatment was observed for any fiber analyses ($P \geq 0.07$). A variety × treatment interaction occurred for leaf lignin content with 7.01, 8.69, 8.85, and 6.59 ± 1.17% DM for BMR/CON, BMR/FUN, FLY/CON, and FLY/FUN, respectively ($P = 0.04$). Stalks from BMR had lower ADF concentration than stalks from FLY with 43.04 and 47.66 ± 1.02% DM, respectively ($P = 0.04$). Corn silage NDF was lowest when ensiled for 90 or 150 d with 40.64 and 36.84 vs. 43.22 and 43.41 ± 0.99% DM at 0 and 30 d, respectively ($P < 0.0001$). BMR corn silage had lower NDF than FLY with 39.73 and 42.32 ± 0.83% DM, respectively ($P = 0.005$). Corn silage ADF was lowest when ensiled for 90 or 150 d with 20.92 and 19.05 vs. 23.20 and 22.18 ± 0.32% DM at 0 and 30 d, respectively ($P < 0.0001$). BMR CS had lower ADF than FLY with 20.00 and 22.68 ± 0.19% DM, respectively ($P < 0.0001$). Lignin was lowest when ensiled for 90 or 150 d with 2.55 and 2.24 vs. 3.16 and 2.85 ± 0.08% DM at 0 and 30 d, respectively ($P < 0.0001$). BMR corn had lower lignin than FLY with 2.27 and 3.13 ± 0.06% DM, respectively ($P < 0.0001$). In conclusion, BMR variety or longer ensiling times of CS may result in a more favorable fiber content for dairy cows.

Key Words: fungicide, fiber, corn silage

M160 Yield and quality of brown midrib and non-brown midrib corn silage hybrids grown in northern New York over a three-year period. M. D. Miller*, E. O. Young, K. W. Cotanch, C. S. Ballard, and R. J. Grant, *William H. Miner Agricultural Research Institute, Chazy, NY.*

The objective of this study was to evaluate the quality and yield of dry matter and fiber fractions of BMR and non-BMR corn silage hybrids grown in northern New York in 2015, 2016, and 2017. Hybrids [brown midrib-3 (BM3; hybrids 1 and 2), n = 2; brown midrib-1 (BM1; hybrid 3), n = 1; and non-BMR (hybrids 4 and 5), n = 2] were used in a randomized complete block design study and assigned randomly to plots within each block in a 5.7-ha tile-drained field. All hybrids were planted at 83,980 seeds/ha with 0.76-m row spacing with 4 replicates per block. Data were analyzed using MIXED procedure in SAS with fixed effects of treatment, block, year, and treatment by year interaction, and block within year was treated as a random effect. Brown midrib-3 (hybrids 1 and 2) hybrids had lower ($P < 0.05$) undigested neutral detergent fiber at 240-h (uNDF240) than the BM1 and non-BMR hybrids (hybrids 3, 4, and 5; 6.8 and 6.0 vs 9.2, 10.4, and 9.8% DM). Brown midrib-1 (hybrid 3) and non-BMR (hybrid 4) hybrids had higher ($P < 0.05$) starch content than BM3 (hybrid 2; 35.2 and 35.5 vs 32.3% DM). Brown midrib-1 and non-BMR (hybrid 3 and 4) hybrids had lower ($P < 0.05$) potentially digestible NDF (pdNDF) than non-BMR (hybrid 5; 11.4 and 12.1 vs 13.7 Mg/ha). The yield and pdNDF yield had significant ($P < 0.05$) year and treatment by year interactions (Table 1). Results reveal BM3 hybrids had better fiber digestibility than BM1 and non-BMR hybrids, and amylase modified neutral detergent fiber organic matter basis (aNDFom) and pdNDF yields may be a useful metric for corn silage hybrid evaluation.

Key Words: corn silage, brown midrib, potentially digestible neutral detergent fiber

Table 1 (Abstr. M160).

	Hybrid								
	1	2	3	4	5		*P*-value		
Item	BM3	BM3	BM1	Non-BMR	Non-BMR	SE	Trt	Year	Trt × Year
Yield,[1] Mg/ha	38.1[b]	38.8[ab]	39.2[ab]	40.8[ab]	43.0[a]	1.3	0.05	<0.05	0.03
aNDFom,[2] %	40.0[ab]	38.6[c]	38.6[d]	39.8[bcd]	41.3[a]	0.4	<0.05	0.06	<0.05
pdNDF, %	33.2[a]	32.5[ab]	29.4[d]	29.5[c]	31.5[b]	0.4	<0.05	0.56	<0.05
aNDFom yield,[1] Mg/ha	15.2[b]	14.8[b]	15.0[b]	16.1[b]	17.7[a]	0.4	<0.05	<0.05	0.06
pdNDF yield,[1] Mg/ha	12.8[ab]	12.6[ab]	11.4[b]	12.1[b]	13.7[a]	0.4	<0.05	<0.05	0.02

[a–d]Within a row, different superscripts differ at ($P \leq 0.05$).

[1]35% DM basis.

[2]Amylase-modified neutral detergent fiber organic matter basis.

M161 Dry matter yield, nutritive value, and ensilability of triticale harvested at 3 maturity stages and 2 cutting times in Los Altos de Jalisco, Mexico. C. H. Blanco Castellanos*[1], J. J. Olmos Colmenero[1], F. E. Contreras-Govea[2], and M. A. Wattiaux[2], [1]*Departamento de Ciencias Pecuarias y Agrícolas, Centro Universitario de Los Altos de la Universidad de Guadalajara, Tepatitlán, Jalisco, México,* [2]*Department of Dairy Science, University of Wisconsin-Madison, Madison WI.*

The aim of this study was to assess dry matter yield, nutritive value, and ensilability index (EI) of triticale forage when harvested at 3 maturity stages and 2 cutting times. A local variety of triticale was sown in 3 locations on November 25, 2013, and harvested at boot (March 7), early bloom (March 14), and dough stage (March 21) at 0800 (AM) and 1700 (PM) h. At each location, 4 randomly selected plots (2 × 3 m) from a 5.0 ha field, were harvested for dry matter yield determination and about 10.0 kg of fresh triticale was chopped to a TLC of 2.5 cm, wilted for a minimum of 2 h to reach 35% DM, and ensiled in quadruplicate PVC mini-silos targeting 800 kg m^{-3} of fresh material. Water-soluble carbohydrates (WSC) was measured on fresh early bloom and dough stage samples. The experimental design was a split plot with maturity stage as whole plot and harvest time as subplot. Dry matter yield increased from 7.24 to 9.45 t/ha ($P < 0.05$), as maturity increased from boot to dough stage. Surprisingly, WSC was similar for AM and PM (11.3 and 11.1% of DM, respectively). There was no maturity stage by harvest time interaction for CP, NDF, ADF, and ADL. Concentration of CP decreased from 16.1% to 13.4% ($P < 0.05$), but NDF (53.8 vs. 56.3%), ADF (32.5 vs. 34.2%), and ADL (4.34 vs. 5.12%) increased (all $P < 0.05$) as maturity changed from boot to dough stage. On the 5 categories of EI scale (ranging from ≤ −47 to >28), all maturity stages were above 28, indicating high ensilability. In conclusion, regardless of maturity stage at harvest, triticale has excellent characteristics for high quality silage fermentation. Data from this study do not support the common assumption of greater WSC in evening cutting time because of plant photosynthesis during daylight compared with morning cutting time.

Key Words: triticale, ensilability index, maturity

M162 Effects of growth stage on quality characteristics of triticale forages. W. K. Coblentz*[1], M. S. Akins[2], K. F. Kalscheur[3], G. E. Brink[3], and J. S. Cavadini[2], [1]*US Dairy Forage Research Center, Marshfield, WI,* [2]*University of Wisconsin, Madison, WI,* [3]*US Dairy Forage Research Center, Madison, WI.*

The use of triticale (X *Triticosecale* Wittmack) has expanded greatly in recent years, in part to improve land stewardship by providing winter ground cover. Our objectives were to relate the DM yield and nutritive value of triticale forages grown in central Wisconsin with plant growth stage. Replicated 3.7- × 9.1-m plots of triticale were harvested at growth stages ranging from vegetative to soft-dough during spring/early summer of 2016 and 2017. Growth stages on each harvest date were quantified using a linear staging system suitable for use as a continuous, independent regression variable, where assessments during the trial ranged from 30 (vegetative) to 84 d (soft-dough). In 2016, the mean DM yield at boot stage (3,804 kg DM/ha) was only 30% of soft-dough (12,642 kg DM/ha). Although DM yields were reduced in 2017 due to spring flooding, the percentage of forage DM harvested at boot stage compared with soft-dough was similar (26.9%; 1,453 vs. 5,399 kg DM/ha). Regressions of DM yield (kg/ha) on growth stage for 2016 were explained by a cubic model ($Y = 0.0663\ x^3 - 9.44\ x^2 + 595\ x - 9810$; $n = 30$; $R^2 = 0.985$) compared with a simple linear response for 2017 ($Y = 103\ x - 3024$; $n = 24$; $R^2 = 0.934$). Many nutritional characteristics, such as NDF, were affected by the juxtaposition and balance of 2 generally competing factors: (1) increased concentrations of structural plant fiber as plants matured; and (2) the accumulation of highly digestible carbohydrate during seed-head development. As such, the relationship between NDF and growth stage was best fitted to higher-ordered models for 2016 ($Y = 0.0000369\ x^4 - 0.00886\ x^3 + 0.745\ x^2 - 25.4\ x + 342.5$, $n = 30$; $R^2 = 0.975$) and 2017 ($Y = -0.000570\ x^3 + 0.0828\ x^2 - 3.14\ x + 74.9$, $n = 24$; $R^2 = 0.950$). Single-endpoint estimates of in vitro NDF digestibility after 24, 30, or 48-h incubations declined with plant maturity, and were closely related ($R^2 = 0.906$) to growth stage by linear or quadratic regression models that were similar across production years. Typical recommendations for harvesting triticale at boot stage to facilitate the planting of double-cropped corn are supported by the extensive 30-h in vitro disappearance of NDF at that growth stage, which comprised 63.1 ± 1.79 and 64.8 ± 7.39% of NDF for 2016 and 2017, respectively.

Key Words: growth stage, triticale

M163 Yield and nutritional value of forage sorghum varieties for dairy cattle. E. E. Corea Guillén*[1], J. M. Flores Tensos[1], F. M. López Hernández[1], E. A. Martínez Aguilar[1], M. Mendoza[1], J. A. Elizondo Salazar[2], and G. A. Broderick[3], [1]*Departamento de Zootecnia, Facultad de Ciencias Agronómicas, Universidad de El Salvador, San Salvador, El Salvador,* [2]*Estación Experimental Alfredo Volio Mata, Facultad de Ciencias Agroalimentarias, Universidad de Costa*

Rica, Cartago, Costa Rica, [3]*Broderick Nutrition and Research LLC, Madison, WI.*

Sorghum is an important forage source for dairy farms in warm and dry areas of the Pacific coast of Central American because of its tolerance to drought and pests. Its nutritional value is comparable to corn and management strategies including variety selection can affect its performance and feeding value. In this study 3 regional varieties of sorghum (*Sorghum bicolor*) from the Centro Nacional de Tecnología Agropecuaria of El Salvador (CENTA), RCV, BMR and S2, were compared. Sixteen replicates of each variety were planted in 10 × 6.4 m plots at a density of 125,000 plants/ha with 0.8 m between rows and 0.1 m between plants in 4 replicates during the rainy season. Yield of fresh matter was measured in 2.4 × 3 m area in each plot at 85 d after planting at the milky stage of the grain, and whole plant material was separated into leaves, stems, and panicles. Samples of fractions were dried, milled (1 mm screen) and analyzed for DM, CP, and NDF concentration; nutrient yield also was computed. Data were analyzed with Infostat using a completely random model. Greater DM and CP yield was found in RCV and S2 varieties compared with the BMR variety, while more CP in the leaves and less NDF in the panicle were found in the RCV variety (Table 1). Results of this study indicate that improved nutritional composition and greater yield make RCV a better forage sorghum variety for feeding dairy cattle.

Table 1 (Abstr. M163). Yield and nutritional composition of 3 varieties of forage sorghum (*Sorghum bicolor*)

	Variety				
Item	RCV	BMR	S2	SEM	*P* > *F*
% DM	19.0[b]	21.2[a]	20.7[a]	0.65	<0.001
Yield, ton/ha					
Green matter	54.3[a]	31.1[b]	51.6[a]	5.68	<0.001
DM	10.30[a]	6.57[b]	10.39[a]	1.05	<0.001
CP	0.88[a]	0.49[b]	0.77[a]	0.10	0.001
CP, % of DM					
Whole plant	8.53	7.39	7.44	0.37	0.076
Stem	4.91	4.29	4.49	0.18	0.450
Leaves	16.3[a]	13.8[b]	14.9[b]	0.73	< 0.001
Panicle	11.0	12.0	10.8	0.36	0.436
NDF, % of DM					
Whole plant	54.6	50.0	53.1	1.36	0.670
Stem	63.0	61.5	66.1	1.37	0.141
Leaves	52.5	50.8	50.7	0.57	0.645
Panicle	47.4[b]	65.8[a]	53.8[b]	5.39	0.003

[a,b]Means with different superscripts differ ($P < 0.05$).

Key Words: yield, sorghum variety, nutritional composition

M164 Nutrient composition and fermentation characteristics of sorghum preserved as silage in California. J. M. Heguy*[1], J. P. Martins[3], N. E. Clark[3], and D. Meyer[2], [1]*University of California Agriculture & Natural Resources, Modesto, CA,* [2]*University of California, Davis, Davis, CA,* [3]*University of California Agriculture & Natural Resources, Tulare, CA.*

The aim of this study was to determine sorghum silage quality from dairy farms (n = 16) located in California's San Joaquin Valley. Herd size of the participating dairies ranged from 320 to 5,500 lactating dairy cows (median = 2,013). Sorghum fields were characterized by variety as grain (n = 5) or forage (n = 11) type sorghum, and by presence (n = 10) or absence (n = 6) of sugarcane aphid (SCA) infestation during the growing season. Chopped sorghum was ensiled as wedge or pile structures, and in bags. Each silage structure was visited once and sampled from the exposed face for wet chemistry, nutrient and fermentation analyses. Descriptive statistics were calculated using PROC MEANS of SAS, and effect of sorghum type and SCA infestation on forage quality was analyzed by ANOVA using the GLM procedure of SAS. At the time of sample collection, average ensiled time was 238 d (range = 99–439 d). Nutrient composition of the 16 samples of fermented sorghum silage is presented in Table 1. No differences in key nutritional criteria were found between grain and forage type sorghum. In addition, there was no effect of SCA infestation on parameters of nutritional quality. Sorghum silage pH mean was 3.95 (range = 3.68–4.27). Lactic acid averaged 7.5% (range = 3.5–9.8%), acetic acid averaged 4.0% (range = 1.6–6.6%), and propionic acid averaged 0.03% (range = 0.0–0.3%); butyric acid (mean = 0.1%) was only detected in 3 samples and ranged from 0.0 to 1.3%. The current data set is small and highly variable. There is a need to improve sorghum silage quality and determine best management practices, as sorghum may replace corn as an agronomic crop in areas of California in years of water shortages.

Table 1 (Abstr. M164). Sorghum silage nutrient composition (n = 16) in California's San Joaquin Valley

		% of DM						NDFD30,
Item	DM	CP	ADF	NDF	Starch	NFC	Ash	% of NDF
Mean	27.9	9.7	34.0	48.7	8.0	26.3	13.2	30.1
Median	27.6	9.5	34.2	49.1	6.9	26.5	12.9	29.9
Min	22.4	5.9	29.7	44.7	0.3	19.1	10.3	26.9
Max	35.2	12.8	39.2	55.9	18.7	34.3	16.9	34.9
SD	3.5	1.7	2.2	3.2	5.4	4.8	2.1	2.4

Key Words: California, sorghum silage, sugar-cane aphid

M165 Effect of bacterial inoculants containing *Lactobacillus buchneri* and/or *Lactobacillus hilgardii* on the fermentation and quality of sorghum silage. K. G. Arriola*, D. Vyas, D. Kim, M. C. Agarussi, V. P. Silva, J. M. Flores, Y. Jiang, A. A. Pech-Cervantes, and A. T. Adesogan, *University of Florida. Department of Animal Sciences, Gainesville, FL.*

The objective of this study was to determine the effect of inoculation with *Lactobacillus buchneri* and/or *Lactobacillus hilgardii* on the fermentation and chemical composition of sorghum silage. Sorghum plants were harvested at 27% DM from 4 different plots, chopped and ensiled separately in vacuum-sealed nylon-polyethylene bags (40 × 61 cm) for 30 and 90 d. Treatments included application of (1) deionized water (CON) or inoculants containing (2) *Lactobacillus buchneri* (1.5×10^5 cfu/g, LB); (3) *Lactobacillus hilgardii* (1.5×10^5 cfu/g; LH); and (4) *Lactobacillus buchneri* and *L. hilgardii* (1.5×10^5 cfu/g of each inoculant, LB+LH). Samples were collected from each replicate on d 0, 30, and 90 and analyzed for chemical composition, microbial counts, and aerobic stability. Data from each ensiling period were separately analyzed with the GLIMMIX procedure of SAS for a completely randomized design. Fresh forage samples had 6.6 log cfu/g of LAB and a pH of 5.74. Inoculation did not affect ($P > 0.05$) DM loss, pH, VFA concentration or aerobic stability (>500 h) of d 30 samples. However, inoculation increased LAB counts compared with CON after 30 (9.02 vs. 6.88 log cfu/g) or 90 d (8.49 vs. 7.08 log cfu/g) and greater counts were for LB and LB+LH. Concentrations of NDF and ADF were increased

by LB+LH after 30 or 90 d and by LH after 30 d. Inoculation had no effect ($P > 0.05$) on DM loss, pH, or lactic acid concentration after 90 d but it increased ($P = 0.004$) acetic acid concentration, decreased ($P = 0.0014$) lactic to acetic acid ratio (1.9 vs. 3.7) and increased aerobic stability relative to CON (>576 vs. 346 h). Inoculating sorghum forage with LB, LH, and LB+LH improved LAB counts after 30 or 90d of ensiling and improved aerobic stability after 90 d of ensiling; however, fermentation profile was not improved.

Key Words: *Lactobacillus buchneri*, *Lactobacillus hilgardii*, sorghum silage

M166 Comparing leaf:stem ratio and stem characteristics between reduced lignin and conventional alfalfas over a growth cycle. D. M. Donnelly*[1], J. R. R. Dórea[1], C. W. Karls[2], D. M. Schaefer[2], D. J. Undersander[3], and D. K. Combs[1], [1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI,* [2]*Department of Animal Science, University of Wisconsin-Madison, Madison, WI,* [3]*Department of Agronomy, University of Wisconsin-Madison, Madison, WI.*

The objective of this study was to compare how maturity affects leaf:stem ratio (L:S), fiber composition [NDF, indigestible NDF (iNDF), acid detergent lignin (ADL) and in situ total-tract NDF digestibility (ISTTNDFD)] of 4 alfalfa varieties. Two conventional alfalfa varieties developed for high digestibility (C1, C2), a transgenic reduced lignin (RL) variety (GMO) and a RL alfalfa developed by conventional breeding (CB) were compared. First cutting alfalfa at the University of Wisconsin Arlington research station (43°18′9.47″ N, 89°20′43.32″ W) were sampled twice weekly from early vegetative to full flower from May 4 to June 19, 2017. Samples were harvested 7 cm above soil level by hand. Each of the 4 fields (each approximately 8ha) were split into 3 units for replication. Samples were immediately dried at 60°C for 48 h. Leaves were manually stripped from dried samples, and L:S determined. Stems were ground to 1 mm by Wiley mill and weighed (0.5 g) into Ankom F-57 bags. Duplicates were placed into rumens of 2 lactating dairy cows for 24, 30, 48, and 240 h (iNDF). Calculation of ISTTNDFD was according to Lopes et al. (2015). Stem ADL analysis were in triplicate with Ankom bags that contained ADF residue soaked in 72% H_2SO_4 for 3 h. Data were analyzed by split plot design in PROC MIXED in SAS, with variety, day (D), and their interaction as fixed effects. Field section was a random effect and D a repeated measure. Both RL varieties had higher L:S than conventional. The GMO stems had a lower ADL than the other stems. The RL stems had higher ISTTNDFD compared with C2 stems.

Key Words: alfalfa, forage

M167 Evaluation of a microbial inoculant isolated from ruminal contents on corn silage fermentation parameters. E. Castillo-Lopez*[1], S. Rohde[2], S. C. Fernando[2], and P. J. Kononoff[2], [1]*Universidad Nacional Autonoma de Mexico, Cuautitlan, Mexico, Mexico,* [2]*University of Nebraska-Lincoln, Lincoln, NE.*

The objective of this experiment was to test the effects of a microbial inoculant with or without glucose addition on corn silage fermentation parameters. The microbial inoculant was isolated from ruminal contents after animal donors were induced to ruminal acidosis, then the inoculant was grown in vitro. Treatments evaluated were (1) CONT, corn silage mixed with deionized water with no inoculant, (2) BC, corn silage mixed with $1,600 \times 10^5$ cfu of *Bacillus coagulans*; (3) BC+GLU; corn silage mixed with $1,600 \times 10^5$ cfu of *Bacillus coagulans*, and with 0.25% glucose. The microbial inoculant was diluted in deionized water before combination with the corn silage samples. Air was removed from bags by automatic suction; ensiling was conducted during 60 d in plastic bags and using 400 g of forage for each silo (as-is basis). Data were analyzed with the MIXED procedure of SAS as a completely random design considering treatment as a fixed effect and replication load as a random effect. Statistical replications were achieved by replicating the procedures independently 2 times using corn silage samples from different plots, with 3 experimental silos per treatment within each run. Silage pH ($P < 0.05$) was affected by treatment with means of 3.90, 3.86, and 3.84 ± 0.009 for CONT, BC, and BC+GLU, respectively. Ammonia concentration ($P = 0.43$) was not affected by treatment and averaged 2.96 ± 0.338 mg/dL. Total volatile fatty acid concentration was not affected ($P = 0.42$) and averaged 11.5 ± 1.00 mM. Averages for acetate ($P = 0.18$) and butyrate ($P = 0.22$) concentrations were 96.2 ± 3.27% and 0.7 ± 0.97%, respectively. There was a tendency for a treatment effect on propionate concentration ($P = 0.09$) with means of 0.5, 8.9, and 1.2 ± 2.75% for CONT, BC and BC+GLU, respectively. Overall, although total volatile fatty acid concentration was not affected, inoculation of corn silage with *Bacillus coagulans* with or without glucose decreased substrate pH, indicating its potential as a microbial inoculant of corn silage.

Key Words: corn silage, fermentation, microbial inoculant

M168 Nutritional value of the tropical legumes cowpea, lablab, and canavalia. E. E. Corea Guillén*[1], J. M. Flores Tensos[1], E. A. Martinez Aguilar[1], E. A. Perez Medina[1], G. S. Acevedo Cuellar[1], E. A. Alas García[1], D. Vyas[2], K. G. Arriola[2], and G. A. Broderick[3], [1]*Departamento de Zootecnia, Facultad de Ciencias Agronómicas, Universidad de El Salvador, San Salvador, El Salvador,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*Broderick Nutrition and Research LLC, Madison, WI.*

Table 1 (Abstr. M166). Leaf:stem ratio and fiber analysis of stems of 2 conventional alfalfa varieties (C1, C2) and 2 reduced lignin alfalfas (GMO and CB)

	Alfalfa variety					*P*-value		
Variable	C1	C2	GMO	CB	SEM	Variety	D	Variety×D
L:S ratio, DM	0.57[b]	0.59[b]	0.64[a]	0.65[a]	0.01	<0.01	<0.01	<0.01
Stem composition								
ADL, % of DM	7.61[b]	7.95[a]	6.74[c]	7.42[b]	0.08	<0.01	<0.01	<0.01
NDF, % of DM	46.0	48.4	45.7	45.8	0.8	0.06	<0.01	0.24
iNDF, % of NDF	45.6[ab]	48.8[a]	41.3[b]	45.2[ab]	1.6	<0.04	<0.01	0.12
ISTTNDFD, % of NDF	39.5[ab]	37.3[b]	41.1[a]	41.5[a]	1.0	<0.01	<0.01	0.04

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

Tropical forages often have poor nutritional value for dairy cattle, necessitating concentrate feeding to meet animal needs. Feeding tropical legume forages is a strategy for improving nutritional value of diets at reduced cost and external dependence. A study evaluated relative nutritional value of 3 tropical legumes: cowpea (*Vigna sinensis* L.), lablab (*Dolichos lablab* L.), and canavalia (*Canavalia ensiformis* L.) as potential forages. Crops were grown in 4 replicated plots during the dry season under irrigation and harvested 70 d after planting; yield was estimated in a 9-m^2 area in each plot. Whole plant was separated into leaves and stems; fractions were dried, ground (1 mm screen) and analyzed for CP and NDF. Ruminal DM digestibility (DMD) was estimated after 48 h by incubating samples in vitro in Ankom bags in triplicate in gas-tight culture bottles at 39°C and by incubating samples in situ in nylon bags in triplicate in a cannulated dairy cow. Data were analyzed in a random model using Infostat; results are in Table 1. No difference was detected in DM from whole plant, whereas leaves from canavalia and lablab had greater CP content than cowpea. Content of NDF was lower, and ruminal DMD greater, in all cowpea fractions. Leaves from lablab tended to have greater DM digestibility. Composition and digestibility differences among these species reflect differences in nutritional value. Cowpea was the most promising species because of higher DMD, probably due to lower NDF content.

Table 1 (Abstr. 168). Forage composition and digestibility

Item	Cowpea	Lablab	Canavalia	SEM	*P*-value
DM, %					
Whole plant	16.2	16.9	16.4	0.20	0.743
Stem	14.9^{b}	15.9^{b}	34.0^{a}	6.19	<0.001
Leaves	19.2^{b}	19.7^{b}	28.2^{a}	2.9	<0.001
CP, % DM					
Whole plant	17.9	16.4	18.6	0.64	0.429
Stem	9.9	9.2	9.6	0.21	0.579
Leaves	23.7^{b}	29.6^{a}	27.6^{a}	1.74	<0.001
NDF, % DM					
Whole plant	50.0^{b}	54.2^{a}	51.8^{ab}	1.23	0.020
Stem	52.7^{c}	62.2^{b}	66.6^{a}	4.10	<0.001
Leaves	33.7^{b}	38.6^{a}	35.4^{b}	1.45	0.090
Ruminal in vitro DMD, %					
Whole plant	70.4^{a}	61.4^{b}	59.1^{b}	3.44	<0.001
Stem	68.1^{a}	49.5^{b}	41.3^{b}	7.93	<0.001
Leaves	68.2	77.4	63.1	4.2	0.065
Ruminal in situ DMD, %					
Whole plant	76.2^{a}	62.0^{b}	67.7^{b}	4.13	<0.001
Stem	68.1^{a}	58.3^{b}	48.1^{c}	5.75	<0.001
Leaves	84.4^{a}	83.6^{a}	80.3^{b}	1.26	0.003

$^{a–c}$Means with different superscripts differ ($P < 0.05$).

Key Words: dry matter digestibility, tropical legume

M169 Growth rate and biomass accumulation of mucuna (*Mucuna pruriens*), centrosema (*Centrosema pubescens*), gliricidia (*Gliricidia sepium*), and leucaena (*Leucaena leucocephala*). A. B. Afe, A. H. Ekeocha*, and A. A. Aganga, *Federal University Oye-Ekiti, Oye-Ekiti, Ekiti State, Nigeria.*

Forage legumes have played important roles in crop-livestock systems and in forage research. Research conducted to test the effect of forage legumes on livestock productivity in the sub humid zone of Nigeria is scanty. This research aimed at developing a model that can correctly predict the growth rate and biomass accumulation of *Mucuna pruriens*, *Centrosema pubescens*, *Gliricidia sepium*, and *Leucaena leucocephala.* The experiment was conducted at the screen-house of the Faculty of Agriculture, Federal University, Oye-Ekiti, Ikole campus (07°48.308 N, 005°29.573 E; 548.4m above ground level). Planting was done using a completely randomized design (CRD) in 4 rows with 4 replicates of 8 pots of each legume species and a spacing of 1 m apart was applied between each bed. The soil used for this study contained 39.83% organic matter before planting and 57.72% after harvesting. The highest growing legume was *Mucuna pruriens* during the experiment. Plant heights of the 4 legume species were different from each other. *Leucaena leucocephala* had the highest N and CP contents and this was observed in all the cuttings, at wk 2 (2.83, 2.28, 1.95, 1.77), 4 (3.05, 2.30, 1.69, 1.46), 6 (3.04, 2.70, 2.22, 1.77), and 8 (2.51, 2.21, 1.89, 1.53). Moisture content (MC) varied between the legume species and the times of cuttings; *Leucaena leucocephala* had the highest MC throughout the 8 wk of study. Crude protein, crude ash (CA), and crude fat (CF) varied among the 4 species with values ranging from 15.71 to 9.59% CP, 16.16 to 9.86% CA, and 2.41 to 1.47% CF at wk 8 of the experiment with *Leucaena leucocephala* having the highest values and *Mucuna pruriens* the least. There were fluctuations in the nutrient contents of the legume species due to cutting times. Growth rate of legumes was observed throughout the course of undertaking this study and varietal differences were observed with *Mucuna pruriens* the fastest. The observed biomass accumulation was 39.26 to 80.51% whereas the growth rate of the legume was 87.39 to 88.98%.

Key Words: *Mucuna pruriens*, *Centrosema pubescens*, *Gliricidia sepium*

M170 Chemical composition and kinetics of in vitro ruminal degradation of savoy grass (*Panicum maximum* Jacq.) silage with the tropical fruit residues. M. L. Medina Villacis*, I. F. Espinoza Guerra, L. F. Espinosa Delgado, A. R. Sánchez Laiño, M. J. Romero Romero, C. A. Vallejo Torres, and L. B. Montenegro Vivas, *Universidad Tecnica Estatal de Quevedo, Quevedo, Los Rios, Ecuador.*

The objective of this research was to study the effect of combining 3 residues of tropical fruits (passion fruit, pineapple, and banana) with savoy grass, before silage, into the chemical composition and in vitro ruminal degradation of the silage material obtained. The forage and residues were chopped and homogenized. After that, 4 combinations were prepared: T1 = savoy grass as the only material to be ensilaged, T2 = savoy grass mixed with 15% (fresh basis) passion fruit, T3 = savoy grass mixed with 15% (fresh basis) pineapple, and T4 = savoy grass mixed with 15% (fresh basis) banana residue. The silage was ensiled in experimental mini-silos, opened after 60 d of storage and representative samples were taken. The chemical composition for DM, ash, CP, NDF, ADF, and in vitro ruminal degradation of the DM was analyzed; and the ADF of the samples were determined at 0, 3, 6, 12, 24, 48, and 72 h. The experimental design was completely randomized with 6 replicates. The DM results did not show significant differences between treatments ($P > 0.05$) T1, T2, T3, and T4 (17.95, 17.04, 17.34, 16.73%); The content of ash differ ($P < 0.05$) in T1 (16.29%) compared with treatments T2, T3 and T4 (13.85, 14.60, and 12.96). However, the NDF and ADF contents were higher ($P < 0.05$) in T3, T2, and T1 (72.06 and 39.59, 70.78 and 35.63, 70.35 and 35.18%) in relation to T4 (68.3 and 32.49%). The deg-

radation potential of the DM and NDF did not show differences between treatments ($P > 0.05$) T1 (59.95 and 52.61%), T2 (64.98 and 51.82%), T3 (59.99 and 52.05%) and T4 (61.41 and 61.14%), The degradation potential of the ADF present differences ($P < 0.05$) with T1 (43.69%) while T2, T3 and T4 (49.04, 47.84 y 46.14%. These results suggested that the combination of savoy grass with passion fruit, pineapple, and banana residues for production of the silage could be an efficient way to properly dispose of these tropical fruit residues, reducing environmental risk and contributing to the improvement of the cattle feeding.

Key Words: mini-silo, silage, degradability in vitro.

M171 Effect of treating alfalfa fibrous residue silages with corn flour or apple pomace on fermentation quality, nutritive value, and proteolysis. Y. L. Xue*[1], Y. Jiang[2], D. Vyas[2], L. Sun[1], G. M. Yin[1], Y. Y. Zhang[1], S. B. Liu[1], Z. Yu[3], Q. Z. Sun[4], and A. T. Adesogan[2], [1]*Inner Mongolia Academy of Agriculture and Animal Husbandry Science, Hohhot, Inner Mongolia, China,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*Department of Animal Sciences, China Agricultural University, Beijing, China,* [4]*Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Hohhot, Inner Mongolia, China.*

Alfalfa fibrous residues (AFR) are the primary by-product of alfalfa leaf protein (LP) extraction after blending chopped whole plant alfalfa. The AFR is typically difficult to ensile due to high moisture and low water-soluble carbohydrate (WSC) concentrations. The objective of this study was to determine the effect of adding corn flour (CF) or apple pomace (AP) to AFR on the ensiling characteristics, fermentation quality, microbial population, nutritive value, and proteolysis. The AFR was made from second cutting alfalfa harvested at a vegetative stage. After extracting LP, AFR was mixed without additives (Control) or with corn flour (CF) or apple pomace (AP) at 5%, 10%, and 15%, on a fresh weight basis in triplicate and packed in laboratory silos (40 cm high × 30 cm) at a density of 750 g L^{-1} and was stored in the dark for 60 d. Data were analyzed using the ANOVA procedure of SAS. The DM, lactic acid concentration, and in vitro DM digestibility (IVDMD) of ensiled AFR linearly increased whereas pH, buffering capacity, and acetate concentrations linearly decreased with incremental inclusion of CF or AP ($P < 0.01$). The counts of lactic acid bacteria and fungi linearly decreased with inclusion of CF or AP ($P < 0.01$). The WSC concentration and total bacteria counts linearly reduced ($P < 0.01$) with incremental inclusion of CF but linearly increased ($P < 0.01$) with inclusion of AP. Crude protein, ammonia-N/total-N, and non-protein N/total N (NPN/TN) decreased with incremental inclusion of CF or AP ($P < 0.01$). Concentrations of NPN/TN were lower when AP was applied instead of CF ($P < 0.05$). Ensiling AFR with CF or AP improved fermentation quality and IVDMD of silages, and reduced proteolysis. Adding AP was more effective than CF at inhibiting proteolysis in AFR.

Key Words: alfalfa fibrous residues silage, fermentation quality, proteolysis

M172 SESN2 negatively regulates cell proliferation and casein synthesis by inhibition the amino acid-mediated mTORC1 pathway in cow mammary epithelial cells. C. Luo[1,2], S. Zhao[1,2], M. Zhang[1,2], Y. Gao[1,2], J. Wang*[1,2], M. D. Hanigan[3], and N. Zheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*Department of Dairy Science,Virginia Tech, Blacksburg, VA.*

Amino acids (AA) are one of the key nutrients that regulate cell proliferation and casein synthesis in cow mammary epithelial cells (CMEC), but the mechanism of this regulation is not yet clear. In previous reports, sestrin2 (SESN2) protein was found to respond to AA depletion resulting in negative effects on the mTORC1 pathway in human cells. In this study, the effect of SESN2 on AA-mediated cell proliferation and casein synthesis in CMEC was assessed. After 12 h of AA starvation, CMECs were cultured in the absence of all AA (AA-), in the presences of only essential AA (EAA+), or of all AA (AA+). Cell proliferation was texted by MTT method, expression of 4 kinds of caseins, SESN2, and the mammalian target of rapamycin complex 1 (mTORC1) pathway associated proteins in protein level was tested by Western blotting, and the lysosomal localization of mTOR was tested by immunofluorescence. The results showed that cell proliferation, casein expression, and activation of mTORC1 pathway were increased; but SESN2 expression was decreased in response to increased EAA or AA supply. Cells were transfected with SESN2 overexpressed or inhibited plasmid. Cell proliferation, expression of 4 kinds of caseins, SESN2 and mTORC1 pathway associated proteins in protein level, and the lysosomal localization of mTOR was tested. The results showed that cell proliferation, casein expression, and activation of the mTORC1 pathway were all controlled by SESN2 expression. Furthermore, the increase in cell proliferation, casein expression, and activation of the mTORC1 pathway in response to AA supply was inhibited by overexpressing SESN2, and those effects were reversed by inhibiting SESN2. These results indicate that SESN2 is an important inhibitor of mTORC1 in CMEC blocking AA-mediated cell proliferation and casein synthesis.

Key Words: sestrin2, casein synthesis, amino acid

M173 CRISPR/Cas9-mediated stearoyl-CoA desaturase 1 (SCD1) deficiency by NHEJ pathway affects fatty acid metabolism in goat mammary epithelial cells. H. Tian* and J. Luo, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

Stearoyl-CoA desaturase 1 (SCD1) is a kind of fatty acid desaturase which could catalyze the cis double bond in Δ9 position to produce monounsaturated fatty acid (MUFA). MUFA, which has a high nutritive value, is one of the most important content in goat milk. Recently, CRISPR/Cas9 technology has been developed as a useful tool for genome editing in many types of cells, but there is no report about applying it in goat mammary gland epithelial cells (GMEC). To explore the effect of SCD1 on fatty acid synthesis, we used CRISPR/Cas9 technology through NHEJ pathway to induce knockout of *SCD1* gene in GMEC. Three sgRNAs targeted to *SCD1* exon 2 were designed by online website (CRISPR design: http://crispr.mit.edu/). sgRNA oligonucleotides were inserted into pSpCas9 (BB)-2A-Puro vector. The control group was the cells transfected with empty vector, which did not contain any sgRNA sequence. After extraction of genome DNA and detection of T7EN1 assay, sgRNA1 and sgRNA3 showed 8.3 and 19.4% cleavage efficiency, respectively. sgRNA2 showed no significant cleavage efficiency. Therefore, the Cas9/sgRNA3 expression vector was used for cell transfection and single clone selection. One single clone with 24 nucleotides deletion and one nucleotides mutation in *SCD1* exon 2 in one allele was selected for the following experiments. The mRNA level of *SCD1* decreased by 80% and the protein level decreased by 50%. Monoallelic knockout of *SCD1* significantly decreased ($P < 0.01$) the mRNA level of genes related to de novo fatty acid synthesis, fatty acid elongation, transportation, desaturation, and TAG synthesis, including *FASN, ACACA, ELOVL6, FABP3, FABP4, FADS1, DGAT1*, and *AGPAT6*. However, *DGAT2* showed significant increase ($P < 0.01$) after *SCD1* monoallelic knockout compared with control group. Moreover, the fatty acid desaturation index of C16:1 and C18:1 decreased dramatically ($P < 0.05$). In conclusion, CRISPR/Cas9-mediated monoallelic knockout of SCD1 through NHEJ pathway affected the expression of genes related to fatty acid metabolism, and decreased the triacylglycerol synthesis and MUFA desaturation in goat mammary gland.

Key Words: CRISPR/Cas9, stearoyl-CoA desaturase 1 (SCD1), goat mammary epithelial cells (GMEC)

M174 Tea polyphenols protect bovine mammary epithelial cells from hydrogen peroxide-induced oxidative damage by activating the NFE2L2/HMOX-1 pathway. Y. Ma[1], L. Zhao[1], M. Gao[1], and J. J. Loor*[2], [1]*Institute of Animal Nutrition and Feed, Inner Mongolia Academy of Agriculture and Animal Husbandry Sciences, Hohhot, Inner Mongolia, China,* [2]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL.*

Nuclear factor erythroid 2-related factor 2 (NFE2L2) is a critical transcription factor in maintaining cellular redox balance and metabolism, which can induce an adaptive response against oxidative stress (OS). Tea polyphenols (TP) is a potent antioxidant that exerts protective effects on cells by scavenging free radicals and increasing the levels of NFE2L2 and phase II detoxifying enzymes. Thus, we used NFE2L2 short interfering siRNA (siRNA) to downregulate NFE2L2 expression in cultured bovine mammary epithelial cells (BMEC) and to investigate the cytoprotective effects of TP in BMEC exposed to hydrogen peroxide- (H_2O_2-) induced OS. The content of reactive oxygen species (ROS) and malondialdehyde (MDA), activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione-S-transferase (GST) were determined to identify the oxidative damage of BMEC. All statistical tests were carried out using SAS 9.0. To establish an OS, isolated BMEC were exposed to increasing concentrations of H_2O_2 (0, 100, 200, 400, 600, 800, 1,000 μ*M*) for 0, 2, 4, 6, 8, 12, and 24 h. Optimal doses of TP (0, 60, 80, 100 μg/mL) were evaluated by pretreatment of BMEC for 0, 2, 4, 6, 8, 12, and 24 h, followed by a H_2O_2 (600 μ*M*) challenge for 6 h. BMEC were transfected with NFE2L2-siRNA, pretreated with 100 μg/mL TP for 12 h, followed by 600 μ*M* H_2O_2 challenge for 6 h. The results revealed that treatment with 600 μ*M* H_2O_2 for 6 h induced oxidative damage of BMEC, indicating this model could be used to establish an OS injury model. A concentration of TP of 100 μg/mL during 12 h incubation increased cell viability, protein and mRNA abundance of NFE2L2, and decreased intracellular ROS accumulation after H_2O_2 exposure. In addition, transfection with NFE2L2-siRNA confirmed that mRNA abundance of NFE2L2 and

heme oxygenase-1 (HMOX-1) in response to TP was decreased, while ROS production was increased with H_2O_2 (600 μ*M*). Overall, TP had beneficial effects on BMEC redox balance, TP can slow down cellular OS-related injury, decrease ROS production, and increase the mRNA abundance of NFE2L2 and HMOX-1 by activating the NFE2L2/HMOX1 pathway. TP may potentially serve as an antioxidant against OS in dairy cows.

Key Words: tea polyphenols, bovine mammary epithelial cells, oxidative stress

M175 Effect of temporary cessation of milking for 3 days on innate immune components in goat milk. N. Isobe*, J. Ueda, and Y. Yoshimura, *Hiroshima University, Hiroshima, Japan.*

Temporary cessation of milking for 3 d following intramammary infusion of antibiotics was established as a new treatment method for mastitis in Japan. Innate immunity shows quick response after pathogen invasion and has broad spectrum of antimicrobial activity. Innate immune components (antimicrobial peptides) are synthesized in the alveolar epithelial cells and leukocytes and are secreted into milk. We hypothesized that innate immunity has an important role on the mammary gland during treatment with cessation of milking. Therefore, we investigated the concentration of innate immune components during and around the use of this treatment in goat milk. Ten Tokara goats in the mid lactating stage were used. Milking of both udders was stopped temporarily for 3 d followed by the resumption of milking once a day. Milk was collected from 3 d before milking cessation until 7 d after resumption of milking. After milk yield was measured, somatic cells were counted. Concentration of lactoferrin and cathelicidin-7 (innate immune components) and lactoperoxidase activity in defatted milk were measured. The Kolmogorov-Smirnov test was used to check the normal distribution of milk yield, lactoferrin, cathelicidin-7 and LPO data. A non-parametric Kruskal-Wallis test followed by Steel-Dwass test was used to compare the data among different days. One-way ANOVA followed by Tukey multiplex analysis was used to compare SCC among different days. Probability at <0.05 was considered significant. Milk yield was decreased significantly on d 2 and 3 after the end of milking cessation compared with that before milking cessation ($P < 0.05$). Conversely, there was significantly higher SCC on d 2 after the end of milk cessation than that before milk cessation ($P < 0.05$). Lactoferrin and cathelicidin-7 concentrations were increased temporarily at d 2 and 3 after the end of milking cessation compared with that before cessation ($P < 0.05$). These results suggested that 3-d cessation of milking induces temporal augmentation of lactoferrin and cathelicidin-7 that is contribute in the treatment of mastitis.

Key Words: mastitis, innate immune component

M176 Heat stress and OmniGen-AF alter mammary gland gene expression and endocrine responses in the dry period. T. F. Fabris*[1], A. Skibiel[1], J. Laporta[1], D. J. McLean[2], D. J. Kirk[2], J. D. Chapman[2], and G. E. Dahl[1], [1]*University of Florida, Gainesville, FL,* [2]*Phibro Animal Health Corp., Teaneck, NJ.*

Dry period (DP) heat stress (HT) exposure impairs subsequent milk yield in cows. Our objective was to evaluate the effect of HT and diet (OmniGen-AF; OG) on mammary gene expression and cortisol responses in the DP. Sixty days before dry-off, cows were cooled (i.e., shade, fans and soakers) and split in 2 groups: control (56 g/d AB20; CON) and OG (56 g/d). Cows were dried-off 45 d before expected parturition and split into 2 more groups: cool (shade, fans and soakers; CL) or HT (only shade) resulting in 4 groups: HT (n = 17), CL (n = 16), HTOG (n = 19) and CLOG (n = 14). Blood samples were taken at 0 to 7 d, and 20 ± 2 d relative to dry-off and at 0 to −6 d relative to calving to evaluate cortisol. Mammary gland biopsies (n = 6–7/treatment) were collected in late lactation (−3 d relative to dry-off) and the DP (3, 7, 14, and 25 d relative to dry-off) to evaluate gene expression of apoptotic markers (Caspase 3 and 8), heat shock proteins (HSP70 and 90), heat shock factor 1 (HSF1) and prolactin receptor, short and long form (PRLR-S, and PRLR-L) by RT-PCR. CL cows tended to have higher cortisol concentrations versus HT and OG tended to have higher cortisol versus CON (0.39 ± 0.04 μg/dL vs. 0.31 ± 0.03 μg/dL, $P = 0.10$; 0.39 ± 0.04 μg/dL vs. 0.31 ± 0.03 μg/dL, $P = 0.07$; respectively) before calving. Cortisol was significantly increased the day before calving ($P < 0.01$). HSP70 was lower in OG cows versus CON in late lactation ($P < 0.05$). HT cows tend to have higher HSP90 gene expression than CL cows when dry ($P = 0.08$). OG cows had lower HSF1 gene expression versus CON cows ($P = 0.05$) when dry. In late lactation, OG cows tend to have lower PRLR-S and PRLR-L gene expression versus CON cows ($P = 0.09$ and $P = 0.10$; respectively). During DP, OG and HT had higher PRLR-LF gene expression versus CON and CL cows, respectively ($P < 0.05$). OG cows had lower PRLR-S gene expression versus CON when dry ($P < 0.01$). Lower cortisol concentrations of HT during the week before calving may be an adaptive response to chronic HT. Lower HSP90 and HSF1 expression in CL and OG cows, respectively, is consistent with a reduction in respiration rate during HT of CL and OG cows.

Key Words: cortisol, prolactin receptor

M179 In-depth discovery of milk proteomes and detection of biomarkers using SWATH mass spectrometry. L. E. Hernández-Castellano*[1,2] and E. Bendixen[2], [1]*Department of Animal Science, Aarhus University–Foulum, Tjele, Denmark,* [2]*Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, Aarhus, Denmark.*

Sequential window acquisition of all theoretical mass spectra (SWATH) mass spectrometry is a new proteomic approach that combines the advantages of shotgun-MS (high throughput) with those of selected reaction monitoring (SRM)-MS (high reproducibility and consistency). This study aims to show for the first time the potentials of SWATH-MS for the detection and quantification of immune and metabolism related proteins in milk from dairy cows. In this study we used individual non-fractionated milk samples from 21 healthy Holstein dairy cows (SCC < 100,000 cells/mL), which were individually analyzed with a 6600 TripleTOF-MS. Data were analyzed using Skyline 4.1, interrogating a bovine milk proteome spectral library of 1,500 unique proteins. A selected subset of 34 unique proteins present in all milk samples were relatively quantified, based on validated peptide signatures (those with at least 4 ion transitions and dopt > 0.90), which were selected as optimal candidates for protein quantitation. Thus, we pooled all milk samples and the resulting sample was analyzed 10 times (10 technical replicates) to test the reproducibility of targeted peptides detection for each of the 34 proteins. From the 34 proteins, we consistently measured 27 proteins based on 2 or more peptides signals; however, 7 proteins could only be measured based on one single proteotypic peptide. Notably, both low and high abundant proteins were characterized in this study. These include CD-14, CD-9, osteopontin, apolipoproteins A-I, A-IV and E, lactadherin, mucin-1, profilin-1 and β-2-microglobulin, as well as the major milk proteins such as lactoperoxidase, xanthine dehydrogenase, lactotransferrin, β-lactoglobulin and caseins, reflecting the high resolution and sensitivity of SWATH-MS over traditional shotgun-MS methods. In conclusion, SWATH-MS is a powerful tool that allows the

identification and quantitation of biomarkers and other low abundant proteins within the complex protein background of non-fractionated milk from dairy cows.

Key Words: dairy cow, milk proteomics, sequential window acquisition of all theoretical mass spectra (SWATH)-MS

M180 Palmitic acid increased the gene expression of lipogenic genes from the de novo milk fat synthesis pathway in cultured mammary explants from lactating dairy ewes. P. C. Carraro, E. D. Da Silva, M. Camêra, and D. E. Oliveira*, *Santa Catarina State University, Lages, Santa Catarina, Brazil.*

Lipid supplementation with palmitic acid (C16:0) has been shown to increases the milk yield and milk fat content. Also, changes in the fatty acid profile have been shown variable reducing or not short and medium-chain fatty acid from the de novo synthesis pathway. However, the molecular mechanisms have not been fully investigated. The objective of this study was to evaluate the effect of palmitic acid (C16:0) on the expression of lipogenic genes involved in the milk fat synthesis [(acetyl CoA carboxylase α; ACC-α) isoforms from promoters II and III and fatty acid synthase (FASN)], fatty acid internalization (CD36 molecule; CD36), intracellular transport of fatty acids (fatty acid binding protein; FABP 3 and 4) and fatty acid desaturation (stearoil CoA desaturase; SCD). Mammary explants weighing 30 ± 0.005 mg from 3 Lacaune ewes with 40 ± 6 DIM and BCS of 3.25, obtained through biopsies were cultured in sextuplicates for 24h with one of the following treatments: Control [culture medium + bovine albumin (98%; BSA fatty acid free) at 75 μM] and Palmitic Acid [culture medium + C16:0 (99%) at 75 μ*M*]. Subsequently, total RNA was extracted, complementary DNA (cDNA) was synthesized and the real-time quantitative PCR analysis (RT-qPCR) carried out. The data were analyzed using the MIXED procedure of SAS assuming treatment as a fixed effect and the explant as random. Palmitic acid increased ($P < 0.05$), respectively, ACC-α PIII, ACC-α PII, and FASN gene expression 1.5-, 1.3-, and 1.3-fold compared with the Control. However, palmitic acid did not stimulate the gene expression of CD36, FABP3, FABP4, and SCD. According the literature, the observed increase in the gene expression of the de novo pathway lipogenic genes suggests that short and medium-chain fatty acids are needed to be esterified on the triacylglycerol for preserving milk fat fluidity.

Key Words: lactation biology, lipid metabolism, sheep milk

M181 Impact of different methods at dry-off on cure rate and new intramammary infections in the dry period. A. I. de Prado-Taranilla*[1], K. Krogh[1], A. Pearn[2], and A. Antona[2], [1]*Ceva Sante Animale, Libourne. France,* [2]*Dairy Data Warehouse, Assen, the Netherlands.*

The level of milk production at dry-off (DO) is a risk factor for new Inframammary infections during the dry period and may negatively affect cure rates (CR). The objectives of this study were to investigate the CR and the new infection rate (NIR) when applying at DO 2 methods: gradual or abrupt (no intervention to decrease milk production before DO). Data from 42,633 dairy cows from 656 Dutch commercial herds were analyzed. Discrimination between gradual and abrupt DO was obtained by analyzing daily milk yield and frequency of milking in the last 60 d before DO. A multi-layer perceptron classification algorithm was trained on a subset of lactation curves using an expert-based labeling approach based on prior knowledge and experience in the field and subsequently run over the 42,633 animal data set to predict the respective DO method. The impact on CR and NIR was studied in 28,566 cows with eligible milk recording data by comparing the last SCC recording before DO and the first SCC recording after calving at cow level. Cows were classified as infected if the level of SCC was ≥200,000 cells/mL. Considering this threshold, CR was calculated as the proportion of non-infected cows after calving of the infected cows at DO. NIR was calculated as the proportion of infected cows at calving of the non-infected cows at DO. Information of intramammary antibiotics at DO was not available. According to the classification algorithm, 32% of the animals involved in the analysis underwent a gradual DO and the remaining 68% an abrupt DO. Only 26% of herds applied the same dry-off management to all cows. Higher producing herds were associated with a greater share of gradually dried off animals. The CR and NIR were 71.2% and 19.0% respectively in animals with gradual DO and 72.5% and 17.1% in animals with abrupt DO. A 2-tailed proportion z-test with $\alpha = 0.05$ indicated that reducing the milk production at dry-off gradually resulted in higher NIR (z-score = 3.3) and did not increase CR (z-score = −1.1) during the dry period compared with animals dried-off abruptly.

Key Words: cure rate, dry period, milk production

M182 Distribution of prevalence of cows leaking milk after dry-off in different countries. A. I. de Prado-Taranilla*[1], M. Holstege[2], A. Bach[3,4], Y. H. Schukken[2], and A. Velthuis[2], [1]*Ceva Sante Animale, Libourne, France,* [2]*GD Animal Heath, Deventer, the Netherlands,* [3]*ICREA, Barcelona, Spain,* [4]*Department of Ruminant Production, IRTA, Barcelona, Spain.*

The mammary gland experiences drastic changes during the dry-off transition. Immediately after milk cessation the cisternal spaces, ducts, and alveoli of the gland are engorged with milk that is no longer removed from the gland. The engorged mammary gland may become more prone to milk leakage (ML), thereby allowing bacteria to colonize the gland. The objectives of this study was to record the prevalence of cows leaking milk during the first 2 d after dry-off (DO) and to investigate the potential relationship between ML and teat-end score. The study was conducted in 59 commercial dairy farms from Mexico, Brazil, USA, Europe (Spain, France, Germany, Denmark, Belgium, Italy, Czech Republic, the Netherlands) and Russia. A total of 3,308 cows were included in the study. Cows were eligible for enrollment if they were in good health and had no signs of clinical mastitis, had 4 functional quarters and were not treated with an internal or external teat seal. Occurrence of ML was determined by direct observations at quarter level between DO and 48 to 52 h after DO. Each observation lasted at least 30 s per cow. Teats were assessed and scored according to Standard Operating Procedure for the Scoring of Teat End Hyperkeratosis in Dairy Cattle. A multi-variable logistic regression model was used to investigate the association between teat-end-score and ML in 1,142 cows. Prevalence of ML within 2 d after DO was 24, 31, 26, 24, and 50% in Mexico, Brazil, USA, Europe, and Russia, respectively. The teat end score was not associated with ML ($P > 0.1$). It is concluded that ML prevalence ranges between 24 and 50% in the participating countries. As ML is considered to increase the probability of acquiring new IMI after DO, strategies to reduce ML should be considered to improve udder health.

Key Words: epidemiology, milk leakage, teat-end score

Physiology and Endocrinology I

M183 Adaptive responses of Mérinos d'Arles adult ewes submitted to nutritional and β-adrenergic challenges. E. González-García*[1], M. Alhamada[1], N. Debus[1], J.-B. Menassol[2], J. G. Vero[3], B. Barboza[3], and F. Bocquier[2], [1]*SELMET (Systèmes d'Élevage Méditerranéens et Tropicaux), INRA, Montpellier SupAgro, CIRAD, Univ Montpellier, Montpellier, France,* [2]*SELMET, Montpellier SupAgro, CIRAD, INRA, Univ Montpellier, Montpellier, France,* [3]*Universidade Estadual de Londrina (UEL), Centro de Ciências Agrárias, Londrina, Paraná, Brazil.*

Shortage and refeeding situations lead to switches in metabolic pathways induced by undernutrition and body reserves (BR) replenishment cycles. In a 122-d experiment, we studied adaptive mechanisms in 36 adult non-pregnant Mérinos d'Arles ewes, selected with similar BW and BCS. Ewes were acclimated to diet ingredients (i.e., wheat straw, pelleted alfalfa and sugar beet pulp) and to the facility environment during 22 d. Then, the "diet challenge" (planes of nutrition; 12 ewes each) was applied during the first 50 d (normally fed, Control; underfed, Under; overfed, Over) and an individual monitoring (twice a week) of BW, BCS and energy metabolism was carried out. Then, a "refeeding challenge" was applied during the last 50 d (i.e., diets were adjusted using the same ingredients). The last day, the lipolytic activity was studied with a "β-adrenergic challenge" (4 nmol/ kg BW of isoproterenol) to the same ewes (BCS according to diet; i.e., Normal, Lean and Fat, respectively). The PROC MIXED with repeated measures of SAS package was used for data analyses. The NEFA response at each time after the β-adrenergic challenge was calculated as well as the area under the concentration curve at 5 min. time intervals during 1 h. Anabolic or catabolic responses were accompanied by synchronized metabolic regulations, leading to contrasting metabolic and BR profiles. Average BW and BCS were higher and lower in Over and Under ewes, respectively, which was proportional to BR dynamics (higher and lower BR mobilization in Under and Over ewes, respectively). Higher plasma NEFA were accompanied by lower insulin, leptin and glucose. Differences in leptin were consistent with energy load (Over > Control > Under). After refeeding, a rebound in BW and BCS was observed and NEFA was drastically reduced in Under ewes. No differences were detected in NEFA profiles at the end of the study but lipolytic activity was different and contrasted with adipose tissue mass (Lean > Normal ≈ Fat). Differences in those responses between ewes with the same BR status evidenced diversity among individual adaptive capacities. There is potential for a simplified β-adrenergic challenge protocol helping to characterize intraflock variability in adaptive capacity.

Key Words: energy balance, body reserves, lipolytic activity

M184 Physiologic responses to feeding rumen-protected glucose to lactating dairy cows. J. A. Sauls*[1], S. Banuelos[1], B. Atanasov[1,2], L. H. Baumgard[3], B. J. Bradford[1], and J. S. Stevenson[1], [1]*Kansas State University, Manhattan, KS,* [2]*Ss. Cyril and Methodius University, Skopje, Republic of Macedonia,* [3]*Iowa State University, Ames, IA.*

We hypothesized that supplementing rumn-protected glucose (RPG) would increase concentrations of glucose and insulin resulting in decreased activity of liver cytochromes P450 2C and P450 3A, thus increasing blood progesterone. Estrus and ovulation were synchronized in 62 Holstein cows using GnRH and $PGF_{2\alpha}$ (d 0 = ovulation). Cows were milked thrice daily and assigned randomly to be fed individually a TMR supplemented with 0, 1, 2, or 4 kg of a RPG product (Grain States Soya Inc., West Point, NE) top-dressed into the diet beginning on d −3. Blood was collected pre-feeding and 8 h after feeding on d 0, 2, and 4 to determine glucose and insulin concentrations and daily from d 2 through 12 to assess progesterone. Blood was collected every 4 h for 24 h on d 8 to assess a circadian pattern in progesterone. Diameter of the corpus luteum (CL) was determined by ultrasonography on d 10. On d 8, feed intake (FI; $P = 0.68$), ECM ($P = 0.72$), ECM:FI ($P = 0.52$), somatic cell count ($P = 0.64$) and percentages of milk fat ($P = 0.56$) and lactose ($P = 0.81$) did not differ among treatments. Milk percentages of protein differed ($P = 0.01$) among treatments and percentages of milk solids ($P = 0.04$) and protein ($P = 0.004$) decreased linearly with increasing dose of RPG. Neither pre-feeding ($P = 0.42$) nor post-feeding ($P = 0.57$) concentrations of glucose differed among treatments; however, post-feeding glucose decreased ($P = 0.01$) from d 0 through 4. Pre-feeding insulin ($P = 0.35$) did not differ among treatments, but a post-feeding concave-up quadratic ($P = 0.06$) response of insulin was detected among treatments. Volume of the CL on d 10 did not differ ($P = 0.49$) among treatments. Milk urea nitrogen increased linearly ($P < 0.001$) with dose and pregnancy risk at first AI decreased linearly ($P = 0.01$) with increasing dose. Concentrations of progesterone increased ($P < 0.01$) from d 2 to 11 but were unaffected by treatment ($P = 0.77$). The pattern of progesterone on d 8 fit a 4th-order polynomial curve ($R^2 = 0.97$) with all concentrations during the 24-h period differing ($P < 0.05$) from the last sample concentration. We conclude that the rumen-protected glucose product did not affect progesterone concentrations.

Key Words: glucose, insulin, progesterone

M185 Dose-frequency of prostaglandin $F_{2\alpha}$ treatment of dairy cows exposed to presynchronization and either 5- or 7-d Ovsynch program durations: Ovulatory, luteolytic, and pregnancy risks. J. S. Stevenson*, J. A. Sauls, L. G. D. Mendonça, and B. E. Voelz, *Kansas State University, Manhattan, KS.*

We hypothesized that neither duration of the Ovsynch (OVS) program nor dose-frequency of $PGF_{2\alpha}$ would change the proportion of cows with complete luteolysis and an additional GnRH treatment administered as part of a presynchronization (PRE) program (Double OVS: GnRH–7 d–$PGF_{2\alpha}$–3 d–GnRH) would not alter the proportion of anovulatory cows starting OVS compared with a shorter PRE program with 1 GnRH treatment (PG-3-G: $PGF_{2\alpha}$–3 d–GnRH). Lactating Holsteins (n = 405) milked 3 times daily were enrolled in a 2 × 2 × 2 factorial design (8 treatments) before first postpartum AI. Treatments were employed to test ovulatory, progesterone, and pregnancy outcomes to 3 main effects: (1) 2 PRE programs (PG-3-G vs. Double OVS) administered 7 d before OVS; (2) 2 OVS program durations (GnRH-1–5 or 7 d–$PGF_{2\alpha}$–24 h–$PGF_{2\alpha}$–32 h–GnRH-2–16 h–timed AI); and (3) 2 $PGF_{2\alpha}$ dose-frequency treatments (2 × 25 mg) 24 h apart vs. 1 dose (1 × 50 mg) of $PGF_{2\alpha}$. The PRE treatments of PG-3-G and Double OVS had no effect on the proportion of cows with luteal function (progesterone [P4] > 1 ng/mL) at the onset of the OVS treatments (87.9 vs. 86.2%), respectively. Although ovulation risk was similar after GnRH-1 (65.8 vs. 60.2%), Double OVS cows had greater ($P < 0.05$) ovulatory responses than PG-3-G after GnRH-2 (95.6 vs. 90%), respectively. Two 25-mg doses of $PGF_{2\alpha}$ and 1 × 50-mg dose induced complete luteolysis (P4 < 0.4 ng/mL at 72 h) in both OVS durations, but the 1 × 50-mg dose was less ($P < 0.05$) effective in the 5-d program. More pregnancy per AI (P/AI; 49.2%) tended ($P = 0.07$) to occur in the PG-3-G cows in 7-d program

compared with other treatment combinations (range: 32.4 to 37.4%; OVS × presynch). An OVS × $PGF_{2\alpha}$ dose-frequency interaction (P = 0.08) resulted in the 1 × 50-mg dosed cows in the 7-d program with the greatest (46.1%) P/AI and the least P/AI (30.6%) occurred in 1 × 50-mg dosed cows in the 5-d program. Complete luteolysis occurred less often in the 5-d program after the 1 × 50-mg dose, but both $PGF_{2\alpha}$ dose-frequency effectively induced luteolysis in the 7-d program and luteolysis was related to subsequent P/AI.

Key Words: luteolysis, ovsynch duration, $PGF_{2\alpha}$ dose-frequency

M186 Relationship between air and vaginal temperatures in wild type and slick-haired Puerto Rican Holstein cows. H. L. Sánchez-Rodríguez* and K. Domenech-Pérez, *University of Puerto Rico, Mayaguez Campus, Mayaguez, Puerto Rico.*

Previous studies in cattle have reported a continuous significant increase in body temperature with air temperatures (AT) at or above 25–26°C. However, these studies were performed under environmental conditions that considerably differ from Puerto Rico's tropical weather. The present study evaluated the response of lactating pregnant wild type (WT; n = 10; 2.0 ± 0.01 lactations; 162.70 ± 0.24 DIM; 15.24 ± 1.40 kg/d of milk) and slick-haired (SLICK; n = 10; 2.0 ± 0.01 lactations; 182.4 ± 0.30 DIM; 18.82 ± 1.14 kg/d of milk) Puerto Rican Holstein cows vaginal temperature (VT) to the islands AT values during August 2015. Cows were milked 2x/d (0200–0300 and 1400–1500 h; udders were washed before milking) and grazed the rest of the day in a paddock with natural shade available. The VT and AT were recorded every 5 min for 7 d. Data were averaged by hour for hair coat types' VT comparisons (Proc GLIMMIX, SAS). To evaluate the AT and VT relationship (Procs GLM and REG, SAS), data were averaged to obtain one value by hair coat group every 5 min. The AT values ranged from 20.97 to 34.05°C. Daily VT values were greater for WT than SLICK cows (39.20 ± 0.04 and 38.77 ± 0.03°C, respectively; $P < 0.0001$). For WT cows, the relationship between AT and VT was best described by the quadratic curve: $VT = -0.0098\,AT^2 + 0.6026\,AT + 30.162$ ($R^2 = 0.44$; $P < 0.0001$). For SLICK cows the respective association was also best explained by a quadratic relationship where $VT = -0.0063\,AT^2 + 0.4061\,AT + 32.442$ ($R^2 = 0.45$; $P < 0.0001$). In general, these quadratic trends can be divided into 2 linear segments. The VT values linearly increased in both, WT (0.10°C per 1°C of AT; $P < 0.0001$; $R^2 = 0.42$) and SLICK cows (0.08°C per 1°C of AT; $P < 0.0001$; $R^2 = 0.43$) until the AT reached 30.5°C. After this critical value, VT was no longer affected by AT, for neither WT ($P = 0.0602$; $R^2 = 0.006$) nor SLICK cows ($P = 0.6536$; $R^2 = 0.0003$). After considerably higher AT values than those previously described as critical for thermoregulation in the literature, no further relationship was observed between the AT and VT, suggesting considerable adaptation to tropical weather.

Key Words: slick-haired cows, vaginal temperature, thermotolerance threshold

M187 Sodium propionate and sodium butyrate effects on histone deacetylase (HDAC) activity, histone H3 acetylation, and inflammatory gene expression in bovine mammary epithelial cells. L. Galoro da Silva*[1], B. Ferguson[2], A. S. Avila[3], and A. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Nevada, Reno, NV,* [3]*Universidade Estadual do Oeste do Parana, Marechal Candido Rondon, PR, Brazil.*

Inflammation of the mammary gland is the most costly disease affecting the US dairy sector resulting in costs around $2 billion/year. Histone deacetylase (HDAC) inhibition has anti-inflammatory properties in rodents, and short-chain fatty acids (SCFA) are effective in inhibiting HDACs. We hypothesized that SCFA would inhibit inflammation via HDAC-dependent regulation of gene expression. We aimed to evaluate sodium propionate (SP) and sodium butyrate (SB) effects on HDAC activity, histone H3 acetylation, and inflammatory gene expression. Bovine mammary epithelial (MAC-T) cells were used as the lipopolysaccharide (LPS)-induced inflammatory model. Cells were cultured in basal medium and cell lysates were incubated with increasing doses of SP or SB (0 to 5 m*M*) for 2 h before incubation with HDAC substrates (2 h). HDAC activity was determined by fluorescence detection. Cells were also pretreated with SP or SB (0 to 3m*M*) for 2 h, stimulated with LPS (1 μg/mL) for 2 h, and assessed for histone H3 acetylation by immunoblotting. Next, cells were pretreated with SP or SB (1m*M*) for 24 h, stimulated with LPS (1 μg/mL) for 2 h, and RNA was isolated. PCR array was used to examine the expression of 83 inflammatory genes and quantitative real-time PCR was used for gene validation. One-way ANOVA with Tukey post hoc analysis was used to assess significance ($P \leq 0.05$). SP and SB dose-dependently and selectively inhibited class I HDAC activity, which differed between the SCFAs, where SB inhibited HDACs 2, 3, and 8, while SP inhibited HDACs 2 and 8. SP and SB dose-dependently increased histone H3 acetylation, differing between the SCFAs, where SB increased H3K9/14, H3K18 and H3K27 acetylation, while SP increased H3K9/14 and H3K18 acetylation. SCFAs increased the overall inflammatory gene expression in MAC-T cells. Under our experimental conditions, the findings suggest that SCFAs regulate epigenetic marks on nucleosomal DNA in addition to regulation of inflammatory gene events independent of HDAC activity in MAC-T cells.

Key Words: histone deacetylase inhibitor, inflammation, short-chain fatty acid

M188 Contribution of hormone-sensitive lipase to adipose tissue lipolysis and its regulation by insulin in periparturient dairy cows. J. De Koster[1], R. Nelli[1], C. Strieder-Barboza[1], J. de Souza[2], A. L. Lock[2], and G. A. Contreras*[1], [1]*Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI,* [2]*Department of Animal Science, Michigan State University, East Lansing, MI.*

The aim of this study was to determine how hormone-sensitive lipase (HSL) contributes to adipose lipolysis and how insulin regulates lipolysis in periparturient dairy cows. Subcutaneous adipose tissue (SCAT) samples were taken from multiparous dairy cows (n = 22) at 10 d prepartum (dry) and 10 d (fresh) and 24 d (lactation) postpartum. Adipose lipolysis was determined using a short-term in vitro explant culture (3h). Basal lipolysis was determined without addition of reagents. Stimulated lipolysis was determined by β-adrenergic stimulation with isoproterenol (ISO, 10^{-6} *M*). The inhibitory effect of insulin (1 μg/L) was determined on stimulated lipolysis. HSL contribution to basal lipolysis was determined by adding an HSL inhibitor, CAY10499 (CAY, 2 μ*M*). Statistical analyses were performed in R using a mixed effect linear model. Basal lipolysis was higher in SCAT explants from dry cows (1,295 ± 120 nmol glycerol/10^6 adipocytes) compared with fresh cows (661 ± 114 nmol glycerol/10^6 adipocytes, $P < 0.05$). Inhibition of basal lipolysis by CAY was negligible in dry cows (−3.54 ± 5.05% of basal glycerol release), while in fresh and early lactation cows, CAY inhibited basal lipolysis by 36.05 ± 4.51% and 43.05 ± 4.83%, respectively ($P <$ 0.05). ISO stimulated lipolysis in SCAT explants was not different across periods ($P > 0.1$). Inhibition of stimulated lipolysis by insulin was more pronounced in the dry period (−23.23 ± 3.45%) compared with the fresh

period (−9.64 ± 3.24, $P < 0.05$). Explants with larger adipocytes had higher basal lipolysis ($P < 0.001$) while adipocyte size did not influenced lipolysis in explants cultured with CAY, ISO or insulin ($P > 0.1$). Our results demonstrate that the contribution of HSL to basal lipolysis is negligible in the dry period; however, HSL is the major driver of lipolytic responses in SCAT postpartum. Lower basal lipolysis in early lactation suggests that reduced lipogenesis is an important contributor to early lactation fatty acid release from SCAT. Loss of adipocyte sensitivity to the anti-lipolytic action of insulin develops in the early lactation period, which is suggestive for insulin resistance of the lipolytic activity.

Key Words: lipolysis, adipose tissue, insulin sensitivity

M189 The oxidized linoleic acid metabolite 13-hydroxyoctadecadienoic acid modulates lipolysis in bovine adipose tissue and adipocytes. G. A. Contreras*, S. LaTendresse, J. De Koster, C. Strieder-Barboza, J. De Souza, and A. L. Lock, *Michigan State University, East Lansing, MI.*

During periparturient metabolic stress, excessive adipose lipolysis is a strong predisposing factor for postpartum complications. Linoleic acid is the most abundant polyunsaturated fatty acid (FA) in adipose tissue (AT) of dairy cows and is preferentially released during lipolysis. In the adipocyte, oxidized linoleic acid metabolites (OXLAM) are products of enzymatic and non-enzymatic pathways. Among OXLAM, 13-hydroxyoctadecadienoic acid (HODE) was identified as a lipolysis product, but its role in modulating lipid mobilization in AT is unclear. We hypothesize that 13-HODE reduces lipolytic response to adrenergic stimulation and enhances FA uptake in adipocytes. Subcutaneous AT explants collected from dairy cows (n = 7) at 10 ± 2 d postpartum and cultured bovine adipocytes (n = 4) were incubated with physiological concentrations of 13-HODE: 0 n*M* (CO), 50 n*M* (HL), or 200 n*M* (HH) for 4 h. To stimulate lipolysis, explants were then exposed to the β adrenergic agonist isoproterenol (BAS 0M; ISO, 10^{-6} *M*) for 3 h. Lipolytic responses were evaluated by measuring glycerol release. To evaluate the effect of 13-HODE on lipogenic capacity, adipocytes were treated with insulin (5 µg/mL, 1 h) and the rate of FA uptake was measured with the QBT FA uptake assay. The statistical model included the random effect of animal and the fixed effect of treatment, insulin or ISO stimulation and their interaction. Exposure of AT explants to HL and HH for 4 h did not induce a lipolytic response compared with CO ($P = 0.62$). Glycerol release upon adrenergic stimulation was reduced in HL+ISO and LL+ISO compared with CO+ISO ($P < 0.05$). Incubation with 13-HODE did not affect FA uptake in cultured adipocytes ($P = 0.43$). β-adrenergic agonist stimulation enhanced gene expression of 13-HODE targets GPR132 and testicular orphan nuclear receptor 4 (TR4), key regulators of lipid trafficking in adipocytes. These data suggest that 13-HODE is a modulator of lipolysis but not lipogenesis in AT. Future studies will evaluate the mechanisms by which 13-HODE signals through GPR132 and TR4 to exert its effects on AT lipolytic responses.

Key Words: lipolysis, lipogenesis, periparturient period

M191 Osteopontin expression dynamics link macrophage infiltration and lipolysis intensity in adipose tissues of periparturient cows. E. Thompson*, C. Strieder-Barboza, J. de Souza, R. Nelli, J. De Koster, A. Lock, and A. Contreras, *Michigan State University, East Lansing, MI.*

Lipolysis triggered adipose tissue remodeling (AT) in periparturient cows is characterized in part by macrophage infiltration and aggregation. In cows with metabolic diseases, characterized by intense and protracted lipolysis, macrophage accumulation in AT is exacerbated and may promote tissue dysfunction. In rodent models, lipolysis triggers macrophage recruitment coupled with increased expression of *SPP1,* the gene that encodes osteopontin. This chemotactic cytokine recruits cells expressing *CD44* glycoprotein that include preadipocytes and macrophages. Our study evaluated the transcription of *SPP1*, *CD44*, and additional gene networks related to macrophage infiltration and AT remodeling using qPCR. Subcutaneous AT samples were collected from multiparous dairy cows (n = 22) at 10 d prepartum (dry), 10 d (fresh), and 24 d (lactation) postpartum. Data were analyzed in R using lognormal distributions and pairwise comparisons. *SPP1* expression was higher in fresh compared with dry ($P < 0.05$). A positive relationship in *SPP1* expression with large adipocytes ($P < 0.05$) suggested that cows with greater adiposity, those that are more prone to disease, recruit more macrophages. There was an increase in CD44 gene expression in lactation when compared with fresh ($P < 0.01$). This finding indicates that CD44, a receptor of SPP1, increases when lipolysis and SPP1 are higher. Expression of *SIRPA*, a mononuclear immune cell marker, was higher in dry compared with fresh and lactation ($P < 0.01$). Reduction of SIRPA in fresh and lactation indicates an increase in macrophage phagocytic activity. *IL10* had a positive relationship with adipocyte volume in both fresh and lactation ($P < 0.05$). In human models, IL10 is responsible for the induction of osteopontin in a time- and dose-dependent manner. Upregulation of *SPP1* alongside increases in *IL10* indicate an association between osteopontin and AT macrophage recruitment in periparturient cows. Future studies will evaluate the potential of osteopontin as a biomarker for macrophage infiltration into AT.

Key Words: osteopontin, adipose tissue macrophages, periparturient period

M192 The adipocyte marker *FABP4* is most prominently induced by combined supplementation of ascorbic acid and bovine serum lipids in cultured bovine adipocytes. S. Jurek*[1], M. A. Sandhu[2], M. Kolisek[3], G. Sponder[1], and J. R. Aschenbach[1], [1]*Institute of Veterinary Physiology, Berlin, Germany,* [2]*PMAS-Arid Agriculture University, Rawalpindi, Pakistan,* [3]*Comenius University Bratislava, Bratislava, Slovakia.*

FABP4 is a marker for adipocyte differentiation. It is induced during in vitro transdifferentiation of bovine pre-adipocytes to adipocytes by the presence of bovine serum lipids (BSL) in the absence of fetal bovine serum (FBS). The intention of this study was to test whether ascorbic acid (AA) has an influence on transdifferentiation and *FABP4* expression. Subcutaneous adipose tissue was collected from calves. After induction of differentiation, adipocytes were incubated with or without AA (40 µL/mL), BSL (10 µL/mL) and/or FBS (10%) for 14d. The accumulation of non-polar lipids was evaluated by Nile-red fluorescence and normalized to DAPI fluorescence. Stem cell markers and *FABP4* were investigated by quantitative RT-PCR and immunohistochemistry. Statistics was conducted by 2-way ANOVA. Results: The development of lipid droplets was promoted ($P < 0.001$) by BSL in absence of FBS. The mRNA expression of *ENG* was reduced in all treatments compared with pre-adipocyte values, with lowest values in FBS-free media ($P < 0.05$). The mRNA expression of *NT5E* was not reduced in cells incubated with FBS compared with pre-adipocyte values but was reduced in FBS-free media compared with pre-adipocyte values and most FBS-treated groups ($P < 0.05$). In the presence of AA, the mRNA expression of the adipocyte marker *FABP4* increased 208, 30 and 144 times in media containing BSL, FBS and BSL+FBS, respectively ($P < 0.05$). In the absence of AA, only BSL increased the mRNA expression of *FABP4* (75 times) above pre-adipocyte values ($P < 0.05$). The differential mRNA expression of

FABP4 was mirrored by immunohistochemical staining of the *FABP4* protein, which was most intense in cultures supplemented with BSL and AA in the absence of FBS. Conclusions: The study confirmed that FBS inhibits bovine adipocyte differentiation whereas BSL potently induces such differentiation. A combination of BSL and AA was most effective in inducing the adipocyte marker *FABP4* despite the fact that lipid accumulation was not significantly influenced by the co-presence of AA. *This work was supported by the Elsa-Neumann-Stipendium.*

Key Words: adipocytes, *FABP4*

M193 Effects of fully acidified close-up diets and dietary calcium content on in vitro innate immune function in transition dairy cows. X. Zhang[1], K. M. Glosson*[2], S. S. Bascom[3], A. D. Rowson[3], and J. K. Drackley[2], [1]*Institute of Animal Nutrition, Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Sichuan Agricultural University, Chengdu, Sichuan, China,* [2]*University of Illinois, Department of Animal Sciences, Urbana, IL,* [3]*Phibro Animal Health Corp., Teaneck, NJ.*

A negative dietary cation-anion difference (DCAD) dry cow diet has been used to reduce the risk of clinical and subclinical hypocalcemia (SCH). Innate immune system functions, phagocytosis (P) and oxidative burst (OB) activities of neutrophils (N) and monocytes (M), are believed to be influenced by Ca, which may contribute to immune dysfunction in freshening cows with SCH. The objective of this study was to determine the effects of 3 close-up dry cow dietary strategies on the innate immune system of multiparous Holstein dairy cows (n = 81) during the transition period, blocked by parity and calving date. Cows were assigned to 1 of 3 treatments during the close-up dry period (−28 d to calving): 1) a positive DCAD diet (+6 mEq/100 g DM) with low dietary Ca (0.4% DM; CON); 2) a negative DCAD diet (−24 mEq/100 g DM) with low dietary Ca (0.4% DM; LOW); or 3) a negative DCAD diet (−24 mEq/100 g DM) with high dietary Ca (2.0% DM; HIGH). Ionized Ca (iCa) was measured within 1 h (± 1 h) of calving and 1, 2, and 4 d postpartum. Antibodies were used to tag N, M, and the *E. coli* challenge in whole blood samples for flow cytometry analysis. Blood collected at −10 d (± 3 d) before calving and d 5 and 14 (± 1 d) postpartum were evaluated for PN, PM, OBN, and OBM. The changes in the measured innate immune factors were calculated as the difference between the prepartum sample and the d 5 and 14 samples. The MIXED procedure in SAS was used to contrast: 1) CON vs the average of LOW and HIGH; and 2) LOW vs HIGH. Cows fed LOW or HIGH had greater iCa concentrations than those fed CON, both at calving and 24 h after calving ($P < 0.01$). There were no differences among treatments in changes of PN, PM, or OBM, at either d 5 or 14 ($P > 0.10$). Cows fed LOW tended to have greater OBN at 5 DIM compared with cows fed HIGH ($P = 0.05$), which may indicate a greater immediate reaction to challenges faced at calving. While DCAD level affected iCa concentrations at 24 h postpartum, there was no difference in immune measurements among treatments, indicating no effect on these aspects of the innate immune system.

Key Words: acidogenic close-up diet, innate immunity, calcium

M194 Effects of fully acidified close-up diets and dietary Ca content on urinary mineral excretion in transition dairy cows. K. M. Glosson*[1], X. Zhang[2], S. S. Bascom[3], A. D. Rowson[3], and J. K. Drackley[1], [1]*University of Illinois, Department of Animal Sciences, Urbana, IL,* [2]*Institute of Animal Nutrition, Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Sichuan Agricultural University, Chengdu, Sichean, China,* [3]*Phibro Animal Health Corp., Teaneck, NJ.*

A negative dietary cation-anion difference (DCAD) dry cow diet strategy has been used to reduce the risk of clinical and subclinical hypocalcemia. The acidogenic diet creates compensated metabolic acidosis in late gestating cows, monitored through urinary pH. The objective of this study was to determine the effects of 3 dietary strategies for close-up dry cows on mineral excretion of multiparous Holstein dairy cows (n = 81) through the transition period. Cows were assigned to 1 of 3 treatments during the close-up dry period (−28 d to calving): 1) a positive DCAD diet with low dietary Ca (0.4% DM; CON); 2) a negative DCAD diet (urine pH 5.7) with low dietary Ca (0.4% DM; LOW); or 3) a negative DCAD diet (urine pH 5.7) with high dietary Ca (2.0% DM; HIGH). Urine was sampled for 3 consecutive days at the start of the close-up period and then every second day until calving to monitor urine pH. Urine samples at −21, −14, −7, +1, +2, and +7 d relative to calving were analyzed for mineral and creatinine concentrations. Urine volume, calculated using creatinine and BW, was estimated for mineral excretion. The MIXED procedure in SAS was used to contrast: 1) CON vs the average of LOW and HIGH; and 2) LOW vs HIGH, with a fixed effect of treatment, random effect of block, and sample as the repeated variable. Prepartum urine pH for cows fed LOW or HIGH averaged 5.7 and cows fed CON remained above 8.0. The excretion of Ca in urine prepartum was greater in cows receiving LOW or HIGH (8.4 and 13.4 g/d) than in cows receiving CON (1.0 g/d; $P < 0.01$). Cows fed the HIGH diet had greater urinary Ca excretion than those given LOW ($P < 0.01$). After calving there was no statistical difference in urine volume or urinary Ca concentration averaged over the postpartum samples, but Ca excretion remained greater in cows receiving LOW or HIGH (0.68 and 0.42 g/d) when compared with cows fed CON (0.19 g/d; $P = 0.04$). In conclusion, cows given LOW and HIGH successfully created a Ca sink in the prepartum period to increase the requirement of Ca before calving. Higher dietary Ca did not affect urinary pH but increased urinary Ca excretion.

Key Words: acidogenic close-up diet, urine minerals

M195 Impacts of reducing urine pH prepartum by altering dietary cation-anion difference on physiological and productive responses of Holstein × Gir dairy cows. R. O. Rodrigues[1], R. F. Cooke[2], S. M. B. Rodrigues[1], L. N. Bastos[1], V. F. S. Camargo[1], K. S. Gomes[1], and J. L. M. Vasconcelos*[1], [1]*São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu/SP, Brazil,* [2]*Department of Animal Science, Texas A&M University, College Station, TX.*

This study compared physiological and productive parameters in 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate containing traditional anionic salts to reduce urine pH to 7.0 (CON; n = 17), or an anionic supplement (Animate; Phibro Animal Health, Teaneck, NJ) to reduce urine pH to 6.0 (SUPP; n = 17). Nonlactating, multiparous, pregnant cows were ranked by parity, body weight (BW), and body condition score (BCS), and assigned to receive SUPP or CON for 21 d before expected calving. Cows were maintained in single drylot pen with ad libitum access to corn silage, and individually received their prepartum concentrate once daily before calving. Cows from both treatments completely consumed their concentrate allocation within 30 min after feeding. Cow BW and BCS were recorded weekly, urine pH measured every 3 d, and blood samples collected on d −21, −14, −9, −6, and −3 relative to expected calving. Cows were moved to an adjacent drylot pen with ad libitum access to water and a total-mixed ration after calving (d 0), and were milked twice daily. Cow BW and

BCS were recorded weekly, and daily milk production recorded until 30 d in milk (DIM). Blood samples were collected before each milking during the initial 5 DIM, and at 6, 9, 16, 23, and 30 DIM before the morning milking. Based on actual calving dates, cows receiving SUPP or CON for (mean ± standard error) 19.2 ± 1.2 and 19.0 ± 0.9 d, respectively. Urine pH was less ($P < 0.01$) in SUPP vs. CON cows during the last 15 d of gestation (6.12 vs. 7.15, respectively). Milk yield during the experimental period was greater ($P = 0.04$) in SUPP vs. CON cows (by 14%). Serum concentrations of fatty acids were greater ($P \leq 0.01$) in SUPP vs. CON cows 3 d before and at calving (by 52 and 22%, respectively), whereas SUPP cows had less ($P \leq 0.03$) serum glucose and cortisol concentration at calving (by 23 and 27%, respectively). Hence, SUPP decreased prepartum urine pH to 6.0 in Holstein × Gir dairy cows without depressing concentrate intake, and increased milk yield compared with CON.

Key Words: anionic supplement, dairy cow, prepartum

M196 Calcemia and risk factors for subclinical hypocalcemia in cows at dry off. K. Krogh*, N. Menudier, L. Meppiel, J.-F. Collin, C. Mansanet, G. Pagny, B. Cuminal, A. de Prado-Taranilla, C. Azevedo, and N. Isaka, *Ceva Sante Animale, Libourne, France.*

Blood total calcium in the adult cow is closely regulated. Subclinical hypocalcemia (SCHCa) were in this study defined as blood Ca concentration below 2.2 mmol/L (8.8 mg/dl). The prevalence of subclinical hypocalcemia in dairy cows at dry off is unknown. The objective of this study was to determine the prevalence of subclinical hypocalcemia at drying off in dairy herds in France and Denmark. Additionally, to identify risks factors related to the drying-off management procedures leading to the emergence of hypocalcemia at this stage. This is a multicenter prospective cohort study including 381 dairy cows from 37 herds in France and 345 cows from 21 herds in Denmark. Biochemical parameters were assessed at 2 blood samplings; one in mean (SD) 0.9 (0.7) h (BS1) after the last milking and another in mean (SD) 9.2 (1.3) h later (BS2). Data on feeding and management practices at cow level were collected. Specifically, the interaction between dry-off method and incidence of SCHCa at BS2 was assessed using descriptive statistics and ANOVA. Abrupt dry off implied no prior change in either milking or feeding; 180 and 154 cows were dried off abruptly in France and Denmark respectively. Gradual implied any change in these practices; 201 and 175 cows were dried off gradually in France and Denmark respectively. Calcium concentrations at BS1 and BS2 and relative variation between BS1 and BS2 are shown in Table 1. Cows dried off gradually in France had an incidence of SCHCa at BS2 at 17%, in Denmark 21.8%. Cows dried off abruptly had a lower incidence of SCHCa at BS2 in France 5.5% and in Denmark 2.7%. Relative risk (RR) for SCHCa at BS2 is 3.85 (CI95% 2.18:6.78) ($P < 0.0001$) for cows dried off gradually compared with abrupt. Reduction of blood Ca concentrations after dry-off occurs with varying incidence across countries. A gradual dry off increases the likelihood that cows will have subsequent subclinical hypocalcemia.

Table 1 (Abstr. M196). Mean [minimum; maximum] calcium concentration (mmol/L)

Country	BS1	BS2	Relative variation BS1 − BS2
France	2.4 [1.6; 2.9]	2.3 [1.6; 2.7]	−1.7% [−33.3; 31.3]
Denmark	2.4 [1.9; 3.2]	2.3 [1.5; 3.1]	−4.0% [−37.5; 13.6]

Key Words: dry off, subclinical, hypocalcemia

M197 Hepatic gluconeogenesis is differentially altered by choline and methionine in bovine primary hepatocytes. T. L. Chandler*[1], S. J. Bertics[1], B. A. Barton[2], and H. M. White[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Balchem Corp., New Hampton, NY.*

Coordinated expression patterns of genes controlling gluconeogenesis in primary hepatocytes suggested increased capacity for gluconeogenesis with increasing choline, but not methionine. The objective of this experiment was to quantify glucose export and cellular glycogen in primary bovine hepatocytes exposed to increasing concentrations of choline chloride (CC), D,L-Met (DLM), and added fatty acids (FA). Primary hepatocytes isolated from 3 Holstein calves were maintained as monolayer cultures for 24 h before treatment with CC (61, 128, 2028, 4528 μM) and DLM (16, 30, 100, 300 μ*M*), with or without a 1 m*M* FA cocktail in a 4 × 4 × 2 factorial design. Treatments were applied in triplicate to basal medium with 5.5 m*M* glucose and 1.25 m*M* sodium pyruvate. After 24 h, medium was collected to quantify glucose, and cells were harvested to isolate glycogen and quantify DNA. Total glucose, comprised of medium glucose and glucose exported from cells, and total cellular glycogen were normalized to corresponding total DNA to account for differences in cell plating density between calves, before averaging the normalized values within triplicates. Data were expressed relative to the lowest CC and DLM treatment without FA within each cell prep before data were analyzed using PROC MIXED of SAS 9.4 with linear and quadratic contrasts in a model with fixed effect of treatment, corresponding interactions, and random effect of calf. Interactions were not significant; therefore, only main effects are discussed. Fatty acid treatment did not affect medium glucose ($P = 0.98$) or cellular glycogen ($P = 0.29$). Increasing CC linearly decreased ($P = 0.0019$) relative glucose in medium by up to 13.5%, and linearly ($P > 0.0001$) and quadratically ($P < 0.0001$) increased relative cellular glycogen by up to 26.7%. Medium glucose ($P = 0.86$) and cellular glycogen ($P = 0.69$) were not affected by DLM. Increased cellular glycogen with increasing CC, and a lack of change in medium glucose or cellular glycogen with increasing DLM, supports previous gene expression data. Differential effects of CC on cellular glucose export and cellular glycogen should continue to be investigated.

Key Words: glucose, glycogen, fatty acid

M198 Pyruvate carbon flux in response to choline and methionine in bovine primary hepatocytes. T. L. Chandler*[1], S. J. Erb[1], S. J. Bertics[1], B. A. Barton[2], and H. M. White[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Balchem Corporation, New Hampton, NY.*

Choline and methionine can influence carbon metabolism in bovine hepatocytes. Pyruvate carbon was traced through pathways of gluconeogenesis to elucidate the ability of choline or methionine to alter carbon flux. Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h. At 24 h glucose-free medium was applied and treatments of choline chloride (CC; 0, 0.01, 0.1, or 1.0 m*M*) and D,L-Met (DLM; 0, 0.1, or 0.3 m*M*) were added in a factorial design along with 1.0 m*M* FA, reflecting the blood FA profile at calving. After 21 h, 1.25 m*M* [2-^{14}C]pyruvate was added to medium and CO_2 collected after a 3-h incubation. Cells were harvested to quantify glycogen and ^{14}C enrichment. Parallel treatments were incubated without radiolabeled substrate for 24 h to quantify medium glucose. Data were normalized to DNA, expressed relative to a no FA control within each cell prep, and analyzed by PROC MIXED (SAS 9.4) with fixed effects of CC, DLM, their interaction, and random effect of calf. Contrasts evaluated for CC were 0 m*M* vs. (0.01, 0.1, 1.0 m*M*) and linear contrast

0.01, 0.1, 1.0 m*M*. Two contrasts evaluated for DLM were 0 m*M* vs. (0.1, 0.3 m*M*) and 0.1 vs. 0.3 m*M*. Data are reported as least squares means ± SE with differences declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. No interactions were detected. Medium glucose was decreased by presence of both CC ($P < 0.01$) and DLM ($P < 0.01$), while cellular glycogen was increased by presence of CC ($P < 0.01$), but not DLM ($P = 0.52$). Recovery of pyruvate label as glycogen tended to be decreased ($P = 0.10$) by presence of CC, but not affected ($P = 0.25$) by DLM. Relative enrichment of cellular glycogen by pyruvate label was decreased ($P < 0.01$) by presence of CC and tended ($P = 0.10$) to be decreased by 0.3 m*M* DLM. Recovery of pyruvate label as CO_2 tended to be decreased ($P = 0.10$) by presence of CC, and significantly decreased ($P = 0.02$) by 0.3 m*M* DLM. Pyruvate carbon spared from oxidation by DLM was not recovered as glycogen carbon. Although CC increased cellular glycogen, decreased ^{14}C enrichment suggests glycogen carbon originated from an alternative glucogenic precursor.

Key Words: glucose, glycogen, oxidation

M199 Methionine supply in vitro alters cell proliferation, metabolism, and production of reactive oxygen species in ruminant microglial cells undergoing oxidative stress. I. Martínez-Cortés*[1], J. Stanton[2], J. Muñoz-Gutiérrez[3], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*University of Georgia, Athens, GA,* [3]*University of Wyoming, Laramie, WY.*

Microglia are monocytes (MC) that function as resident macrophages in the central nervous system (CNS). The MC are the most important immune defense in the CNS, responding rapidly to infection in part by releasing pro-inflammatory cytokines and reactive oxygen and nitrogen species (ROS, RNS). Methionine (Met) is the first-limiting amino acid for milk protein synthesis and also serves as substrate for the antioxidants glutathione and taurine through the 1-carbon metabolism and transsulfuration pathways. Previous research demonstrated beneficial effects of enhancing Met supply during the periparturient period on voluntary DMI, inflammatory and oxidative stress status, and innate immune function. Because of the linkage between inflammation, oxidative stress, and satiety centers in the brain, we sought to evaluate the in vitro response of MC to incremental doses of Met. Immortalized ovine MC cultures (n = 5/treatment) were pretreated with 0, 10, or 20 μ*M* Met for 12 h followed by oxidative stress challenge with 150 μ*M* H_2O_2 for 3 h. An ANOVA using the MIXED procedure of SAS was used in the statistical analysis. Concentration of ROS was greater overall ($P < 0.05$) with Met supplementation and 20 μ*M* Met prevented an increase in ROS after challenge with H_2O_2. Although MC not pretreated with Met had greater ($P < 0.05$) IL-6 concentration in culture medium, Met supply prevented an increase in IL-6 even after H_2O_2 challenge. Similarly, compared with MC not pretreated with Met, cellular proliferation assayed with Resazurin was lower ($P < 0.05$) with pretreatment of 10 or 20 μ*M* Met. Challenge with H_2O_2 markedly increased ($P < 0.05$) cellular proliferation to the levels detected in MC not pretreated with Met. Indirect measure of NADPH oxidase activity via Resazurin assay indicated that, compared with MC not pretreated with Met, 10 or 20 μ*M* dampened ($P < 0.05$) metabolic activity of MC when challenged with H_2O_2. Overall, data indicate that enhanced Met supply to MC can help control ROS production, cellular proliferation, and metabolic activity even during oxidative stress. The functional outcomes of these responses in vivo remain to be determined.

Key Words: central nervous system, inflammation, amino acid

M200 Methionine supply during the periparturient period alters transcriptome profiles and enhances function of polymorphonuclear leukocytes in Holstein cows. H. Dai*[1,2], F. Batistel[2], R. R. C. Yambao[2], A. A. Elolimy[2], C. I. M. Garces[2], J. M. Arroyo[3], C. Parys[4], X. Shen[1], and J. J. Loor[2], [1]*Nanjing Agricultural University, Nanjing, China,* [2]*University of Illinois, Urbana, IL,* [3]*Universidad de la Republica, San José, Uruguay,* [4]*Evonik Nutrition & Care GmbH, HanauWolfgang, Germany.*

Polymorphonuclear leukocytes (PMNL) are key cellular components of the immune system and represent the first line of defense against pathogens or other stressors, especially during the periparturient period. The objective was to examine the role of methionine supply during the transition period on PMNL function and mRNA abundance of genes associated with inflammation, oxidative stress, and metabolism. Multiparous Holstein cows were used in a block design and assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH, Germany). Mepron was fed from −28 to 30 d relative to parturition at a rate of 0.09% and 0.10% of the dry matter intake during the prepartum and postpartum period, respectively. That rate ensured a ratio of Lys to Met in the metabolizable protein close to 2.8:1. Blood for PMNL extraction and in vitro oxidative burst and phagocytosis was collected at −10, +10, and +21 d relative to parturition. Data were analyzed by using the mixed procedure of SAS considering block as random effect and treatment, time and its interaction as fixed effect. Although the glucose transporter *SLC2A1* did not differ, there was an overall upregulation of lactate dehydrogenase in response to feeding MET suggesting increased metabolic activity, which agrees with the greater overall phagocytosis and oxidative burst in those cows. That idea is supported by the upregulation of various components of the Toll-like receptor cascade and both the pro-inflammatory (*TLR2*, *TLR4*, *NFKB1*, and *IL1B*), and anti-inflammatory response (*IL10*). Despite greater overall plasma concentration of myeloperoxidase, a microbicidal enzyme, in MET cows, no differences were detected for genes associated with oxidative stress response (*MPO*, *NOX1*, *SOD1*, *SOD1*, and *NOS2*). However, expression of genes associated with glutathione synthesis and peroxidase activity were upregulated (*GPX1* and *GSR*) in the MET cows. Overall, the data suggest that enhanced supply of methionine improves immune function of PMNL at least in part through molecular mechanisms associated with energy metabolism, glutathione metabolism, and inflammatory responses.

Key Words: neutrophil function, phagocytosis, oxidative burst

M201 Assessment of heat and methane production through infrared thermography in mid-lactation dairy cows. A. R. Guadagnin[1], V. Fischer*[1], J. P. Matiello[1], L. G. R. Pereira[2], and F. S. Machado[2], [1]*Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil,* [2]*Empresa Brasileira de Pesquisa Agropecuaria, Juiz de Fora, MG, Brazil.*

The aim of this study was to correlate skin temperature assessed through infrared thermography (IRT) with heat and methane production in lactation cows. Six Holstein and Gir (HG) crossbreed and 4 Holstein (H) mid-lactation cows (all primiparous) were evaluated for daily heat and methane production in respiratory chambers, and the IRT images were taken at 9 different body locations every hour during 8 h after feeding, during 5 d. IRT were taken of the anus, vulva, ribs (on the right side), left flank, right flank, right front foot, upper lip, masseter and eye. During the whole trial, cows received the same diet ad libitum and the daily milk yield and feed intake were recorded. The trial lasted 40 d, including previous adaptations of the animals to the respiratory chambers. Correlation analysis showed that daily methane production has strong correlation with IRT taken at the eye 6 h after feeding in HG cows (r = 0.85, $P < 0.05$) and with IRT taken at the eye 5 h after feeding in H cows (r = 0.88, $P < 0.05$). Also, heat production showed strong correlation with IRT taken at the right front foot 1 h after feeding in HG cows (0.85, $P < 0.05$) and with IRT taken at the eye 5 h after feeding in H cows (r = 0.90, $P < 0.05$). These results were further confirmed using regression analysis. IRT is effective in assessing heat production and methane production in Holstein and Holstein × Gir crossbreed lactation cows (Table 1).

Table 1 (Abstr. M201).

Breed[1]	Regression equation	R^2
HG	Methane production = 31,85 × Eye H6[2] − 723	0.72
	Heat production = 660.7 × Foot H1[3] − 4414.2	0.72
H[2]	Methane production = 40.19 × Eye H5[4] − 1036.2	0.74
	Heat production = 2282.8 × Eye H5[4] − 58478	0.82

[1]HG = Holstein and Gyr crossbred; H = Holstein.

[2]IRT taken at the eye on h 6 after the feeding.

[3]IRT taken at the foot on h 1 after the feeding.

[4]IRT taken at the eye on h 5 after the feeding.

Key Words: methane production, heat production, infrared thermography

M202 Effect of a combination of natural additives to support milk production without harming the environment. A. P. D'Aurea[1], L. B. Fernandes[1], R. F. Godinho[3], G. Desrousseaux*[2], and R. Tozetto[2], [1]*Research Center Premix Company, Patrocínio Paulista, São Paulo, Brazil,* [2]*Laboratoires Phodé, Terssac, France,* [3]*State University of Minas Gerais, Passos, Minas Gerais, Brazil.*

Concerned about sustainable production in Brazilian, Premix Company in partnership with Phodé, developed a combination of specific natural additives for high production dairy cows. To improve production without affecting the environment, a blend of natural additives (NA) was composed by the association of Fator P (Premix, Brazil) + VeO Premium (Phodé, France). Fator P is composed of amino acids, minerals, probiotics and essential fatty acids. VeO Premium is a neuro-sensory solution mainly composed of a specific extract from the *Rutaceae* family. The experiment was carried out in a Brazilian farm during spring, where 104 mid-lactation multiparity Holstein cows were used, with a mean production of 38.28 ± 0.355 kg/d. Milk production, dry matter intake, feed efficiency and temperature and humidity index were evaluated over the days. To follow the effects of the use of NA were used 4 periods of 20 d each. For the first 20 d of the evaluation the group did not receive NA (−20 to 0). Then NA was fed for 60 d, divided into 3 periods (0 to 20, 20 to 40, and 40 to 60 d with additives). The average of periods were compared using Tukey test with 5% significance. Supplementation of NA increased milk yield ($P < 0.001$) and improved feed efficiency ($P < 0.001$) after 20 d of use. Period 0 to 20 may represent the adaptation time of ruminal microbiota to NA. Dry matter intake decreased ($P = 0.005$) in period 20 to 40 while THI tended to be higher ($P = 0.06$; Table 1). The NA can improve milk production even in heat stress. Results suggest that NA has the potential to be a natural alternative to support performance of high-production dairy cows without harming the environment.

Table 1 (Abstr. M202). Effect of natural additives on dairy performance

Days with additives	DMI, kg/d	Milk production, kg/d	Feed efficiency	THI[1]
−20 to 0 (no additive)	25.06[a]	38.28[b]	1.53[b]	73.8
0 to 20	25.14[a]	38.09[b]	1.52[b]	73.7
20 to 40	24.11[b]	40.54[a]	1.69[a]	74.7
40 to 60	25.19[a]	41.36[a]	1.64[a]	71.8
P-value	0.005	<0.001	<0.001	0.06
SEM	0.14	0.328	0.015	0.396

[a,b]Means with different letters are different by Tukey Test (5% significance).

[1]THI = temperature-humidity index.

Key Words: milk production, natural additives, sustainability

M203 Effect of the forest biomass as bedding material on bacterial counts in compost bedded pack for dairy cows. L. Llonch*, L. Castillejos, X. Manteca, and A. Ferret, *Animal Nutrition and Welfare Service (SNIBA), Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

To assess the effect on bacterial counts of a bedding material based on forest biomass used as compost bedded pack (CBP), 8 dry no pregnant Holstein cows were used. Animals were randomly assigned to 1 of 2 treatments (T) in a crossover design with 4 cows per treatment. Treatments were (1) CBP with forest biomass (CBP-*FB*), and (2) CBP with sawdust (CBP-*S*). The study was conducted between October 2016 and March 2017, in 2 periods (P1 = autumn; P2 = winter) of 11 wk with a 4-wk washout period between them. Cows were individually allocated in concrete floor (12.5 m^2) pens filled at the beginning of each period with 30 cm of each bedding material. Aeration of CBP was made twice daily, and an average of 0.8 kg/m^2/day of bedding material was added when humidity was greater than 60%. Bedding material was sampled at wk 11. Data were analyzed using the MIXED procedure of SAS. Model contained T, P, and T × P interaction as fixed effect, and cow as random effect. Temperature was significantly lower ($P < 0.001$) in

CBP-*FB* (24.3°C) than in CBP-*S* (32.2°C). There was a significant T × P interaction in humidity ($P < 0.001$), which was greater in CBP-*FB* in P1, but greater in CBP-*S* in P2. No significant effects in total bacteria counts were found in bedding material. *Klebsiella* spp. was significantly greater ($P < 0.01$) in CBP-*S*, whereas *Streptococcus spp.,* and yeasts and fungi were significantly greater ($P < 0.05$) in CBP-*FB*. There was a significant T × P interaction ($P < 0.05$) in coliforms at 37°C and 44°C, *E. coli* and *Staphylococcus aureus*, being greater in CBP-*S* in P1, but greater in CBP-*FB* in P2. In contrast, coagulase-negative staphylococci was greater in CBP-*FB* in P1, whereas it was greater in CBP-*S* in P2 ($P < 0.01$). Period was significant for temperature, other gram-negative bacteria, other *Streptococcus* spp., yeasts and fungi, and *Bacillus* spp. These results suggest that forest biomass does not work better than sawdust as CBP bedding material to control temperature and humidity. The effect of bedding material on bacterial counts was season-dependent except for *Klebsiella* spp., *Streptococcus* spp., and yeasts and fungi.

Key Words: bedding material, compost bedded pack, bacterial count

M204 Whole-farm economic and environmental impact of feeding strategies to decrease the enteric CH_4 emissions on Canadian dairy farms. J. Velarde-Guillén*[1], D. Pellerin[1], C. Benchaar[2], and É. Charbonneau[1], [1]*Université Laval, Quebec, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Several dietary strategies have been evaluated for their efficacy to mitigate methane (CH_4) emissions, but their effects at the farm level had been scarcely examined. The aim of this study was to simulate the effect of corn dried distiller grains with soluble (DDGS), linseed oil (LO) and brown midrib corn silage (BMR) on net income (NI) and environmental output using the whole-farm model N-CyCLES. Virtual farms, representative of 3 contrasting Canadian dairy regions were built: Maritimes, Central, and Prairies. Enteric CH_4 computation was adapted to fit results of experiments using these feeds. The DDGS and LO were tested in the 3 regions while BMR was evaluated in Central region, which has the appropriate climate for this plant. A constraint on lactating cow diets was added for each feed (DM basis) tested: DDGS at 10%; LO at 4% and BMR, replacing conventional corn silage (CS), at 40% of the diet. Results (Table 1), compared with CS-based diets, show a slight decrease in enteric CH_4 (averages of 2% for DDGS; 4% for LO and 0.1% for BMR) with typical on-farm diets. The whole-farm evaluation shows that most strategies, except DDGS, had a negative impact on NI. Also, LO and BMR, but not DDGS, resulted in a slight reduction of total greenhouse gas emissions. These results suggest that farm NI and environmental cost from feed importation should be accounted for before a modification in the diet is made to decrease greenhouse gas emission.

Key Words: feeding, methane, strategies

M205 Development of an equation to estimate the enteric methane emissions from Canadian Holstein dairy cows. J. Velarde-Guillén*[1], D. Pellerin[1], C. Benchaar[2], M. A. Wattiaux[3], and É. Charbonneau[1], [1]*Université Laval, Quebec, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [3]*University of Wisconsin-Madison, Madison, WI.*

Accurate prediction of methane (CH_4) emission from enteric fermentation is a key factor in assessing mitigation strategies on dairy farms. The aim of this study was to use dietary characteristics and animal performance data to predict CH_4 emissions from dairy cows. A database was built from measurements on individual cows from 6 experiments (56 Holstein cows, 18 treatments, 193 observations) assessing the impact of dietary modification on CH_4 production. Methane, in these trials, was determined using respiration chambers. Preliminary analyses with simple regression and Pearson correlation were performed to assess the relationship of animal variables (milk yield (MY), milk fat, milk protein, DIM, BW, DMI) and diet variables (OM, CP, NDF, ADF, Starch, EE and Rumen-inert fat: as % of DM) with CH_4 emissions and the potential collinearity between the independent variables. Backward multiple regression was then performed using the MIXED procedure of SAS 9.4 to predict CH_4 emissions with cow included as random effect. Five randomization with 5-fold cross validation were used to validate the equation. The equation with the best r (0.84) and the lowest RMSE (39.9) was: CH_4 (g/d) = −1,607.8 + 2.0 × MY (kg/d) + 56.6 × Milk Fat (%) + 11.8 × DMI (kg/d) + 0.2 × BW (kg) + 74.0 × NDF (% of DM) – 1.1 × NDF^2 (% of DM) + 2.6 × Starch (% of DM) + 14.3 × Rumen-inert fat (% of DM). Predicted CH_4 using the proposed equation was 474 ± 63 g of CH_4/cow/d, which agreed with the observed value (476 ± 73 g of CH_4/cow/d), which in turn was substantially lower than predicted by the IPCC equation (535 ± 59 g of CH_4/cow/d; r = 0.62 and RMSE = 57.0). Further analyses showed no mean bias (1.8 g of CH_4/cow/d; $P = 0.53$) or linear bias (−0.03; $P = 0.49$) for the proposed equation. In contrast, the IPCC equation exhibited a mean bias of −59.5 g of CH_4/cow/d ($P = 0.001$) and a linear bias of −0.23 ($P = 0.001$). The maximum bias with this equation (36 g of CH_4/cow/d) was however less than the prediction standard error (58 g of CH_4/cow/d). The proposed model is

Table 1 (Abstr. M204). Economic and environmental impacts of dietary strategies to reduce CH_4 production[1]

	Maritimes			Central				Prairies		
Item	CS	DDGS	LO	CS	DDGS	LO	BMR	CS	DDGS	LO
Net income, $/100 kg of FPCM	10	11	3	21	22	16	20	23	24	16
CH_4 production[2]										
Early lactation	34	33	31	33	32	30	33	34	33	31
Mid-late lactation	48	49	54	56	57	56	57	58	55	56
GHG emissions[2]										
Total	153	160	150	154	162	152	152	147	151	137
Milk allocation	122	127	118	116	119	112	115	115	118	107
Cash crop allocation	6	7	8	15	19	18	13	3	2	2

[1]Maritimes = 63 cows and 513,584 kg/yr; Central = 71 cows and 613,841 kg/yr; Prairies = 144 cows and 1,212,875 kg/yr; FPCM = 4% fat- and 3.4% protein-corrected milk.

[2]In kg of CO_2eq/100 kg of FPCM

superior to IPCC equation to predict CH_4 emission and evaluate mitigation strategies on Canadian dairy farms.

Key Words: equation, methane, dairy cow

M206 Whole-farm nitrogen and phosphorus balance of intensive dairies in central Mexico. O. I. Santana*[1,2] and M. A. Wattiaux[1], [1]*Department of Dairy Science. University of Wisconsin-Madison, Madison, WI,* [2]*INIFAP, Pabellon, Aguascalientes, Mexico.*

Our objective was to determine variables affecting whole-farm balance (WFB) of 2 nutrients [nitrogen (N) and phosphorus (P)] in intensive dairy farms of AguascalientesValley, central Mexico. Eleven farms were selected based on farmer's willingness to participate. During monthly visits (October 2013 to September 2014), herd structure, milk production, feeding practices and crop-related data were recorded, and samples of milk and forages were collected for N and P analysis. Annual WFB were calculated as Purchased inputs (feeds + fertilizers + animals) – sold outputs (milk + animals). Biological N fixation was not considered and none of the farms surveyed exported manure or crops. Explanatory variables included animal unit (AU = 454 kg of liveweight), animal density (AD = AU/ha of tillable land), on-farm forage dry matter (DM) production [OFP (%) = 100 x OFP (Mg)/(OFP (Mg) + forage DM purchased (Mg))], on-farm nutrient production [ONP (%) = 100 x ONP (Mg)/(ONP (Mg) + feed nutrient purchased (Mg))], and nutrient conversion efficiency [NCE (%) = 100 x nutrient output (Mg)/(ONP (Mg) + feed nutrient purchased (Mg))]. Descriptive statistics and multivariate regression using forward stepwise selection were obtained with R. Milking cows was (mean ± SD) 623 ± 582, AU was 1372 ± 1379, tillable land was 109 ± 85 ha (19.7 ± 34.2 ha of alfalfa and 89.5 ± 76.2 ha of corn and other annual crops), AD was 13 ± 6.2 AU/ha, and OFP was 81.2 ± 21.3%. In addition, ONP was 43.6 ± 13.6% and 37.1 ± 15.6%, and NCE was 25 ± 4 and 31 ± 11% for N and P, respectively. Feed nutrient purchased was 70.4 and 83.1% of purchased inputs for N and P respectively. Milk output was 11,229 ± 1,236 kg/cow/yr. The WFB was 80,558 ± 70,604 and 14,080 ± 13,453 kg/yr, 832 and 141 kg/ha, 69 and 12 kg/AU, and 13 and 2.3 kg/Mg of milk, for N and P respectively. For both N and P, WFB (kg/ha, kg/AU and kg/Mg of milk) was associated negatively with OFP and NCE (all $P \leq 0.05$). For the intensive dairy farms of this study, WFB were unusually large, but our findings that increasing OFP and NCE contributed to lowering WFB was consistent with other studies.

Key Words: environment, nutrient

M207 Comparison of Holstein and Jersey milk production with a new stochastic animal model. M. Li*[1], V. E. Cabrera[1], and K. F. Reed[2], [1]*Department of Dairy Sciences, University of Wisconsin, Madison, WI,* [2]*USDA-ARS Dairy Forage Research Center, Madison, WI.*

Holsteins and Jerseys are the most popular breeds in the US dairy industry. We built a stochastic, Monte Carlo life events simulation model in Python to test if Jersey cattle's higher conception rate offsets their lower milk production. The model simulates individual cows and their life events such as the onset of estrus, heat detection, artificial insemination, calving, and culling. Each life event occurs at random according to designated probability distributions. Aggregated data from individual cows represent a herd, which gives the model the ability to simulate management decisions at the farm level. For this study, we assigned different stochastic first service conception rates (CR) for Jerseys and Holsteins based on the literature (McAllister, 2010): 34%, 39%, and 44% for Jerseys and 27%, 32%, and 37% for Holsteins. Subsequent services decreased by 2.6%. (Galvão et al., 2013). We simulated cows in both breeds with voluntary waiting periods (VWP) of 50, 60, and 70 d. All other reproduction-related variables, except CR, VWP were constant in each simulation. We used MilkBot's lactation curve functions for milk production, which had different parameters for Jerseys and Holsteins and distinguished first, second, and later lactations parameters for each breed (Ehrlich, 2011). These lactation curves estimate Jersey milk production to be 67.6% of Holstein production. Simulations had 1000 cows and ran for 3000 d. From the herd, cumulative milk production from the first day of milking until dry-off or culling were sampled from 50 cows for each parity and each breed. Production samples were then divided by the number of days between calvings and multiplied by 365 to get an average milk production/cow/year. Final results represent an average of 5 simulations and indicate that Jerseys still produce less milk than Holsteins but accounting for conception rate differences decreases the gap in milk production slightly. For example, for cows in their second parity with a 50 d VWP, Holsteins produced on average 11,486.8 kg/cow/year, while Jerseys produced 7,869.3 kg/cow/year: 67.9% of Holsteins'.

Key Words: simulation, Jersey, production

M208 An assessment of different modelling strategies to predict milk fatty acid content using Fourier-transform infrared spectroscopy. G. A. Rovere*, G. de los Campos, A. I. Vazquez, A. L. Lock, L. Worden, and R. J. Tempelman, *Michigan State University, East Lansing, MI.*

Inferring composition of dairy cattle milk fatty acids (FA) is of interest for both human nutrition and potentially for dairy management. Fourier-transform infrared spectroscopy (FTIR) has been used to predict milk composition for payment on US dairy farms. Our study aimed to assess prediction abilities for FA content with several models using FTIR data. Milk samples were drawn from 365 early lactation (<90 DIM) cows on 4 Michigan dairy farms. We used FTIR absorbance information on 899 different wavelengths in the mid infrared region generated from 2 different Bentley FTS/FCM NEXGEN spectrometers at NorthStar Cooperative (Grand Ledge, MI) to predict the content of 7 saturated FA (C4:0, C6:0, C10:0, C12:0, C14:0, C16:0, C18:0), 3 unsaturated FA (C14:1 9c, C18:1 9c, C18:2 9c, 12c), and the overall total of saturated (SFA), monounsaturated FA (MUFA) and poly-unsaturated FA (PUFA) all determined using gas liquid chromatography. Content of FA was expressed in g per 100 g of milk. The models tested included partial least squares regression with 5, 10, 15 and 20 components (PLSR5, PLSR10, PLSR15, PLSR20), and 2 Bayesian regression models based on heavy tailed (BayesA) or variable selection (BayesB) specifications. Predictions were assessed by the correlation (COR) between predicted and actual values using a leave-one-herd-out cross-validation approach. The lowest COR was observed for C14:1 9c (~0.36), C10 and C12 (~0.47). The highest COR obtained was with SFA (0.93) and C16 (0.88). For SFA, COR varied between validation herds from 0.87 (PLSR20) to 0.97 (all models except PLSR20); for MUFA, from 0.50 (PLSR20) to 0.92 (BayesA/B) whereas for PUFA, varied from 0.55 (PLSR20) to 0.89 (BayesA/B). We conclude that prediction of FA by FTIR is feasible although predictive performance varied for different FA and for different herds. PLSR models and Bayesian models showed similar prediction performance; however, PLSR model predictions dramatically depended upon the number of components considered. Further developments will be crucial to better utilize widely available FTIR data to predict fine FA

composition of milk for human nutrition, estimate cow genetic merit for FA, and for dairy management applications

Key Words: milk spectral data, fatty acids, Bayesian regression

M209 Use of 3-dimensional camera to predict body weight in pre-weaned dairy calves. J. R. R. Dorea*, A. F. A. Fernandes, V. C. Ferreira, A. Cominotte, D. K. Combs, and G. J. M. Rosa, *University of Wisconsin-Madison, Madison, WI.*

Calfhood diseases can detrimentally affect productive performance during early stages of growth as well as overall lifetime performance of dairy cows. An automated, real-time assessment of body weight (BW) could be used to monitor growth curves in dairy calves. The objectives of this study was to predict BW of dairy calves using image features extracted from a 3D camera. Forty pre-weaned Holstein dairy calves with age varying from 2 to 8 weeks, and BW of 57.0 ± 14.7 kg (average ± SD) were used to develop the models. A 3D camera was positioned to capture images from the dorsal area of the calves. Thirty seconds of video was recorded and at least 10 frames were obtained for each calf. The image was segmented and 28 features were automatically extracted, including projected body volume, dorsal area, dorsal length, dorsal extent, dorsal eccentricity, distance from the camera to the floor, width in 11 points along the dorsal area, and height from the camera to the dorsal area in 11 points. These variables were then used to develop 4 models: (1) linear regression (full model: 28 variables; LRFM), (2) linear regression (stepwise backward selection: including dorsal volume, width 2, width 8, width 11, and dorsal extent; LRSTEP), (3) partial least squares (PLS), and (4) artificial neural network (ANN). The developed models were validated in an independent data set composed of 30 images from 5 pre-weaned calves obtained between the 4th and 8th weeks of age (63.8 ± 6.7 kg of BW). The biometric features automatically extracted from the 3D camera images were consistently associated with body weight. All linear models presented a good fit to body weights (LRFM: $R^2 = 0.93$, LRSTEP: $R^2 = 0.85$, PLS: $R^2 = 0.78$). In the validation data set, the PLS model presented the best prediction quality compared with all other models ($R^2 = 0.80$, mean bias = 6.0 kg, and RMSEP = 6.5 kg). Results indicate that image analysis can be used as a potential tool for real-time prediction of body weight in pre-weaned calves. Monitoring calf growth development in commercial herds can be used to anticipate potential health problems, and hence guide preventive management practices.

Key Words: computer vision, dairy calves, image analyses

M210 Modeling the effects of heat stress in different zones of Spain and the technical and economic impact of cooling systems. O. R. Espinoza* and S. Calsamiglia, *Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

The economic losses of heat stress (HS) and the return on investment in cooling systems (CS) in dairy herds are difficult to evaluate. The objective of the present study was to evaluate the effects of HS and the technical and economic impact of CS in 3 different zones of Spain. To estimate the effects of HS, weather records of southern (SO, Sevilla), central (CT, Toledo) and northern (NO, Lugo) Spain were submitted to the HS biological model (production, intake and reproduction) proposed by St. Pierre et al. (2001). The effects of HS and CS were simulated over 5 years in a stochastic dairy farm model (www.dairyfarm.es). One dairy herd for each zone was created with the same technical and economic characteristics: 300 dairy cows with an average milk production of 30.5 L/cow/d (10,800 L/cow/yr), a pregnancy rate of 15% and a milk price of €0.31/L. Three different scenarios where simulated for each one of the dairy herds: (1) CON (without any HS effects), (2) HS (with the HS effects) and (3) HS+CS (with the HS and CS effects). The initial investment in CS (fans and sprinkles) was calculated from average local prices (€65/cow) with a pay-off time of 7 years and operating cost of €6/cow month of operational time. The temperature-humidity index load calculated above ≥68 units was 12,672, 8,633 and 2,228 U/yr for SO, CT and NO, respectively, which are 2,265, 1,700 and 668 h/yr. Estimated milk production loss was of 852, 651, and 210 L/cow/yr and increase in open days of 27, 18 and 5 d for SO, CT and NO respectively. The effects of HS reduce the herd income in −€125, −€56 and −€16/cow/yr for SO, CT and NO, respectively compared with the CON scenario (average gain of €269/cow/yr). The investment in CS improved income in the SO and CT (+€46 and +€27/cow/yr), but reduced the income in the NO (−€5/cow/yr) compared with the HS scenario. In conclusion, results suggest that the profitability of the investment in fans and sprinkles depends on total heat load, being profitable in southern and central but not in northern of Spain.

Key Words: economics, heat stress, cooling

M211 Using milk somatic cell score to predict of milk yield losses of organic dairy farms in the northeast United States. J. G. B. Galvão Jr.[1], A. H. N. Rangel*[2], A. F. Brito[3], J. B. A. Silva[4], A. F. Benson[5], A. N. Hafla[6], H. M. Darby[7], K. J. Soder[6], R. Kersbergen[8], and V. Brossillon[9], [1]*Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil,* [2]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [3]*University of New Hampshire, Durham, NH,* [4]*Universidade Federal Rural do Semiárido, Mossoró, RN, Brazil,* [5]*Cornell University Cooperative Extension, Cortland, NY,* [6]*USDA-ARS, University Park, PA,* [7]*University of Vermont, St. Albans,VT,* [8]*University of Maine, Orono, ME,* [9]*Ecole Supérieure d'Agricultures, Angers, France.*

The objective of this study was to predict milk yield (MY) losses associated with milk SCS in organically certified dairy herds in the northeast region of the United States. Dairy Herd Improvement records of Holstein (HO), Jersey (JE), and Holstein-Jersey crossbred (HO×JE) cows were obtained monthly (May 2012 to June 2015) from 14 herds in New Hampshire (n = 3), Vermont (n = 3), Maine (n = 3), New York (n = 2), and Pennsylvania (n = 3). A total of 14,246 observations including MY (2 to 60 kg/d), DIM (5 to 320 d) and SCS (0 to 9) were obtained. Milk yield and SCS were analyzed using the PROC MEANS procedure of SAS. Milk yield averaged 21.3 kg/d across breeds, and 23.8, 17.7, and 22.3 kg/d for HO, JE, and HO×JE, respectively. As expected, primiparous cows had lower MY (18.9 kg/d) than multiparous cows (22.4 kg/d). The SCS for all herds averaged 2.54; it was greatest for HO (2.59), intermediate for JE (2.50), and lowest for HO×JE (2.46), and 2.20 and 2.68 for primiparous and multiparous, respectively. The following linear regression model was obtained using the PROC REG procedure of SAS: MY (kg/d) = 22.7 − 0.52 × SCS, with a confidential interval of 1.92 to 2.84 SCS. Based on this linear model, each unit increment in SCS over 1.92 would decrease MY by 0.52 kg. The maximum predicted MY losses corresponded to 0.48 kg/d, which is equivalent to 146 kg of milk in 305 d of lactation across all breeds. HO cows showed the greatest predicted MY losses on an absolute basis (0.32 kg/d), followed by HO×JE (0.28 kg/d) and JE (0.27 kg/d). However, when expressed as a proportion of 305-d MY, predicted MY losses corresponded to 2.83, 2.00, and 1.10% for JE, HO, and HO×JE, respectively. Predicted MY losses were lower in primiparous (0.17 kg/d) than multiparous (0.40 kg/d), corresponding to 1.27% and 2.59% of total lactation production, respectively. Overall, crossbred cows (HO×JE) resulted in less predicted MY losses during

305-d lactation, and multiparous cows require improved management to reduce SCS and MY losses.

Key Words: crossbred, losses, organic

M212 Characterization of dairy farm management practices for mastitis control by use of multiple correspondence analysis. R. C. Serrenho*[1], R. Bexiga[1], T. Nunes[1], and L. Pinho[2], [1]*Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisbon, Portugal,* [2]*Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Vila do Conde, Portugal.*

Correspondence analysis (CA), a multivariate descriptive data analytic method, is a statistical tool for the graphical analysis of contingency tables. Multiple correspondence analyses (MCA) is an extension of the CA technique that allows the analysis to cover more than 2 categorical variables. This exploratory method simplifies and reveals patterns in complex data sets, allowing identification of relationships between variables of interest. The detailed and descriptive analysis obtained with MCA is a potential advantage in studies in which a large amount of qualitative data is collected. Our objective was to describe herd characteristics and management practices that allow pattern recognition in mastitis outbreaks. In the present study, MCA was applied to assess mastitis risk factors in 39 dairy herds in 3 different regions of Portugal. A 38-item questionnaire regarding herd characteristics, milking procedures, mastitis control and biosecurity practices was administered, after which a MCA was performed. The results showed that each region had a particular pattern of management practices and associations between some procedures or routines were identified (3 management practices clusters). The most influential variables were related to the pasture-based farming system studied, frequency of addition of bedding, stall base material, mastitis record-keeping and specific milking procedures such as wearing gloves, pre-dipping, and the method of cleaning and drying teats. Through MCA it was possible to single out region-specific weaknesses which allowed the development and adoption of tailored mastitis control programs. A strong correlation was observed between the presence of a mastitis control program and the utilization of routine management practices. Producers who ask for veterinary advice regarding udder health and mastitis control, acquire a deeper understanding and move toward applying the recommended procedures. This is likely the main reason why a markedly different pattern between regions was observed. MCA technique can be helpful not only mastitis control and milk quality research, but also for other aspects of dairy science.

Key Words: multiple correspondence analysis, mastitis, management practices

M213 Use of a stochastic simulation model to estimate digital dermatitis, sole ulcer, and white line disease cost per case by severity, incidence timing, and parity group in dairy cattle. K. A. Dolecheck*[1], M. W. Overton[2], T. B. Mark[1], and J. M. Bewley[3], [1]*University of Kentucky, Lexington, KY,* [2]*Elanco Animal Health, Greenfield, IN,* [3]*CowFocused Housing, Bardstown, KY.*

The objective of this study was to modify a farm-level stochastic simulation model to estimate the cost per case of 3 hoof diseases by severity, incidence timing, and parity group. Disease expenditures considered within the model included therapeutics, outside labor, and on-farm labor. Disease losses considered within the model included discarded milk, reduced milk production, extended days open, an increased risk of culling, an increased risk of death (natural or euthanized), and disease recurrence. All estimates of expenditures and losses were defined using data from previously published research in stochastic distributions; the program evaluation and review technique and normal distributions were the most commonly used. Stochastic simulation was used to account for variation within the farm model; 1,000 iterations were run. Sensitivity of hoof disease costs to selected market prices and herd specific performance variables was analyzed. Using our model assumptions, the cost per case of disease over all combinations of severity, incidence timing, and parity group was lowest for digital dermatitis (mean ± SD = $137 ± 36), followed by white line disease (mean ± SD = $203 ± 33), and sole ulcers (mean ± SD = $252 ± 39). Disease costs were greater in severe versus mild cases and multiparous versus primiparous cows and were always highest at the beginning of lactation. The greatest contributing cost categories were decreased milk production, increased risk of culling, disease recurrence, and, in severe cases, increased risk of death. The contribution of cost categories to the total cost of disease varied by disease type, severity, incidence timing, and parity group. For all diseases, the average cost per case of disease increased as milk price, rolling herd average milk production, or replacement heifer price increased and decreased as feed price, pregnancy rate, or slaughter price increased. Understanding how hoof disease costs change according to cow- and herd-specific conditions can help improve on-farm decisions about treatment and prevention of hoof diseases.

Key Words: lameness

M214 A survey of United States dairy hoof care professionals on costs associated with treatment of hoof diseases. K. A. Dolecheck*[1], R. M. Dwyer[1], M. W. Overton[2], and J. M. Bewley[3], [1]*University of Kentucky, Lexington, KY,* [2]*Elanco Animal Health, Greenfield, IN,* [3]*CowFocused Housing, Bardstown, KY.*

The objective of this study was to collect information regarding hoof care professionals' billing practices and to gather their opinions about hoof diseases and the value of lameness prevention. Responses were gathered from hoof trimmers through both online (n = 5) and paper (n = 111) survey platforms. Of the 6 hoof diseases included in the survey, the treatment cost per case was greatest for toe ulcers ($20 ± 9.5), sole ulcers ($20 ± 8.6), white line disease ($20 ± 8.1), and thin soles ($18 ± 8.1), and least for infectious diseases (foot rot and digital dermatitis; $8 ± 7.6 and $8 ± 9.6, respectively). Of these diseases, digital dermatitis represented the largest portion of the lameness cases treated by respondents over the past year (44 ± 20.4%) whereas toe ulcers and thin soles represented the least (5 ± 4.1% and 5 ± 5.7%, respectively). Respondents that served mostly large herds (>500 lactating cows) reported a lower prevalence of digital dermatitis (32 ± 4.2% vs. 44 ± 3.4% and 47 ± 3.2% in small and medium herds, respectively) and a higher prevalence of sole ulcers (23 ± 3.0% vs. 13 ± 2.3% and 13 ± 2.4% in small and medium herds, respectively). Region of the US (Northeast, Midwest, or Other) also influenced hoof disease prevalence; respondents from the Northeast reported more sole ulcers than respondents from Other regions (22 ± 2.3% vs. 12 ± 3.3%). When respondents were asked their opinion on which disease was associated with the greatest total cost per case to the producer (e.g., treatment and labor costs plus the reduction in milk yield, reduced reproductive performance), digital dermatitis was ranked as having the greatest and thin soles as having the lowest total cost per case. Finally, respondents indicated that the most important benefits of reducing lameness were enhanced animal welfare and increased milk production whereas the least important benefit was reduced veterinary and hoof trimmer fees. Results from this survey can be used to improve

the accuracy of hoof disease cost estimates and contribute to better decision-making regarding both lameness treatment and prevention.

Key Words: lameness, hoof trimmer, animal health economics

M215 A case study on the effect of barn type on milk yield and income over feed cost in commercial dairy farms in Argentina. F. Bargo*[1], J. L. Monge[3], E. Giugge[2], and C. Chiavassa[2], [1]*Universidad de Buenos Aires, Buenos Aires, Argentina,* [2]*Grupo Chiavassa, Carlos Pellegrini, Argentina,* [3]*Universidad Nacional de Villa María, Villa María, Argentina.*

Improvements in cow comfort through changes in barn type could impact milk yield (MY) and income over feed costs (IOFC). We conducted a case study analysis in a commercial dairy farm of Argentina (Chiavassa Dairy Farm; −32°02′60″ S, −61°47′59″ W) with the objective to investigate the effect of barn type on MY and IOFC: open lot (OL) vs. compost bedded pack (CBP). We analyzed daily MY data from one barn of primiparous cows (lactation = 1) and one barn of multiparous cows (lactation ≥2) during a 6-yr period from June 1, 2012, until July 17, 2017. Barn type changed from OL to CPB barns on January 24, 2014. Primiparous' barn held (mean ± SE) 329 ± 23 and 214 ± 35 SE cows from 0 to 105 DIM at a cow stock density (CSD) of 24.4 ± 2.0 and 15.8 ± 2.5 m^2/cow before and after January 24, 2014. Multiparous' barn held 249 ± 16 and 193 ± 31 cows from 0 to 150 DIM at a CSD of 29.0 ± 1.9 and 17.6 ± 2.9 m^2/cow before and after January 24, 2014. Analysis included a 601-d data set when cows were housed in OL (June 1, 2012 to January 23, 2014) and a 1270-d data set when cows were housed in CBP (January 24, 2014 to July 17, 2017). The effect of barn type (OL vs. CBP) on MY was analyzed using a repeated-measures ANOVA design (InfoStat, 2017) with DIM and year as covariate. For both primiparous and multiparous cows, the effect of covariates were significant ($P < 0.05$); year probably accounted for the genetic progress. When corrected for covariate variable, the effect of barn type on MY was significant ($P < 0.05$). Primiparous cows in CBP yielded 1.21 kg/d more milk than in OL (34.11 ± 0.12 vs. 32.90 ± 0.22). Multiparous cows in CBP yielded 7.37 kg/d more milk than in OL (43.09 ± 0.20 vs. 35.72 ± 0.39). Estimation of IOFC was done using DMI and feed cost data of primiparous and multiparous cows measured during a 12-mo period (July 2016 to June 2017). Farm-gate milk price and feed cost (DM basis) averaged US$0.303 and 0.164 /kg, respectively. The higher MY with CPB increased IOFC from US$6.27 to 6.64/d for primiparous cows and from US$6.63 to 8.86/d for multiparous cows. Our data show that significant improvements in MY and IOFC are possible if cow comfort is improved with better barn facilities.

Key Words: milk yield, barn type, cow comfort

M216 Impact of estrous expression on progesterone concentrations and its association with fertility. A. M. L. Madureira*[1], T. A. Burnett[1], J. L. M. Vasconcelos[2], and R. L. A. Cerri[1], [1]*University of British Columbia, Vancouver, BC, Canada,* [2]*Sao Paulo State University, Botucatu, Sao Paulo, Brazil.*

The objective of this study was to determine the effect of estrous expression, detected by an automated activity monitor, on progesterone concentrations and its association with fertility. Estrous expression (n = 665 events) from 290 lactating Holstein cows were recorded. Cows were monitored continuously by an activity monitoring system attached to the cow's neck. The threshold level to be considered an alert on the monitor was set at SCR's index of 35 or approximately an 80% increase in physical activity. The peak of activity was determined for each event and classified as either high (index ≥80) or low (index 35–79) using the median. Blood sampling was harvested upon detection of estrus (0 d) and analyzed for P4 and E2. A subgroup of 171 events were also sampled at 7, 14, and 21 d post-AI and analyzed for P4. Animals had their ovaries scanned by ultrasound at each collection. Pregnancy was performed at 31 ± 3 d post-AI. Data were analyzed by ANOVA and logistic regression using proc MIXED and GLIMMIX of SAS. Animals that had a higher increase in physical activity had higher P/AI when compared with animals with lower activity (30.7% [48/380] vs 23.6% [64/259]; $P < 0.05$). Animals with low activity had higher concentration of P4 and lower concentrations of E2 upon detection when compared with animals with high activity (1.0 ± 0.2 ng/mL vs. 0.3 ± 0.2 ng/mL; $P < 0.01$ and 4.6 ± 0.3 pg/mL vs. 6.7 ± 0.2 pg/mL; $P < 0.01$). Follicle diameter did not differ between animals with high or low peak of activity ($P = 0.41$). Higher concentrations of P4 at 7 d, 14 d and 21 d post-AI were found in animals that had high estrous expression detected at the time of alert (7 d – High: 3.4 ± 0.2 pg/mL vs. Low: 2.7 ± 0.2 pg/mL; $P < 0.05$; 14 d – High: 4.9 ± 0.4 pg/mL vs. Low: 2.9 ± 0.4 pg/mL; $P < 0.01$ and 21 d – High: 6.8 ± 0.3 pg/mL vs. Low; 5.4 ± 0.3 pg/mL; $P < 0.01$). Size of corpus luteum on 7, 14, and at 21 d post-AI did not differ between animals that expressed high or low activity. In conclusion, animals that had higher expression of estrus had greater P/AI and a P4 profile at and post-AI normally associated with improved early embryonic development.

Key Words: automated activity monitor, progesterone, estrus intensity

M217 Fertility response to commercially available GnRH products in lactating cows synchronized with the Double-Ovsynch protocol. M. Luchterhand[1], C. A. Gamarra[2], R. S. Gennari[2], J. N. Guenther[2], P. D. Carvalho*[2], A. H. Souza[3], and R. V. Barletta[2], [1]*Elusive Hill dairy, Madison, WI,* [2]*Independent Dairy Consultant, Madison, WI,* [3]*Animal Reproduction Department, VRA-USP University, Sao Paulo, SP, Brazil.*

This study was designed to evaluate whether commonly used gonadorelin products that are commercially available in the United States have comparable ovulation and pregnancy per AI (P/AI) results in synchronized lactating dairy cows. A total of 1411 lactating Holstein cows receiving the Double-Ovsynch (DOV) protocol with the addition of a second $PGF_{2\alpha}$ (d 25) treatment (d 1: GnRH, d 7: $PGF_{2\alpha}$, d 10: GnRH, d 17: GnRH, d 24: $PGF_{2\alpha}$, d 25: $PGF_{2\alpha}$, d 26-p.m.: GnRH, d 27: AI 16 h after last GnRH) for first postpartum AI were randomized to receive one of the following GnRH products throughout the Double-Ovsynch protocol: (1) Cystorelin (CYS, gonadorelin diacetate, n = 484); (2) Factrel (FAC, gonadorelin hydrochloride, n = 482); or (3) Fertagyl (FER, gonadorelin diacetate, n = 515). On d 17 and d 24 of DOV, a subgroup of cows (n = 487) were examined by ultrasound to identify whether ovulation had occurred following the 3rd GnRH injection in the DOV protocol (G17). Circulating progesterone concentration was also measured on G17 of DOV (presumably d 7 of the estrous cycle) in a subset of cows (n = 487) to evaluate the impact of circulating P4 on ovulatory responses. Statistical analyses were performed with the procedure Glimmix and Logistic of the SAS software (9.4 version). Proportion of cows ovulating at G17 tended ($P = 0.07$) to differ among GnRH salts (gonadorelin hydrochloride = 61.5% vs gonadorelin diacetate = 72.7%) but was similar across GnRH products (FER = 74.1% vs FAC = 61.5% vs CYS = 72.2%). In a further analysis, the logistic regression model indicated that predicted ovulation response to FAC was consistently lower than the other 2 GnRH products regardless of circulating P4 levels, although greater circulating P4 had a similar detrimental effect on ovulation across all GnRH products. Results on P/AI at 60 d differed across GnRH salts ($P = 0.02$) as well as commercial products ($P = 0.04$), in which P/AI was similar between FER (47.8%^{b}) and CYS (49.8%^{b}), but both were greater than FAC (42.0%^{a}). In conclusion, fertility following Double-Ovsynch was significantly lower for the hydrochloride-based GnRH product likely due to poorer ovulatory responses throughout the synchronization protocol.

Key Words: Double-Ovsynch, GnRH, dairy cow

M218 Reproductive performance of lactating dairy cows managed with the Short-Resynch or the Day25-Resynch protocol. R. Wijma*, M. M. Pérez, E. M. Sitko, M. Scarbolo, F. S. Hernandez, and J. O. Giordano, *Cornell University, Ithaca, NY.*

Our objectives were to evaluate time to pregnancy for dairy cows managed with the Short-Resynch (SR) or Day25-Resynch (D25R) protocol. Lactating Holstein cows not pregnant after first service from 2 farms were stratified by parity (1 vs. >1) and assigned to the SR (n = 1,533) or the D25R (n = 1,555) treatment. Cows in D25R received GnRH 25 ± 3 d after AI. At 32 ± 3 d after AI, nonpregnant cows from SR and D25R with a corpus luteum (CL) ≥ 15 mm and a follicle ≥10 mm received $PGF_{2\alpha}$ (PGF), 24 h later PGF, 32 h later GnRH, and 16 h later TAI. Cows without a CL ≥15 mm and/or a follicle ≥10 mm (NoCL cows) received an Ovsynch protocol with 2 PGF treatments and progesterone (P4) supplementation ($GnRH^{+}$CIDR-7 d-CIDR-out+PGF-1 d-PGF-32 h-GnRH-16 h-TAI). Circulating P4 concentration was determined (n = 659 cows) at the GnRH treatment before TAI. Binomial data were analyzed using logistic regression and time to pregnancy [only cows with 210 d at risk after first AI (D25R = 543; SR = 512)] using Cox's proportional hazards regression. A greater ($P < 0.01$) proportion of inseminations were conducted at detected estrus in SR (60.4%) than in D25R (49.8%) but, P/AI did not differ ($P = 0.14$; D25R = 40.4%; n = 1,191 vs. SR = 37.8%; n = 1,489). More cows had a CL at NPD ($P < 0.01$) in D25R (84.3%; n = 1,139) than SR (78.5%; n = 939). Pregnancies per AI differed for all TAI combined ($P = 0.01$; D25R = 40.7% vs. SR = 35.4%), for CL cows ($P < 0.01$; D25R = 40.9%vs. SR = 32.8%), and tended to differ for NoCL cows ($P = 0.06$; D25R = 39.1% vs. SR = 44.6%). Treatment did not affect pregnancy loss ($P > 0.10$). The proportion of cows with P4 < 0.5 ng/mL at the GnRH before TAI did not differ ($P > 0.10$). The hazard of pregnancy was not affected by treatment ($P = 0.77$; HR = 0.98, 95% CI: 0.85–1.13) or parity ($P = 0.71$; HR = 1.01,

95% CI: 0.87–1.16). Median days to pregnancy were 75 and 74 for SR and D25R, respectively. The proportion of nonpregnant cows 210 d after first service was similar ($P = 0.20$) for SR (23.6%) and D25R (20.3%). In conclusion, the Short-Resynch protocol resulted in more inseminations at detected estrus and similar time to pregnancy than the D25-Resynch protocol in spite of a reduction in P/AI for TAI services for CL cows. *Supported by NYFVI Project FVI17–013 and USDA Hatch NYC127434.*

Key Words: Short-Resynch, resynchronization, dairy cow

M219 Effects of intrauterine infusion of seminal plasma at artificial insemination on fertility of lactating Holstein cows. W. G. Ortiz*[1], J. A. Rizo[1], L. R. Carvalheira[2], E. C. Estrada[1], B. R. Harstine[3], J. J. Bromfield[1], and P. J. Hansen[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Dept. de Clínica e Cirurgia Veterinárias, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil,* [3]*Select Sires Inc., Plain City, OH.*

An inflammatory response is induced in the reproductive tract by the deposition of semen during natural mating. It has been proposed that this response could facilitate establishment and maintenance of pregnancy by modifying the microenvironment of the reproductive tract. The best evidence for this idea comes from mice where females mated with males lacking seminal vesicles experience reduced conception rates, embryos with poor development to the blastocyst stage, low implantation rates, and offspring with altered postnatal phenotype. Here we hypothesized that intrauterine infusion of 0.5 mL of seminal plasma at the time of artificial insemination (AI) in first-service lactating Holstein cows will improve pregnancy rate per insemination. The experiment was conducted at a commercial dairy farm in north-central Florida during the winter (November to February); voluntary waiting period was 82 d. Cows were inseminated [multiparous cows (n = 692), conventional semen; primiparous cows (n = 322), X-sorted semen] using the Double Ovsynch protocol. Cows were randomly assigned to receive intrauterine infusion of either 0.5 mL seminal plasma (prepared as a single pool from 44 bulls) or vehicle (saline) immediately after AI. Pregnancy diagnosis was performed at d 32 after AI. Data were analyzed by the GLIMMIX procedure of SAS. Pregnancy rate per AI was affected by parity ($P = 0.0030$) and treatment ($P = 0.0586$) but not by the interaction. Least squares means for multiparous cows were 51.7 + 2.8% for vehicle and 49.0 + 2.6% for seminal plasma. Least squares means for primiparous cows were 45.3 + 3.9% for vehicle and 35.4 + 3.8% for seminal plasma. Results do not support a beneficial effect of seminal plasma on pregnancy rate per AI at first service. Further research to evaluate effects of seminal plasma on pregnancy loss and characteristics of the offspring is warranted. *Study supported by Select Sires.*

Key Words: seminal plasma, dairy cattle, Holstein

M220 Association among calving season, milk yield, and body condition score during the dry period and pregnancy at first artificial insemination in Argentinian dairy herds. P. Melendez*[1], F. Bargo[2], G. Tuñon[3], and J. Grigera[4], [1]*College of Veterinary Medicine, University of Missouri, Columbia, MO,* [2]*Universidad de Buenos Aires, Buenos Aires, Argentina,* [3]*INIA, Uruguay,* [4]*Private consultant, Argentina.*

The objective was to identify factors for pregnancy at first artificial insemination (P/1AI) in dairy herds from Argentina. A data set of 7,202 lactations from 31 dairies from central Argentina, with records of BCS at dry-off and at calving, type of parturition (normal, or dystocia), calving season (fall-winter vs spring-summer), milk yield up to 90 d (<2,200, ≥ 2,200 kg) and incidence of diseases (milk fever, RFM, metritis, mastitis, and lameness) were analyzed. Data were collected by researchers. For dichotomous variables, logistic regression models were conducted. Models with the best deviance were retained. For continuous variables GLM ANOVA were conducted, testing for interactions. From the total, 60.2% gained or maintained BCS from dry-off to parturition and 39.8% lost BCS. Normal parturitions were 87.9% and dystocia 12.1%. Incidence of milk fever was 6.0%, RFM 8.5%, metritis 4.3%, mastitis 20.8%, and lameness 9.0%. From the total, 63.0% were fall-winter parturitions and 37.0% spring-summer parturitions. Primiparous cows were 32.5% and multiparous 67.5%. Overall, P/1AI was 18.1% and not statistically different between parity ($P > 0.05$). Within multiparous, those cows that calved in spring-summer had a lower P/1AI than cows calving in fall-winter ($P \leq 0.05$). The variable most associated with P/1AI was accumulated milk yield within 90 d pp. Higher producers (>2,200 kg) had a 23.6% P/1AI and lower producers 8.54%. Milk yield for pregnant cows at first service was 2,737 kg and for non-pregnant 2,313 kg ($P \leq 0.05$). This trend was the same within calving season and parity. Cows gaining or maintaining BCS during the dry period had a P/1AI of 20.2% and 2,644 kg of milk yield, while cows losing BCS had a P/1AI of 16.4% and 2,569 kg of milk yield ($P \leq 0.05$). Higher producers had 16% of dystocia and lower producers 19.9%. Higher producers had 6.7% of lameness and lower producers 11.1%. It is concluded that lower milk yield was associated with increased incidence of diseases, greater loss of BCS during the prepartum period, more dystocia, and lower P/1AI than those cows with higher milk yield

Key Words: BCS, prepartum, diseases

M221 Maximizing inseminations at detected estrus for second and greater services in lactating dairy cows. M. Masello*[1], B. Ceglowski[2], M. J. Thomas[2], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Dairy Health and Management Services, Lowville, NY.*

Our objective was to compare time to pregnancy after first service for dairy cows managed for second and greater AI services with a program that either promotes insemination at detected estrus (AIE) or timed AI (TAI) after nonpregnancy diagnosis (NPD). After first service, Holstein cows were blocked by parity and randomly assigned to the Day 32 Resynch (D32R; n = 567) or the AIE Resynch (AIER; n = 597) group. Cows in D32R received a GnRH treatment 32 ± 3 d after AI and NPD was conducted 39 ± 3 d after AI by transrectal ultrasonography (TUS). Nonpregnant cows with a corpus luteum (CL) ≥ 15 mm (CL cows) completed the Resynch protocol ($PGF_{2\alpha}$-56h-GnRH-16h-TAI). Cows without a CL present at NPD (NoCL cows) were enrolled in a PreG-Resynch protocol (GnRH-7d-GnRH-7d-$PGF_{2\alpha}$-56h-GnRH-16h-TAI) to receive TAI. For the AIER group, nonpregnant cows based on TUS 32 ± 3 d after AI and a CL ≥ 15 mm received a $PGF_{2\alpha}$ (PGF) treatment to induce estrus. Cows not AIE within 7 d were enrolled in Resynch (GnRH-7d-$PGF_{2\alpha}$-56h-GnRH-16h-TAI). No CL cows in AIER were enrolled in PreG-Resynch. Cows remained in AIER and D32R until pregnancy or herd exit. Binomial data were analyzed with logistic regression and time to pregnancy [only cows at risk for 200 d after first AI (D32R = 426; AIER = 475)] with Cox's proportional regression. At NPD, the proportion of CL cows was greater ($P < 0.01$) for D32R than for AIER (70.3% vs. 62.7%). A greater ($P < 0.01$) proportion of CL cows were AIE in AIER than D32R (50.9% vs. 5.7%) and P/AI did not differ ($P = 0.79$; AIER = 36.0% vs. D32R = 35.1%). A greater ($P = 0.05$) proportion of NoCL cows were AIE in AIER (16.8%) than D32R (9.6%) and P/AI was greater ($P = 0.02$) for AIER (41.8%) than D32R (31.0%). Overall, P/AI for all AIE and TAI did not differ [$P = 0.13$;

D32R = 34.0% vs. AIER = 38.2%]. The hazard of pregnancy (HR = 1.00; 0.87–1.16) and the proportion of cows pregnant 200 d after first service was similar ($P > 0.10$) for D32R (88.2%) and AIER (88.0%). In conclusion, a program aimed at increasing the proportion of cows re-inseminated at detected estrus through treatment with PGF at NPD resulted in similar time to pregnancy during lactation than a program that favored TAI after NPD.

Key Words: resynchronization, dairy cow, estrus

M222 Association between hoof lesions and fertility of dairy cows. B. O. Omontese*[1], R. Bellet-Elias[1], A. Molinero[1], G. D. Catandi[1], R. Casagrande[1], Z. Rodriguez[1], R. S. Bisinotto[2], and G. Cramer[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN,* [2]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.*

Objectives were to evaluate associations between hoof lesions (HL), estrous cyclicity, and fertility in dairy cows. Jerseys were enrolled at 20 ± 3 DIM (d20). At d20, cows were evaluated for body condition score (BCS) and presence of claw horn HL (CLW; sole ulcer, toe ulcer, white line disease), sole hemorrhage (SH), infectious HL (INF; digital dermatitis, foot rot), and injury (INJ). Lesion status at d 20 was categorized into (1) no HL (n = 1,197) or with HL (n = 442) and (2) HL category as CLW (n = 113), SH (n = 280), INF (n = 36), and INJ (n = 13). Ovaries were scanned at 27 and 41 ± 3 DIM and cows with corpus luteum >20 mm were considered cyclic. Estrus was synchronized with $PGF_{2\alpha}$ given 14 d apart starting at 27 ± 3 DIM. Cows not inseminated in estrus after 3 $PGF_{2\alpha}$ were subjected to a 5-d Cosynch protocol. Cows were reinseminated in estrus or via timed AI after a nonpregnancy diagnosis. Pregnancy was diagnosed 32 and 60 ± 3 d after AI. At 120 ± 3 DIM (d120), cows were reevaluated for HL and BCS. To assess the relationship between HL development and fertility, cows were classified as healthy (no HL at d20 and d120; n = 308), cured HL (any HL at d20 and no HL at d120; n = 72), new HL (no HL at d20 and any HL at d120; n = 597), or chronic HL (any HL at d20 and d120; n = 226). Binary variables were analyzed by logistic regression (results presented as odds ratio, 95% CI) and time to pregnancy was analyzed using Cox's proportional hazard regression (results presented as hazard ratio, 95% CI). Open cows were censored at 150 DIM. Cows with SH (0.60, 0.45–0.81), CLW (0.42, 0.26–0.66), INF (0.32, 0.14–0.70), and INJ (0.04–1.07) at d20 were less likely ($P \leq 0.06$) to be cyclic compared with healthy cows. Cows with HL at d20 had smaller ($P < 0.01$) hazard of pregnancy (0.83, 0.73–0.96) and more days open (91 vs. 77) compared with healthy cows. Cows with SH had smaller ($P = 0.04$) pregnancy hazard compared with those without HL (0.84, 0.71–0.99). Compared with healthy animals, cows with cured HL tended to have a greater ($P = 0.09$) pregnancy hazard (1.30, 0.96–1.76). In conclusion, HL at d20, but not new HL, were associated with decreased odds of cyclicity and reduced pregnancy hazard in cows.

Key Words: lameness, foot trim, pregnancy

M223 Prevalence of purulent vaginal discharge in dairy herds depends on timing but not method of diagnosis—A meta-analysis. A. Ricci*[1], K. Reed[2], and O. Pascottini[3], [1]*DPT Scienze Veterinarie, University of Torino, Torino, Italy,* [2]*USDA-Agricultural Research Service, US Dairy Forage Research Center, Madison, WI,* [3]*Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

A systematic meta-analysis of existing literature was conducted to determine the prevalence of purulent vaginal discharge (PVD) and factors influencing global prevalence estimates. Four databases (PubMed, Google Scholar, Web of Science, and Scopus) were queried with keywords "endometritis," "PVD,"and "dairy." Abstracts of initial search results were scanned to ensure dairy cows and one of the 3 most common methods for PVD diagnosis (gloved hand, vaginoscopy, or Metricheck) were used. This resulted in collection of 42 manuscripts, 36 of which reported sufficient information about their methodology and PVD prevalence to be included in the analysis. Reports were from 5 geographic regions: North America, South America, Europe, Asia, and the South Pacific and included 85 observations on PVD prevalence. Proportion of population positively diagnosed with PVD ([0, 1]) was used as the measure of prevalence. The *metafor* package (Viechtbauer et al., 2010, J Stat. Softw. 36:1–48) in R statistical software was used to fit fixed, random, and mixed effects models to estimate average PVD prevalence and factors influencing this prevalence using a logistic transformation of the original proportion values weighted by the number of animals in the cohort. The fixed effects model estimated a prevalence of 0.23 [95% (CI): 0.23–0.23] while the random effects model estimated a slightly higher effect size of 0.24 (95% CI: 0.21 – 0.28). The full mixed effects model included fixed effects for parity, calving strategy (seasonal or continuous), region of the world, method of diagnosis, and days in milk (DIM) at diagnosis. Only DIM at diagnosis had a significant ($P = 0.07$) effect on the prevalence of PVD with a decrease of 0.4% per day in lactation. Average PVD prevalence estimated by the mixed-effects model was 0.249 [95% CI: 0.20 – 0.30]. All models included a large degree of heterogeneity indicating factors not included here account for most of the variation in PVD; however, our results show PVD prevalence does not vary with region of the world, parity, or method of diagnosis.

Key Words: purulent vaginal discharge (PVD), meta-analysis, dairy cows

M224 Bellowing and vaginal discharge as secondary symptoms of estrus detection for successful insemination of dairy cattle in tiestall barns in a tropical environment. S. Kanwichai*[1], S. Panasophonkul[2], P. L. A. M. Vos[3], and W. Suriyasathaporn[1], [1]*Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chaing Mai, Thailand,* [2]*Department of Companion Animal and Wildlife Clinic,Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand,* [3]*Departments of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands.*

Detection of standing heat is difficult for cattle in tiestall barns and many farmers use secondary signs of estrus behavior including bellowing and vaginal discharge for AI. The aim of this study was to determine pregnancy success to AI after detection of estrus in cattle in tie-stall barns using by secondary signs. The study was performed using reproductive data from 175 cattle of Thai-Holstein dairy cattle in tiestall barns in Chiang Mai Province, Thailand, during February to July 2017. After observing secondary signs of estrus, farmers notified the researchers who performed AI within 12, 24, or 36 h after estrus exhibition. Data on season (cool, summer, rainy), signs of estrus (bellowing, vaginal discharge, redness of vulva, swollen vulva), duration between observed estrus and AI time (12, 24, 36 h) were independent variables. Pregnancy checks performed at 60 d after AI was defined as a dependent variable. Multiple logistic regressions were used to determine the factor relating to pregnancy. Results showed that cattle with combination of bellowing and having vaginal discharge had the highest pregnancy rate (68%) and significantly higher than cattle with other signs (OR = 2.02).

The pregnancy rate in cool and summer seasons were higher than rainy season with OR = 3.67 and OR = 1.63, respectively. Cattle inseminated at 24 h after expression had significantly higher pregnancy rate than 12 h (OR = 2.67). Results indicated that highest pregnancy rates were achieved with cattle displaying bellowing and vaginal discharge, with insemination interval of 24 h after signs of estrus, and in the cool season.

Key Words: bellowing, vaginal discharge, pregnancy rate

M225 Feeding regime does not affect estimation of daily urine output but it affects estimation of total purine derivatives excretion in spot urinary sampling. K. Kljak*[1], F. Pino[2], and A. J. Heinrichs[2], [1]*Department of Animal Nutrition, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia,* [2]*Department of Animal Science, The Pennsylvania State University, University Park, PA.*

Estimation of urine output and purine derivatives (PD) excretion from spot samples is possible because daily creatinine excretion is proportional to animal BW. Feeding regimen was so far omitted in the evaluation of effects on this estimation, and this study aimed to explore the effect of ad libitum or restricted intakes on estimation in spot urinary sampling. Eight Holstein heifers (457 ± 27 kg BW) were randomly assigned to a split-plot 4 × 4 Latin square experimental design with 18-d periods (14 d of adaptation and 4 d of sampling). Heifers were offered ad libitum and restricted intakes (85% of ad libitum intake) of diets differing in forage quality (high, corn silage; low, grass hay) and fiber content (high, 48% NDF; low 40% NDF). In each period, spot urine samples were taken 4 h after feeding on d 14 while total urine samples were composited from total urine collected during 4 sampling days. Creatinine excretion was affected by dietary treatment with lower values in heifers fed restricted intakes (70.06 vs. 79.55 mmol/day; $P < 0.05$). However, when expressed per BW, it was similar among treatments and lower than in previous studies (25 vs. 28 mg/kg BW). Restricted intake resulted in higher urine output and lower PD excretion ($P < 0.05$) regardless if estimated from spot sample (14.22 vs. 8.14 kg for urine output and 116.9 vs. 144.6 mmol/day for PD excretion) or observed in total urine collection (16.01 kg vs. 9.61 kg for urine output and 146.4 vs. 171.2 mmol/day for PD excretion). However, relationship between estimated and observed values for urine output was linear regardless of feeding regimen ($R^2 = 0.75$, RMSE = 2.73, mean bias = 5.22%) while the relationship for PD excretion was linear only for ad libitum intake ($R^2 = 0.53$, RMSE = 14.83, mean bias = 18.34% vs. $R^2 = 0.01$, RMSE = 24.35, mean bias = 29.34% for restricted). Spot urinary sampling could be used for daily urine output estimation in both ad libitum and restricted feeding regimens while it is suitable for estimation of PD excretion only in ad libitum intake.

Key Words: spot urinary sampling, daily urine output, purine derivative excretion

M226 Rumen-protected lysine prototype supplementation increased milk production in lactating dairy cows. H. R. Bailey*[1], J. D. Kaufman[1], B. Barton[2], C. Zimmerman[2], K. Estes[2], and A. G. Ríus[1], [1]*University of Tennessee, Knoxville, TN,* [2]*Balchem Corp., New Hampton, NY.*

Lysine is typically a limiting AA for milk production in North American dairy cow corn-based diets. The objective of this study was to evaluate the effects of a rumen-protected lysine prototype (RP-Lys; Balchem Corp., New Hampton, NY) on milk production in dairy cows. Ten multiparous Holstein cows (94 ± 25 DIM) were used in a 5 × 5 replicated Latin Square with 18-d treatment periods. Treatments were 1) control (CTL; 0 g of Lys fed as RP- Lys), 2) 17.5 g of Lys fed as RP-Lys (low dose; RPLL), 3) 21.8 g Lys fed as RP-Lys (medium dose; RPLM), 4) 31.51 g of Lys fed as RP-Lys (high dose; RPLH) and 5) 30 g of Lys fed as rumen protected Lys (APL, Aji-Pro L, generation 2, Ajinomoto, Chicago, IL). The basal diet was composed of 52% forage and 48% concentrate (DM basis) and was balanced assuming 27 kg/d DMI, 54 kg/d milk, 3.55% milk fat, 3.06% milk protein, and 669 kg BW. The basal diet was formulated to have MP and MP Lys balances of −93 and −15 g/d, respectively. Feed samples were collected to determine chemical and nutritional compositions. Milk samples were collected on d 15–18 of each period and analyzed for milk components. Data were analyzed using the Mixed procedure in SAS 9.4. ANOVA indicated no treatment effects (Table 1). Regression analyses showed linear increases in milk and lactose yields of 9 and 0.5 g for every 1 g of supplemental Lys prototype ($P \leq 0.05$). There was a trend for a quadratic effect on milk protein percent. The results of this study indicate that supplementation with RP-Lys prototype promoted an increase in milk and lactose yields in mid-lactation multiparous cows.

Table 1 (Abstr. M226). Effects of rumen-protected Lys on milk production and milk composition

	Treatment					
Item	CTL	APL	RPLL	RPLM	RPLH	SEM
DMI, kg/d	29.5	29.8	30.2	29.3	30.0	0.52
Milk yield, kg/d	44.2	44.8	45.9	46.3	45.3	1.18
ECM, kg/d	50.0	49.7	52.3	51.4	50.4	1.81
Lactose, %	4.70	4.73	4.75	4.72	4.71	0.02
Lactose, kg/d	2.10	2.10	2.21	2.19	2.12	0.07
True protein, %	3.15	3.12	3.10	3.12	3.15	0.03
True protein, kg/d	1.39	1.38	1.44	1.43	1.40	0.07
Fat, %	4.40	4.40	4.37	4.21	4.26	0.22
Fat, kg/d	1.91	1.91	2.00	1.95	1.93	0.10

Key Words: dairy cow, rumen-protected lysine, milk production

M227 Ingestive behavior of Holstein dairy cows grazing temperate pasture versus TMR fed in confinement: daily pattern. J. P. Soutto*[1], P. Giles[2], A. L. Astessiano[1], M. Carriquiry[1], P. Chilibroste[1], and A. I. Trujillo[1], [1]*Facultad de Agronomia, UDELAR, Montevideo, Uruguay,* [2]*Facultad de Agronomia, UNCPBA, Azul, Argentina.*

To assess the effect of feeding strategy (pasture vs. total mixed ration, TMR) on diurnal feeding pattern a study was conducted in a randomized block design for 20 d (15 d of adaptation and 5 d of measurements). Fourteen Holstein dairy cows (DIM = 148 ± 4.7; BW = 535 ± 10.9 kg; BCS = 2.6 ± 0.08; milk yield = 28.9 ± 0.9 kg) were allowed to graze an oat pasture (herbage allowance = 45 kgDM/day; 165 d from seeding, DM = 38% at ground level, NEL = 1.56 Mcal/kgDM) and were supplemented at milking parlor (0.9% BW, corn and barley grain as energy source and soybean meal as protein source), PAS treatment, or were confined and individually fed ad libitum TMR based on corn silage (55:45 forage to concentrate ratio, as dry basis; DM = 40%, NEL = 1.73 Mcal/kgDM), offered once daily in the morning (TMR treatment). Cows were milked twice daily and the access time to each diet was 16 h. Individual cows were observed during light hours every 5 min on 3 consecutive days and time spent eating, ruminating or other (idling and drinking) was recorded assuming that the activity was maintained during each 5 min. An event was defined when the activity was maintained for at least 2 consecutive observations. Data were analyzed using a mixed model and repeated measures, with treatment as fixed effect and block and day as random effects. The time spent eating was greater while time spent in other activity was lower ($P < 0.001$) for PAS than TMR

cows (283 vs. 227 ± 14 min for grazing and 110 *vs*. 183 ± 12 min for other, respectively). Duration of the first meal events after the am and pm milking did not differ between diet types and averaged 69 ± 8 min and 43 min ± 7, respectively. However, the first non-eating event after the am milking (85 *vs*. 55 ± 13 min) and the last meal event before pm milking (43 vs. 27 ± 8 min) were greater ($P < 0.01$) for PAS than TMR cows. Results suggest that different ingestive behavior performed by TMR and PAS cows could be associated with different dietary signals that would affect short-term feed intake.

Key Words: feeding pattern, pasture vs. TMR, dairy cow

M228 Ingestive behavior of Holstein dairy cows grazing temperate pasture vs. TMR fed in confinement: First daily eating event. A. I. Trujillo[1], J. P. Soutto*[1], P. Giles[2], A. L. Astessiano[1], M. Carriquiry[1], and P. Chilibroste[1], [1]*Facultad de Agronomia, UDELAR, Montevideo, Uruguay,* [2]*Facultad de Agronomia, UNCPBA, Azul, Argentina.*

A greater understanding of the factors determining short-term feed intake under grazing and TMR (total mixed ration) feeding conditions would increase our knowledge of short-term mechanism regulating dry matter intake. Thus, multiparous Holstein cows (n = 14 cows; DIM = 147.8 ± 12.7; BW = 560 ± 27.1 kg; BCS = 2.8 ± 0.2; milk yield = 28.9 ± 3.3 kg) were used to assess the effect of type of diet on short-term feed intake during the first a.m. meal event. Cows were in a randomized block design assigned to 2 treatments: (PAS) grazing on a vegetative oat pasture (herbage allowance = 45 kg DM/cow/day; DM = 38%, NEL = 1.56 Mcal/kgDM) or (TMR) ad libitum TMR (70:30 forage to concentrate ratio, as-fed basis; DM = 60%, NEL = 1.73 Mcal/kgDM) in confinement. Cows were milked twice daily and access time to each diet was 16 h. The experiment lasted 20 d (15 d of adaptation and 5 d of measurements). Short-term feed intake was measured by weighing cows before and after grazing with correction for insensible weight loss in PAS and as the difference between feed offered and refused in TMR. The intake rate was calculated by dividing short-term feed intake by the time spent in the first a.m. meal event. Data were analyzed using a mixed model including treatment as fixed effect and block as a random effect. The short-term intake, (expressed as kgDM) and short-term energy intake (expressed as a proportion of estimated NEL requirements; NRC 2001) were greater ($P < 0.001$) for PAS than TMR cows (6.88 *vs*. 4.25 ± 0.48 kgDM and 0.36 *vs*. 0.22 ± 0.19, respectively). Time spent in the first a.m. meal event tended ($P = 0.06$) to be greater and intake rate was greater ($P < 0.001$) also for PAS than TMR cows (95.3 vs. 74.2 ± 7 min and 56.7 vs. 71.3 ± 0.12 gDM/min, respectively). The results indicate that, depending on diet type, cows used different strategies in the first a.m. meal event, which could be associated with both different dietary signals and cow energy requirements.

Key Words: dairy cow, nutrition, short-term feed intake

M229 Effects of pulse-dose intraruminal butyrate infusion on glucagon-like peptide 2 in dairy calves. B. Hatew*[1], Y. Inabu[1,2], T. Sugino[2], and M. Steele[1], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*Hiroshima University, Graduate School of Biosphere Science, The Research Center for Animal Sciences, Higashi-Hiroshima, Hiroshima, Japan.*

Administration of exogenous glucagon-like peptide 2 (GLP-2) has recently been shown to be a strategy to improve gastrointestinal tract health in dairy calves. However, this strategy may not be a feasible approach in the current dairy calf production system because of practical application and animal welfare issues. An alternative approach is to stimulate the release of endogenous GLP-2 through ingestion of nutrients. Thus, the objective of this study was to evaluate effects of pulse-dose intraruminal butyrate infusion on dynamic change in plasma GLP-2 concentration in dairy calves. Five rumen-cannulated male calves (330 ± 16.0 kg) were used in a 5 × 5 Latin square design with 4-d periods. On d 1 of each period at 0800 h, calves were ruminally infused with 1 of 5 treatments: 0 (saline), 0.3, 0.6, 0.9, and 1.2 g butyrate per kg of BW. Infusion of butyrate was preceded by 12 h of fasting to ensure a steady-state condition. Sequential blood and rumen fluid samples after infusion were taken on d 1 of each period. Data were analyzed using the MIXED procedure of SAS where the model included fixed effect of period, square, treatment, time, and interaction of treatment with time, and the random effect of calf within square. The effects of treatment (pulse-dose of butyrate infusion) were further analyzed using the orthogonal polynomial contrast. Rumen butyrate ($P < 0.001$) and total VFA ($P < 0.001$) concentrations increased linearly in a dose-dependent manner while propionate ($P = 0.047$) and iso-butyrate ($P = 0.040$) increased quadratically. There was a cubic effect of dose of butyrate infusion on plasma GLP-2 concentration (1.59, 2.15, 0.93, 1.62 and 1.68 ng/mL; $P < 0.001$); whereas, the concentrations of rumen ($P < 0.001$) and plasma ($P < 0.015$) butyrate, and BHB ($P < 0.001$) increased linearly. Rumen and plasma concentrations of butyrate, BHB, glucose, GLP-2, and insulin were all affected by time ($P < 0.001$) and interaction of time with treatment ($P < 0.001$). Plasma GLP-2 was not correlated with BHB, plasma or rumen fluid butyrate levels. This study provides a basis for future calf studies investigating the mechanisms of action of butyrate and other biomolecules to stimulate GLP-2 secretion.

Key Words: butyrate infusion, glucagon-like peptide 2 (GLP-2), calf

M230 Effect of corn silage with highly digestible starch on dry matter intake, daily gain, milk production and blood component of lactating cows. N. Nishizawa*[1], K. Obata[1], H. Kubozono[1], A. Saegusa[1], and Y. Murai[2], [1]*ZEN-RAKU-REN, Nishi-shirakawa, Fukushima, Japan,* [2]*Kaneko Seeds Co. Ltd. Kunisada Breeding Station, Isesaki-City, Gunma, Japan.*

The objective of this study was to evaluate the effect of feeding corn silage with highly digestible starch (HD) on productivity of lactating cows. Twelve Holstein cows (BW: 563 ± 46 kg; mean ± SD in mid- lactation (days in milk: 121 ± 52 d) were assigned to one of experimental diets containing HD or a conventional corn silage (CONT) at 35% of diet DM in a switch-back design with 3 21-d periods. Concentrations of neutral detergent fiber (NDF), crude protein (CP), and starch were 37.3, 8.8, and 33.1% for the HD corn silage, and 41.6, 8.1, and 30.9% for the CONT corn silage on a DM basis, and 7-h in situ starch digestibility was greater for HD compared with CONT corn silage (73.8 vs. 64.5%; $P < 0.05$). Dietary contents of NDF, CP, and starch were 31.8, 14.7, and 26.8% for the HD diet, and 33.3, 14.5, and 26.0% for the CONT diet on a DM basis. Treatment did not affect DMI (23.3 vs. 22.0 kg/d; HD vs. CONT, respectively), milk yield (34.0 vs. 32.6 kg/d), and concentrations of milk fat (4.00 vs. 4.04%), protein (3.34 vs. 3.30%), and lactose (4.53 vs. 4.55%). However, cows fed the HD corn silage decreased milk urea N (10.2 vs. 12.3 mg/dL; $P < 0.05$), tended to increase milk protein yield (1.13 vs. 1.06 kg/d; $P < 0.10$), and increased BW change (0.59 vs. −0.07 kg/d; $P < 0.05$) compared with those fed the CONT corn silage. Consistent with these results, cows fed the HD corn silage had lower ($P < 0.05$) concentrations of serum free fatty acids (78.3 vs. 94.2 μEq/L) and serum urea nitrogen (12.6 vs. 16.1 mg/dL). These results suggest that feeding HD corn silage can improve the efficiency of N utilization

and energy balance of lactating dairy cows possibly by increasing starch fermentation in the rumen.

Key Words: corn silage, starch digestibility, lactating cow

M231 Effects of feeding purple corn silage (*Zea mays* L.) on productivity and antioxidant function of lactating cows. T. Matsuba*[1], H. Kubozono[1], A. Saegusa[1], K. Obata[1], K. Gotoh[2], K. Miki[2], and T. Akiyama[3], [1]*ZEN-RAKU-REN, Nishi-shirakawa, Fukushima, Japan,* [2]*Nagano Animal Industry Experiment Station, Nagano, Japan,* [3]*Takii & Co. Ltd., Kyoto, Japan.*

The objective of this study was to evaluate effects of feeding purple corn silage (*Zea mays* L.) on productivity and antioxidant function of lactating cows. It was hypothesized that feeding a purple corn silage (AX-152; Nagano Animal Industry Experiment Station and Takii & Co. Ltd.) would increase milk production as it contains more anthocyanin, an antioxidant, which removes active oxygen from the body. Sixteen Holstein cows (8 primiparous cows and 8 multiparous cows, BW: 574.5 ± 10.0 kg, mean ± SD) in mid-lactation (DIM: 136.7 ± 10.4 d) were assigned to one of 2 treatments with an effort for balanced parity, BW and DIM between the treatments. Experimental diets contained either AX-152 corn silage (ACS; 31.2% DM, 8.4% CP, 40.2% NDF, and 26.6% starch) or a conventional corn silage (CONT; 30.5% DM, 8.7% CP, 42.1% NDF, and 26.5% starch) at approximately 32% of diet DM. The ACS and CONT were ensiled for more than 3 mo before the study. Treatment diets were fed as TMR ad libitum for a 12-week period from February 1, 2016 to April 25, 2016. Cows fed the ACS increased ($P < 0.05$) milk yield (31.7 vs. 29.2 kg/d) and concentration of blood superoxide dismutase, an antioxidant enzyme (9,588 vs. 8,946 U/mL), compared with those fed CONT. In addition, the ACS treatment decreased serum total protein concentration (6.9 vs. 7.3 g/dL; $P < 0.05$) and increased serum aspartate aminotransferase concentration (90.8 vs. 81.7 U/L; $P < 0.05$) compared with CONT although anthocyanins was not detected in plasma or milk. However, it is noteworthy that anthocyanin concentration in the ACS decreased over the 12-week experiment period; 70 mg/kg DM for the first 4-week period, 20 mg/kg DM for the second 4-week period, and not detectable for the last 4-week period. Anthocyanins in the CONT were not detected at any time points. These results suggested that feeding purple corn silage may increase antioxidant capacity and milk production of dairy cows, but the long-term feeding effects need to be confirmed in future studies as anthocyanins may be degraded over time in silos.

Key Words: anthocyanin, antioxidant, corn silage

M232 Change in feeding strategy affects intake, rumination behavior, and ruminal pH pattern in dairy cows. D. Cavallini*[1], L. Mammi[1], A. Palmonari[1], M. Fustini[1], J. Heinrichs[2], and A. Formigoni[1], [1]*University of Bologna, Ozzano Emilia, BO, Italy,* [2]*Pennsylvania State University, University Park, PA.*

The objective of this study was to investigate feeding behavior of dairy cows fed restricted or ad libitum total mixed rations (TMR) with abrupt changes between. Eight multiparous Holstein cows were assigned to a double crossover design, with treatment 1 (T1) corresponding to ad libitum feeding and treatment 2 (T2) to restricted feeding with TMR available for 19 h/d. After 4 wk of adaptation, data were collected for 2 d before (d −2 and −1), on d 0, and for 2 d after (d 1 and 2) the switch of feeding strategy. Rumination time, ruminal pH, and dry matter intake (DMI) were recorded continuously. Statistical analysis was performed with a factorial arrangement of treatments using the MIXED procedure of JMPpro (v13.1.0, 2016, SAS). The change from T1 to T2 decreased DMI dramatically on d 0 (−8 kg; $P < 0.01$). The DMI of the first meal after feed delivery was higher on d 1 after switching from T1 to T2 (5.11 vs 2.19 kg; $P < 0.05$). On d 2, animals showed the same feeding pattern. In T2 during feed restriction time (1500 to 2000 h), rumination time and pH increased (+19 min in d 0 vs 1; $P < 0.05$, and +0.24 pH in d 0 vs 1; $P < 0.01$). When animals were moved from T1 to T2, the daily rumination pattern changed from 1 peak observed early in the morning (0200 to 0400 h) to a bimodal pattern with a second peak of rumination during the hours of restriction (1500 to 2000 h). Similar changes occurred in the ruminal pH, which shifted its maximum from 0500 to 0600 h to 1800 h during the restriction. When animals were moved from T2 to T1, pH, rumination, and DMI quickly changed to the usual pattern. In conclusion, our results confirm that an abrupt change in availability of TMR affects feeding and rumination behavior and ruminal pH pattern; however, cows adapt within 1 d after the change.

Key Words: TMR, feed restriction, feeding behavior

M233 Effect of age and physical form of oats within calf starter on hepatic enzyme expression in pre-weaned dairy calves. G. A. Chishti*[1], I. Salfer[1], J. Suarez-Mena[2], and A. J. Heinrichs[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Provimi North America, Brookville, OH.*

The objective was to determine effects of calf age and physical form of oat grain in calf starter on gene expression of hepatic energy metabolism enzymes in pre-weaned dairy calves. In 3 experiments, either ground or whole oats were used in the calf starter. Seven calves were slaughtered at 5 wk of age in experiment 1; 6 were slaughtered at 6 wk in experiment 2; and 7 in experiment 3 were slaughtered at 7 wk of age. Liver tissue was harvested immediately and stored at −80°C for further processing. Calves from experiment 1 and 2 were cannulated and their rumen pH measured. Liver RNA was extracted by a phenol-chloroform method and reverse transcribed to cDNA. The mRNA expression of gluconeogenic enzymes pyruvate carboxylase (*PC*), cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (*PEPCK-C* and *PEPCK-M*) and fatty acid oxidation enzyme carnitine palmitoyl transferase I (*CPT-1*) was measured by RT-PCR. The statistical model included fixed effects of experiment and physical form of oats and the geometric mean of normalization genes (β-Actin and GAPDH) as a covariate. The mRNA expression for all enzymes *PC, PEPCK-C, PEPCK-M,* and *CPT-1* was not affected ($P > 0.10$) by the physical form of oats; however, all enzymes were affected by experiment ($P < 0.10$). Expression of *PC*, *PEPCK-C,* and *CPT-1* was greater in experiment 2 than experiment 1 and 3 calves ($P \leq 0.10$). Expression of *PEPCK-M* was greater experiment 2 and 3 than experiment 1 ($P < 0.05$). For all enzymes, expression differed between experiment 1 and 2 calves. Similarly, rumen pH was also different, with experiment 1 calves averaging 5.69 and experiment 2 calves averaging 4.81 ($P < 0.05$). Except for *PEPCK-M*, mRNA expression of all enzymes was similar between experiments 1 and 3. We conclude that the influence of age on liver gluconeogenic and fatty acid oxidation enzyme expression was not consistent and physical form of oats in calf starter did not affect their expression in pre-weaned dairy calves. However, lower rumen pH may be related to the upregulation of these enzymes in experiment 2 calves.

Key Words: age, enzyme expression, oat

M234 The factors affecting the milk urea nitrogen concentration in Chinese Holstein cows. H. Jiang, M. Li, Z. Chen, Z.P. Yang, and H. Zhang*, *College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China.*

To investigate the factors affecting the milk urea nitrogen (MUN) in Chinese Holstein cows, a large commercial dairy farm participated in a 30-mo study. 103 593 test-day records were used for the analysis. In this study, the mean MUN concentration was 11.75 mg/dL (Table 1). The MUN reached the maximum value on 90 d of lactation for the first parity and the third or greater parities ($P < 0.05$), but it peaked at the end of lactation during the second parity. The MUN of the first parity was lower than other parities. The MUN showed its minimum level in January ($P < 0.05$), and reached its maximum in July ($P < 0.05$). The MUN of cow calving in summer was higher than other seasons at the first month of lactation, while the MUN of cow calving in autumn was significantly lower than other seasons at the fourth month of lactation ($P < 0.05$). The positive correlations were observed between daily milk yield, net energy for lactation, crude protein and MUN for the first and third parities, but negative correlations were observed in the second parity. The MUN showed significantly positive correlations with fat content, total solid content, and dry matter intake for all parities ($P < 0.01$). The negative correlation was observed between MUN and protein content, with the exception of the second parity. For all data, as MUN concentration increased, milk protein content decreased. It has been recommended that MUN concentration should be evaluated in association with parity, days in milk, season (or month), day matter intake and dietary nutritional components, to improve the management and economic benefits of dairy farm.

Table 1 (Abstr. M234). The mean and SD of DHI variable for Chinese Holstein cows

Variable	N	Minimum	Maximum	Mean	SD
DIM	103,384	1.00	999.00	181.57	129.56
Parity	103,593	1.00	5.00	1.59	0.81
Milk yield, kg/d	103,593	8.00	65.00	29.63	9.46
Fat, %	103,593	2.00	7.00	4.27	0.81
Protein, %	103,593	2.00	6.00	3.37	0.41
SCS	103,593	0.00	9.00	2.13	1.60
Lactose, %	103,593	1.96	5.65	4.94	0.24
Total solid, %	103,593	7.86	20.38	13.76	1.32
MUN, mg/dL	103,593	5.00	30.00	11.75	2.81
DMI, kg	103,384	13.13	23.83	20.35	2.25
NE_L, Mcal/d	103,384	1.32	60.13	33.52	5.89
NE_L, Mcal/kg	103,384	1.40	2.91	1.64	0.14
CP, %	103,384	13.20	18.40	16.66	1.18

Key Words: Chinese Holstein cow, milk urea nitrogen, different factors

M235 Assessing three levels of a rumen-protected methionine prototype on dairy cow performance. A. M. Barnard*[1], M.K. Conklin[1], K. Estes[2], B. A. Barton[2], C. Zimmerman[2], and T. F. Gressley[1], [1]*Department of Animal and Food Sciences, College of Agricultural and Natural Resources, University of Delaware, Newark, DE,* [2]*Balchem Corp., New Hampton, NY.*

Differences in the encapsulation technology used to protect methionine products may affect their rumen degradation and intestinal availability. The objective of this study was to determine the effect of a lipid encapsulated methionine prototype on milk, milk protein and casein yields. The study was conducted as a replicated 5 × 5 Latin square design with 5 multiparous and 5 primiparous Holstein cows assigned to either a negative control ration (NC) deficient in metabolizable (MP) methionine (−16.2 g/d), NC supplemented with Smartamine M (SM, Adisseo, Antony, France) or the NC supplemented with a methionine prototype containing 70% D,L-methionine at 3 different inclusion levels (M1, M2, M3, Balchem Corporation, New Hampton, NY) containing 100, 125, and 150%, respectively, of methionine that is in Smartamine M. The ration was balanced using NDS assuming 28 kg/d DMI, 50 kg/d milk, 3.80% fat, 2.90% protein, and 707 kg BW. Periods were 14 d and milk samples were collected on d 11–14 of each period. Data collected during the last 4 d of each period were averaged by cow and analyzed using the Glimmix procedure of SAS. The model included a covariate (data collected during a 2-week standardization period), fixed effects of treatment, period, parity and the interaction of treatment × parity and the random effect of cow. Treatment affected protein percentage ($P = 0.03$) and casein percentage ($P = 0.05$; Table 1). Compared with the NC, SM, M2 and M3 treatments increased protein percentage and casein percentage suggesting that the M2 and M3 treatments were equally as effective as Smartamine M.

Table 1 (Abstr. M235). Effect of methionine supplementation on production measures (kg/d unless otherwise noted)

	Treatment (T)								
			Methionine prototype				*P*-value		
Item	NC	SM	M1	M2	M3	SEM	T	Parity	T× Parity
DMI,	26.4	26.1	26.1	26.2	25.0	0.9	0.37	0.80	0.14
Milk,	39.9	39.9	38.8	40.5	39.4	0.9	0.55	0.04	0.44
ECM,	40.0^{AB}	40.1^{AB}	38.7^{B}	41.0^{A}	40.0^{AB}	0.7	0.08	0.004	0.27
Protein									
%	2.89^{B}	2.98^{A}	2.94^{AB}	2.95^{A}	2.96^{A}	0.03	0.03	0.42	0.75
kg/d	1.14	1.19	1.13	1.19	1.16	0.03	0.29	0.06	0.60
Casein									
%	2.26^{B}	2.34^{A}	2.31^{AB}	2.32^{A}	2.32^{A}	0.03	0.05	0.35	0.84
kg/d	0.89	0.93	0.89	0.93	0.91	0.02	0.29	0.07	0.51

Key Words: bovine, methionine, milk protein

M236 Validating and optimizing spot sampling of urine to estimate urine output using creatinine in dairy cows. C. Lee*, D. L. Morris, and P. A. Dieter, *Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH.*

An experiment was conducted to validate urinary creatinine as a marker for urine output and optimize spot urine sampling in dairy cows. Total 12 lactating cows were used in a randomized block design. Cows were grouped and randomly assigned to 2 diets: a diet with corn silage (49% of DM) or a diet with alfalfa silage (48% of DM) with supplemental potassium (3.0% K in dietary DM). The experiment lasted for 21 d and total collection of urine (TC) was conducted for the last 3 d. Twelve spot samples of urine were also collected to represent every 2 h in a 24-h cycle during TC. Urine from total collection and individual spot samples from cows were analyzed for creatinine concentrations. Creatinine concentrations from individual spot samples were averaged to simulate equally spaced 12, 6, 4, and 2 time points of composite urine (12TPs, 6TPs, 4TPs, and 2TPs, respectively; starting at feeding). The coefficient of 29 (creatinine excretion, mg/kg of BW d^{-1}) was used to estimate urine output from TPs. All data were analyzed using the

MIXED procedure of SAS with group as random and diets, methods, and their interaction as fixed. Urinary output of cows fed the diet with alfalfa silage and potassium increased (38.5 vs. 26.8 kg/d; $P < 0.01$) compared with the corn silage diet. Creatinine concentrations from TC varied from 36 to 121 mg/dL and coefficient from 16.7 to 34.5 with an average of 28.5. Creatinine concentration from 12TPs was greater (75 vs. 65 mg/dL; $P = 0.05$) when compared with TC, resulting in underestimating ($P = 0.025$) urine outputs. When compared among TPs, creatinine concentrations and urine outputs were different ($P \leq 0.09$) between 12TPs and 2TPs but not different among 12, 6, and 4TPs. In the current study, creatinine concentrations from composite spot samples (even 12TPs) failed to represent true creatinine concentration (TC), resulting in inaccurate estimation of urine output. This occurred due to the large diurnal variation of urine creatinine: urinary creatinine concentration largely decreased after feeding and gradually increased 6 h after feeding for both diets.

Key Words: spot sampling, urine output, creatinine

M237 Effect of protein supplementation on performance of crossbred dairy cows grazing tropical pasture. J. A. Freitas*[1], C. A. Bittencourt[1], A. M. Herzog[1], and V. L. Daley[2], [1]*Federal University of Parana, Palotina, Brazil,* [2]*University of Kentucky, Lexington, KY.*

Concentrate supplementation has been an important strategy to improve dairy cow production and farm profitability. The aim of this study was to evaluate the effect of different protein supplements on intake, performance, and feed efficiency of crossbred dairy cows grazing on tropical pasture. Twelve crossbred (Holstein × Gyr) dairy cows were allocated to 4 treatments in a 4 × 4 Latin square design. Treatment periods were 14 d of adaptation and 7 d used for sample and data collection. Crossbred dairy cows (BW = 500 ± 10.0 kg, body condition score = 3.5 ± 0.20, parity = 3.5 ± 1.30, milk yield = 20 ± 1.5 kg/d) were kept in Tanzania grass (*Panicum maximum*). The treatments consisted in different sources of supplement: T1: soybean meal (SM, control); T 2: sunflower meal (SFM); T3: peanut meal (PM); and T4: cottonseed meal (CSM). Cows individually received concentrate (1.0 kg for each 3 kg of milk produced) according to their milk production. Protein supplements were offered immediately after each milking (6:00 a.m. and 4:00 p.m.) in individual stalls (12.5 m^2). The DMI, CP, NDF, and total digestive nutrient, as well as the efficiency of concentrate (milk kg/kg DMI concentrate) and feed conversion efficiency (milk kg/kg DMI) were not affected by treatments ($P > 0.05$). On the other hand, cows fed PM had higher 3.5% fat-corrected milk compared with cows fed SBM, CSM, and SFM (22.96 vs. 21.06, 20.57, and 21.05 kg/d, respectively, $P < 0.01$). Milk total solids, protein, fat, and lactose contents were similar between groups ($P > 0.01$). The overall average somatic cell count of milk for crossbred dairy cows was 318,000 cells/mL. Dairy cows fed PM showed higher levels of milk urea nitrogen (MUN) compared with SBM, CSM, and SFM groups (14.4 vs. 12.7, 12.9, and 13.2 mg/dL, respectively, $P < 0.01$). The higher MUN of dairy cows fed PM might be related to its rumen degradability compared with diets based on SBM, SFM, and CSM. The protein supplements evaluated in this study can be used to replace soybeans meal in concentrates for dairy cows grazing on tropical pasture.

Key Words: crossbred dairy cow, supplementation

M238 The effect of fructose infusion on dry matter intake in dairy cattle. R. Yair*[1,2], S. J. Mabjeesh[2], and M. S. Allen[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Department of Animal Science, Faculty of Agriculture, The Hebrew University, Rehovot, Israel.*

Loading of fructose (F) in mammals prevented ATP synthesis, and as a consequence, increased feed intake; phosphate (Pi) loading prevented the effect of F. The objective of this work was to link hepatic ATP synthesis and feeding behavior. Accordingly, 4 ruminally cannulated multiparous cows (15–26 d postpartum) were used in a 4 × 4 Latin square. Periods were 24 h, including 1 h for infusions and 23 h for recovery. Cows were infused to the abomasum with 0.6 mol/h of F or glucose (G) and 0.3 mol/h of Pi (as NaH_2PO_4) or NaCl (Cl). Liver biopsies were taken 30 and 60 min post-infusion and examined for Pi content and expression of genes involved in the TCA cycle (α-ketoglutarate dehydrogenase; KD), fructolysis (Fructokinase), oxidative phosphorylation (ATP5b) and gluconeogenesis (cytosolic Phosphoenolpyruvate carboxykinase; PEPCK). Ribosomal protein S9 was used as a housekeeping gene. Effects of treatments were analyzed by ANOVA, the fixed effects were F infusion, Pi infusion, period, and their interactions. Cow was included as a random effect. The F+Cl treatment increased DMI in the first 4 h after infusion began by 28–47% (F-Pi interaction, $P = 0.003$; 5.14, 3.50, 4.00 and 3.59 kg for the F+Cl, F+Pi, G+Cl and G+Pi, respectively), demonstrating that Pi infusion prevented the effect of F. Moreover, the G+Pi treatment increased hepatic Pi 30 min into the infusion by 49–89% (F-Pi interaction, $P = 0.07$; 8.19, 10.4, 10.1 and 15.5 μmol/g for the F+Cl, F+Pi, G+Cl and G+Pi, respectively). F also increased hepatic RNA expression of KD 60 min into the infusion by 2.25-fold ($P = 0.01$). Pi tended to increase Fructokinase expression 30 min into the infusion by 2.9-fold ($P = 0.09$). F increasing DMI while Pi preventing the effect of F, support a connection between ATP synthesis and DMI in cows postpartum. The increase in hepatic KD expression may suggest a shift toward ATP producing pathways (e.g., TCA cycle) as a response to a reduction in ATP rather than reducing ATP consuming pathways (e.g., gluconeogenesis). The increase in Fructokinase expression suggest that fructolysis is regulated by the availability of Pi rather than the availability of F. These hypotheses needs to be further examined.

Key Words: fructose, phosphate, DMI

M239 Stability of different rumen-protected lysine products in total mixed rations. T. Sugino*[1], S. Ishimaru[1], M. Nakamura[2], H. Funo[2], and T. Obitsu[1], [1]*The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan,* [2]*Shimane Prefectural Livestock Technology Center, Izumo, Japan.*

This study aimed to evaluate the releasing rate of lysine from 3 rumen-protected lysine products (RPL) during storage in 2 total mixed rations (TMR) diets or water. Three commercial PRL products in Japan (A: AjiPro-L; B: FeedtechTM Bypass Lysine; C: LysiPearlTM) were used. Each RPL (2 g) was mixed with 1 kg each of 2 types of TMR (TMR 1: corn- and grass-silage based TMR as the major forage sources, 52.9% DM content; TMR 2: fermented TMR comprising rice whole crop silage, 58.6% DM content). The treated TMRs were stored in the plastic bags (6 replicates) at 20°C for 0, 1, 3, 6, 12, 24, and 48 h. TMRs without RPL were used as control samples. At each time point, the solubilized free Lys in the treated TMR was extracted with 1 L of ion-exchanged water containing 0.5% Arg as internal standard. Additionally, as a simplified method to evaluate Lys release from RPL, 2 g of RPL was stored in 20 mL ion-exchanged water at 20°C for 0, 1, 3, 6, 12, 24, and 48 h. After filtration (0.45 μm) of the extracted solutions, free Lys content in

the extract was analyzed, and Lys release rate (LR, %) during storage in the TMR or water was calculated. Data were analyzed by ANOVA with mixed model. The results (Table 1) showed that all RPL exhibited increased LR over storage time for both TMRs and water treatments ($P < 0.01$). However, RPL by time interaction was observed ($P < 0.01$), the LR of the products B and C was greater than that of the product A for both TMRs and water treatments. Our results indicate that there is greater lysine loss from some RPLs into the TMR, and a simple LR test with water can be used to evaluate the stability of RPL in TMR.

Table 1 (Abstr. M239). Lysine release (%) of RPL stored in TMR or water with time

	Store time (h)							
Trt/RPL	0	1	3	6	12	24	48	SEM
TMR1								
A	0.8	2.0^{c}	2.4^{c}	3.3^{c}	3.8^{c}	5.1^{b}	7.1^{c}	0.43
B	0.4	7.0^{b}	46.3^{a}	47.0^{a}	53.2^{a}	50.0^{a}	42.9^{b}	0.43
C	2.0	17.7^{a}	28.0^{b}	37.3^{b}	45.0^{b}	51.9^{a}	54.4^{a}	0.43
TMR2								
A	1.4	1.7^{c}	2.9^{c}	2.9^{c}	3.4^{c}	4.2^{b}	6.7^{c}	0.43
B	1.2	9.5^{b}	52.1^{a}	49.4^{a}	51.8^{a}	48.9^{a}	46.6^{b}	0.43
C	2.0	17.4^{a}	27.2^{b}	25.9^{b}	40.4^{b}	46.2^{a}	54.4^{a}	0.43
Water								
A	1.2	2.6 ^{c}	3.7 ^{c}	4.8 ^{c}	6.6 ^{c}	8.9 ^{c}	12.3^{c}	0.39
B	1.0	28.1^{a}	63.7^{a}	67.2^{a}	76.5^{a}	76.9^{a}	74.5^{a}	0.39
C	0.8	11.6^{b}	17.9^{b}	41.9^{b}	56.8^{b}	58.1^{b}	67.8^{b}	0.39

$^{a–c}$Values in the same treatment and time with different superscripts are different ($P < 0.05$).

Key Words: rumen-protected Lys

M240 Use of indirect calorimetry to study energy utilization in lactating Jersey dairy cattle consuming distillers dried grains with solubles or canola meal. M. A. Myers[1], J. V. Judy[1], K. J. Herrick[2], A. L. Knoell*[1], and P. J. Kononoff[1], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*POET Nutrition LLC, Sioux Falls, SD.*

The use of co-products as an alternative feed source is a common practice when formulating dairy rations. A study using 12 multiparous (79 ± 16 DIM; mean ± SD) lactating Jersey cows, was conducted to evaluate the feeding effects of dried distillers grains with solubles (DDGS) or canola meal on energy utilization. A replicated 4 × 4 Latin square design was used to compare 4 different dietary treatments. Treatments were composed of a control (CON) containing no co-products, a treatment diet containing 10% (DM basis) reduced fat DDGS (RFDDGS), 10% high fat DDGS (HFDDGS), and a 10% canola meal (CAN). The crude fat content of the RFDDGS, HFDDGS, and canola meal was 6.05 ± 0.379%, 10.0 ± 0.134%, and 3.46 ± 0.085%. Co-products were included in partial replacement for corn and soybean meal. Indirect headbox-style calorimeters were used to estimate heat production. Daily dry matter intake and milk yield were similar ($P > 0.55$) across treatments averaging 17.5 ± 0.78 kg/d and 24.1 ± 0.80 kg. Heat production per unit of metabolic body weight tended ($P = 0.058$) to be affected by treatment and was lowest for CON and diets containing co-products were similar (192, 200, 215, and 204 ± 5.91 kcal per kg of metabolic body weight for CON, LFDDGS, CAN, and HFDDGS respectively). The ME concentration of the diet was affected ($P = 0.034$) by dietary treatment specifically, HFDDGS did not differ from CON, but was higher than RFDDGS and CAN (2.58, 2.46, 2.29, and 2.27 ± 0.089 Mcal/kg for HFDDGS, CON, RFDDGS and CAN respectively). Lastly, the NEL concentration of the diet tended to be affected ($P = 0.062$) by dietary treatment. Although HFDDGS did not differ from CON and RFDDGS, it was higher than CAN (1.38, 1.36, 1.14, and 1.06 ± 0.111 Mcal/kg for HFDDGS, CON, RFDDGS, and CAN respectively). Results of this study suggest that the energy content of co-products is influenced by the crude fat content and that RFDDGS and canola meal have a similar energy content when consumed by lactating dairy cattle.

Key Words: dairy cow, dried distillers grains with solubles, energy

M241 Palatability of total mixed rations containing 3-nitrooxypropanol for lactating dairy cows. A. Melgar*[1], K. Nedelkov[2], C. M. M. R. Martins[3], K. C. Welter[3], X. Chen[4], M. T. Harper[1], S. Duval[5], and A. N. Hristov[1], [1]*The Pennsylvania State University,, University Park, PA,* [2]*Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria,* [3]*University of Sao Paulo, Pirassununga, Brazil,* [4]*College of Pastoral Agriculture Science and Technology, Lanzhou University, China,* [5]*Research Centre for Animal Nutrition and Health, DSM Nutritional Products, France.*

The objective of this study was to investigate the effect of 3-nitrooxypropanol (3NOP), a methane inhibitor, on palatability of TMR for Holstein dairy cows. Following a 1-wk covariate period, 12 multiparous cows averaging (±SD) 74 ± 22 DIM, 53 ± 12 kg/d milk yield, and 630 ± 146 kg BW were fed a basal TMR containing increasing levels of 3NOP during 5, 6-d periods. The basal TMR (16.5% CP and 39.4% NDF) contained, on DM basis, 39% corn silage, 11%, alfalfa haylage, 4% grass hay, and 46% concentrate feeds and mineral-vitamin premix. Feed bins were split in half by a solid divider and cows simultaneously received the basal TMR supplemented with (1) a placebo (Period 1), or (2) 3NOP included in the TMR at 30, 60, 90, or 120 mg/kg DM (Periods 2–5). 3NOP was incorporated in the ration through a premix containing ground corn grain, soybean oil, and molasses; the inclusion rate of the premix was adjusted according to the targeted 3NOP concentration for each experimental period and DM of the TMR. Cows were fed twice daily: 60% of the daily feed DM allowance at 0800 h and 40% at 1800 h. Feed offered and refused was recorded at each feeding. During the morning feedings, each cow was offered either control or 3NOP-treated TMR at 150% of her normal intake. After collection of the evening refusals, cows received only the basal TMR without the premix until the next morning feeding. Location (left or right) of the control and 3NOP treated TMR within a feed bin was switched every day during each period to avoid feed location bias. Compared with the control (6.93 kg/10 h) DMI was increased ($P < 0.001$) by 3NOP inclusion rates of 30, 60, and 90 mg/kg DM (8.75, 8.78, and 9.35 kg/10 h, respectively). DMI was similar between control and 120 mg/kg 3NOP (7.23 kg/10 h). There was no effect of feed location (right vs left) within feed bin on DMI ($P > 0.17$). There was a treatment × day of TMR offering interaction ($P < 0.001$), which appeared to be due to a slight decrease over time in DMI with the 90 and 120 mg/kg 3NOP inclusion rates. In this short-term experiment, 3NOP did not affect DMI in lactating dairy cows.

Key Words: 3-nitrooxypropanol, palatability, dairy cattle

M242 Exogenous enzymes on performance and rumen function of mid-lactation dairy cows. E. M. de Castro Zilio[1], T. A. Del Valle[1], L. G. Ghizzi[1], M. S. S. Dias[1], A. T. Nunes[1], N. T. S. Grigoletto[1], G. Gomes da Silva[1], T. Barrera de Paula e Silva[1], J. A. Marques[1], D. Graugnard[2], and F. P. Rennó*[1], [1]*Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil,* [2]*Alltech Inc., Nicholasville, KY.*

Addition of fibrolytic and amylolytic enzymes in dairy cow diets aims to increase digestibility of NDF and starch, leading to better performance of the animals. The aim of this study was to evaluate the effect of exogenous fibrolytic (FIBROZYME, Alltech Inc., Nicholasville, KY) and amylolytic (AMAIZE Alltech Inc.) on performance and ruminal fermentation. Eight multiparous Holstein cows 133 ± 44.1 (mean ± SD) DIM, 532 ± 83.2 kg of BW and 31.8 ± 5.70 kg/d of milk blocked according to milk yield were used in a 4 × 4 Latin square experimental design. Treatments were as follows: 1) Control, basal diet without exogenous enzymes; 2) Fibrolytic enzyme, provision of 12 g/d (51 IU of xylanase activity/kg diet DM); 3) Amylolytic enzyme, provision of 8 g/d (203 FAU/kg diet DM); and 4) Both fibrolytic and amylolytic enzymes added at the same rate in individual treatments. Each 21-d experimental period included 14 d adaptation and 7 d sampling. Individual corn silage and orts samples were analyzed for DM, crude protein, ether extract, ash, NDF and starch. Milk yield was recorded and milk samples were analyzed for fat, lactose and crude protein. Ruminal liquid was collected (5 different sites within rumen) on 20th day of each experimental period, before the morning feeding (time 0), and after 2, 4, 6, 8, 10, 12, 14 and 16 h relative to the morning feeding, and was evaluated for pH, ammonium N and SCFA. Data were analyzed using the MIXED of SAS, means were adjusted by the LSMEANS procedure and interaction effect $F_l \times A_m$ were decomposed using Fisher's protected LSD means test. Exogenous enzymes no effect ($P \geq 0.104$) on intake or milk production (kg/d). Amylolytic enzyme decreased ($P = 0.022$) milk protein (kg/d) but had no effects ($P \geq 0.247$) on lactose or fat production. Enzymes showed no effect ($P \geq 0.112$; Table 3) on most ruminal characteristics. Amylolytic enzymes raised butyrate concentration in the absence of fibrolytic enzymes (interaction, $P = 0.043$). In summary, exogenous enzymes did not affect productive performance; however, amylolytic enzymes increased butyrate concentration, without other ruminal alterations.

Key Words: amylolytic, fibrolytic, short-chain fatty acids

M243 Effects of microalgae on intake and milk yield, composition and fatty acids profile of dairy cows. J. A. Marques[1], T. A. Del Valle[1], L. G. Ghizzi[1], M. S. S. Dias[1], A. T. Nunes[1], N. T. S. Grigoletto[1], L. S. Gheller[1], T. Barrera de Paula e Silva[1], E. M. de Castro Zilio[1], G. Gomes da Silva[1], L. Kuritza[2], and F. P. Rennó*[1], [1]*Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil,* [2]*Alltech do Brasil, Araucária, Paraná, Brazil.*

Omega-3 fatty acids intake has been credited with many benefits to animal and human health. However, lipid addition to dairy cow diets may reduce dry matter intake and milk fat. The objective of the study was to evaluate the effects of increasing levels of microalgae rich in docosahexaenoic acid (DHA; All-G Rich, Alltech, Nicholasville, KY) in the diet of dairy cows on feed intake and milk yield, composition, and fatty acids profile. Twenty-four Holstein dairy cows were used in 6 replicated 4 × 4 Latin squares to evaluate the following treatments: basal diet, with no algae addition (A0); 2g (A50), 4 g (A100) or 6 g (A150) /kg of DM. Each experimental period included 14 adaptation days and 7 sampling days. Individual dry matter intake and milk production were recorded daily; milk samples were collected for 3 consecutive days, during the morning and afternoon milking. Data were analyzed using the MIXED procedure of SAS Inst (2001). Dry matter intake and milk fat, protein and lactose content were linearly reduced ($P < 0.001$) with increasing algae, while production efficiency was linearly increased ($P < 0.001$). Milk production quadratically increased ($P = 0.053$) by algae dietary inclusion, with maximal milk production of 29.77 kg on A50. Algae inclusion also quadratically increased ($P \leq 0.041$) 3.5% FCM production and milk fat yield, with maximal increases on A50. DHA was detected in only 4 of 96 samples, at the higher algae doses. The ratio of saturated and unsaturated fatty acids was linearly reduced ($P = 0.021$) with increasing algae, while the proportion of PUFAs was linearly increased ($P < 0.001$). Algae inclusion reduced linearly ($P \leq 0.001$) C18:0, C18:1 *cis*-9, C18:2 *cis*-9,12, and C18:3 *cis*-9,12,15 proportion, and increased linearly ($P < 0.001$) C18:2 *cis*-9, *trans*-11 proportion. Also, algae inclusion increased quadratically ($P \leq 0.044$) C18:1 *trans*-9 and C18:1 *trans*-11 proportion, with the maximal increases on A150. The A50 dietary algae dose may maximize milk production and milk fat yield. Algae dietary inclusion modified milk fatty acids profile; however, there was limited DHA incorporation into milk fat.

Key Words: docosahexaenoic acid (DHA), performance, dairy cow

M244 Intake, digestibility and milk production in mid-lactation dairy cows fed exogenous enzymes. E. M. de Castro Zilio[1], T. A. Del Valle[1], L. G. Ghizzi[1], A. T. Nunes[1], M. S. S. Dias[1], N. T. S. Grigoletto[1], G. Gomes da Silva[1], L. S. Gueller[1], A. Koontz[2], and F. P. Rennó*[1], [1]*Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil,* [2]*Alltech Inc., Nicholasville, KY.*

Lactating cow diets are comprised mostly of carbohydrates, which are not fully fermented by rumen microbes, limiting energy release from feed. The aim of this study was to evaluate exogenous fibrolytic (FIBROZYME Alltech Inc., Nicholasville, KY) and amylolytic (AMAIZE Alltech Inc.) enzymes in mid-lactation dairy cows. Thirty-two multiparous Holstein cows 181.3 ± 35.3 DIM, 571 ± 72.7 kg BW and 29.6 ± 5.24 kg/d of milk yield, blocked according to milk yield were randomly allocated to treatments in a 4 × 4 Latin square design. Treatments were (1) Control (CON), basal diet without exogenous enzymes; (2) Fibrolytic enzyme (FIB), provision of 12 g/d (51 IU of xylanase activity/kg diet DM); (3) Amylolytic enzyme (AMY), provision 8 g/d (203 FAU/kg diet DM); and (4) Both fibrolytic and amylolytic enzymes (FIB+AMY), enzymes added at the same rate as in individual treatments. Each 21-d experimental period included 14 d of adaptation and 7 d of sampling. Individual TMR and orts samples were collected and assessed for particle size distribution. From 17 to 19-d of each period feces were collected every 9 h to determine total-tract digestibility. Feeds, orts, and feces were analyzed for DM, crude protein, ether extract, ash, NDF, and starch. Indigestible NDF was used as internal marker to estimate daily fecal DM excretion. Milk yield was recorded and samples analyzed for fat, lactose, and crude protein. Data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Enzymes addition had no effect ($P \geq 0.111$) on intake. Cows fed amylolytic enzymes selected against large feed particles (>19 mm; $P = 0.031$), and slightly in favor of smaller particles (8 to 19 mm) only in the absence of fibrolytic enzymes (interaction; $P = 0.024$). Enzyme addition showed no effect on digestibility, milk yield, fat-corrected milk, or milk fat concentration. Either enzyme product alone reduced daily milk lactose and protein production (interaction, $P \leq 0.053$). In summary, addition of exogenous enzymes had no effect on performance, intake, or digestibility but modified selection of feed particle sizes, lactose and protein production.

Key Words: amylase, carbohydrate, xylanase

M245 Supplementing aluminosilicate clay on the reduction of aflatoxin M_1 in milk and biomarkers of liver function in dairy cows. E. H. Branstad*[1], C. S. McCarthy[1], B. C. Dooley[1], S. M. Rous[1], C. Domenech[2], J. Pie[2], G. E. Rottinghaus[3], E. Bowers[1], L. H.

Baumgard[1], and H. A. Ramirez-Ramirez[1], [1]*Iowa State University, Ames, IA,* [2]*Biovet S.A., Tarragona, Spain,* [3]*University of Missouri, Columbia, MO.*

Aflatoxin M_1 (AFM_1) is harmful metabolite that is transferred to milk; therefore, effective mitigation strategies are needed to reduce exposure. Consequently, the objectives were to evaluate an aluminosilicate clay, Alquerfeed Antitox, as a sequestering agent in dairy diets contaminated with aflatoxin (AF), and to determine liver enzyme response to an AF challenge. Twelve primiparous Holstein cows (279 ± 87.8 DIM and 632 ± 51 kg BW) were used in replicated 3 × 3 Latin squares with 21-d periods (d 15–21 were considered for data collection). Treatments were: 1) Control (CON), consisting of a basal TMR; 2) aflatoxin diet (AF), consisting of CON + AF challenge (100 µg of AFB_1/kg DM); and 3) AF + aluminosilicate clay (AF+C), consisting of AF + clay at 0.10% dietary DM. Feed intake and milk yield were recorded daily, blood samples were collected on d 21 of each period, and milk samples were collected on the last 2 d of each period. Data were analyzed using the MIXED procedure of SAS with square, period within square and treatment as fixed effects and cow within square as a random effect. Milk yield and DMI were similar ($P \geq 0.25$) across treatments averaging 26.8 ± 1.34 kg/d and 24.0 ± 0.92 kg/d, respectively; milk composition was also similar across treatments ($P \geq 0.18$). Compared with CON, addition of AF and AF+C had no effect on blood concentration of alanine aminotransferase (61.1 ± 3.54 IU/L; $P = 0.93$), aspartate aminotransferase (72.6 ± 5.46 IU/L; $P = 0.26$), and γ-glutamyltransferase (77.4 ± 6.98 IU/L; $P = 0.34$). No AFM_1 was detected in CON cows; addition of clay reduced ($P \leq 0.01$) concentration of AFM_1 in milk from 1.57 to 1.14 ± 0.10 µg/L for AF and AF+C, respectively. Excretion of AFM_1 was reduced ($P \leq 0.01$) by 11.64 ± 4.36 µg/d, transfer was also reduced with AF+C ($P \leq 0.01$; 1.65 *vs* 1.19 ± 0.13%) compared with the AF diet. Lack of response on liver enzymes after prolonged exposure suggests that evaluating liver function earlier in the challenge phase may be a better approach. These results demonstrate that the inclusion of aluminosilicate clay reduces absorption and transfer of dietary aflatoxin to milk.

Key Words: binder, mycotoxin, liver enzymes

M246 Effects of molasses and corn grain at 2 levels of ruminally degradable protein on lactating cow ruminal fermentation and rumen content mass. G. I. Zanton* and M. B. Hall, *USDA-Agricultural Research Service; Dairy Forage Research Center, Madison, WI.*

The objective of this study was to evaluate lactating dairy cow ruminal fermentation and rumen content mass as affected by diets containing molasses (M) or finely ground dry corn grain at 3 levels of M (0, 5.25, 10.5% DM) and with differing levels of ruminally degradable protein (+RDP or –RDP). Twelve ruminally cannulated, multiparous Holstein cows (initial mean ± SD: parity 2.25 ± 0.62; 185 ± 56 DIM; 41.3 ± 6.3 kg milk) were allocated to treatment sequences in a split-plot, replicated 3x3 Latin square design with a 2x3 factorial arrangement of treatments. Experimental diets were individually fed to cows for ad libitum consumption and formulated to contain 35% corn silage and 20% alfalfa silage with +RDP formulated with a greater concentration of solvent soybean meal (SBM) and expellers SBM replacing a portion of solvent SBM in -RDP. Each period lasted 28 d; on d 21 and 28 of each period, rumen contents of all cows were evacuated, subsampled, and replaced beginning 6 h after feeding. On d 25–28 of each period, samples of rumen contents were taken at 4 or 6 h intervals to represent every 2 h. Data were analyzed in SAS with significance was declared at $P < 0.05$ and tendencies at $0.05 < P \leq 0.10$. Dry matter intake and production responses were not different among diets. Ruminal pH, organic acid concentration, and ammonia concentration were not affected by diet and followed similar temporal patterns. Ruminal acetate molar percent decreased linearly and butyrate molar percent increased linearly with increasing levels of M. Ruminal free AA concentration was greater for +RDP and branched chain VFA concentration declined linearly with increasing M. Rumen content mass was greater for –RDP, which was due to greater quantities of rumen liquid. Ruminal acetate pool size was greater for -RDP and butyrate pool size increased linearly with M. Lactate pool size tended to be higher with +RDP. Under the conditions of this experiment, ruminal fermentation or rumen content mass were affected by level of M or RDP individually, but these diet characteristics were not detected to interact with each other.

Key Words: molasses, protein degradability, rumen fermentation

M247 Effects of molasses and corn grain at 2 levels of ruminally degradable protein on lactating cow performance. M. B. Hall* and G. I. Zanton, *U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

The objective of this study was to compare responses of 59 lactating Holstein cows to molasses (M) vs. dry corn grain (CG) at 3 levels of M (0, 5.25, 10.5% of dry matter; DM) and 2 levels of ruminally degradable protein (+RDP or -RDP) in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Cows were individually fed a common diet during a 2 wk covariate period followed by 8 wk on experimental diets. Diets were formulated to be isonitrogenous and provide similar amounts of starch+water-soluble carbohydrates (ST+WSC). All experimental diets contained 35% corn silage, 20% alfalfa silage and 16.6% crude protein (DM basis); the 0, 5.25, and 10.5% M diets respectively contained, 19, 14.5, and 10% CG, 28, 25, and 22% ST, and 5.5, 8.5, and 11.5% WSC. Linear M effects were evaluated with contrasts. Significance was declared at $P < 0.05$ and tendencies at $0.05 < P < 0.10$. Table 1 contains least squares means and standard errors of the difference (SED). DM intake, and production of milk, milk protein, and 3.5% fat- and protein-corrected milk (FPCM) declined linearly with increasing M, and there was a tendency for N efficiency (milk N/intake N; MN/IN) to do so. Differences among diets were not detected for milk fat production and FPCM/DM intake. No RDP or interaction effects were detected. On a DM basis, M and CG respectively contain 17.0 and 1.6% ash, approximately 60.4 and 80.4% ST+WSC as free monosaccharides, and 0 and 10% NDF. That milk production efficiency did not differ between M and CG suggests that DM intake was a primary driver of performance. The similar efficiencies observed would not have been predicted based on M and CG composition but may relate to differences in fermentation rates, digestion products, or sites of digestion.

Table 1 (Abstr. M247).

	+RDP			−RDP			
Diet	0M	5.25M	10.5M	0M	5.25M	10.5M	SED
DMI, kg	29.0	27.9	26.7	28.8	27.8	28.0	1.09
Milk, kg	47.7	44.8	42.3	48.3	44.7	44.2	1.81
Fat, kg	1.59	1.68	1.65	1.69	1.57	1.57	0.107
Protein, kg	1.51	1.38	1.36	1.49	1.37	1.32	0.054
FPCM, kg	46.5	45.8	44.7	47.9	44.3	43.8	2.07
FPCM/DMI	1.62	1.65	1.68	1.67	1.61	1.59	0.080
MN/IN	0.310	0.292	0.302	0.308	0.294	0.283	0.013

Key Words: molasses, milk production, sugar

M248 The effect of dietary supplementation of yeast culture on the lactation performance of crossbred dairy cattle. E. M. Jimenez*[1], L. A. Kinman[1], W. B. Smith[1], S. A. Armstrong[3], and B. W. Jones[1,2], [1]*Department of Animal Science and Veterinary Technology, Tarleton State University, Stephenville, TX,* [2]*Texas A&M AgriLife Research, Stephenville, TX,* [3]*Phibro Animal Health, Teaneck, NJ.*

The objective of this study, conducted at the Southwest Regional Dairy Center, was to evaluate the effect of dietary supplementation of 2 different yeast culture concentrations on lactation performance of dairy cows. Multiparous crossbred cattle (n = 46) averaging 55 d in milk (DIM) at the start of a 14 d adaptation period were used in a randomized complete block design with a 42 d treatment period. Cows were balanced by parity, DIM, and milk yield (MY) and randomly assigned 1 of 3 dietary treatments: (1) Treatment A, 56 g of Cellerate Culture Classic (Phibro Animal Health Corp., Teaneck, NJ), plus a basal total mix ration (TMR) and 58 g of ground corn (A + TMR); (2) Treatment B, 14 g of a concentrated dried yeast product (not commercially sold; Phibro Animal Health Corp.) plus a basal TMR and 100 g of ground corn (B + TMR); and (3) Control (CON) consisting of a basal TMR plus 114 g of ground corn. Cows were housed in a free-stall barn equipped with a Calan Broadbent feeding system (American Calan, Inc., Northwood, NH) to allow for individual feedings daily. Milk yield and dry matter intake (DMI) were recorded daily and averaged/wk. Milk samples and TMR were collected for 3 consecutive d/wk and analyzed for components and DMI. The MIXED procedure of SAS (Cary, NC,) was used to evaluate fixed effects of period, treatment, and period × treatment with cow as random effect on MY (kg/d) and DMI (kg/d). Stepwise backward elimination was used to remove non-significant interactions ($P \geq 0.10$). All main effects were kept in the model regardless of significance. No statistically significant differences ($P \geq 0.10$) were observed for any of the 3 treatments on MY or DMI. Milk yield for A + TMR, B + TMR, and CON were, 43.76 ± 0.54, 43.09 ± 0.56, and 44.04 ± 0.55 kg/d, respectively. Dry matter intake for A + TMR, B + TMR, and CON 27.91 ± 0.47, 26.76 ± 0.48, and 27.98 ± 0.48 kg/d, respectively. This data suggests that supplementing cattle with 2 different concentrated yeast cultures shows no differences on MY and DMI.

Key Words: dry matter intake, milk production, yeast culture

M249 Effect of betaine supplementation on total-tract digestibility and production performance in mid-lactating Holstein dairy cows. H.-C. Hung*[1], C.-Y. Tsai[1], G. Chibisa[1], M. Chahine[1,2], M. McGuire[1], and P. Rezamand[1], [1]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*Twin Falls Research and Extension Center, Twin Falls, ID.*

Betaine, also called trimethylglycine, is either produced endogenously by choline oxidation or found naturally in wheat or sugar beets. We hypothesized that betaine supplementation improves the total-tract digestibility and production performance in mid-lactation dairy cows. There were 20 mid-lactation dairy cows assigned to a 3 × 3 Latin square design with 3 periods of 28 d each and 3 treatments of betaine (0, 100, and 200 g/d). Milk yield and feed intake for each cow was recorded daily. During d 21–28, cows were fed with chromic oxide (15 g/d per cow). On d 26–28, fecal samples were collected and analyzed for digestibility via chromic oxide concentration analysis by inductively coupled plasma – atomic emission spectrometry (ICP-AES). Milk samples were collected on d 21 and d 28 for components analysis and fatty acids profile via gas chromatography. Data were analyzed using the Proc Mixed of SAS with significance declared at $P \leq 0.05$ and trends at $P < 0.1$. Apparent total-tract digestibility tended to increase by supplementing 100 g betaine as compared with that with no dietary betaine (0.61 vs 0.58 ± 0.01; $P = 0.1$). Milk fat percent (3.16 vs 3.36 ± 0.08%, for 0 g betaine/d and 200 g betaine/d, respectively) and C20:1 (0.005 vs 0.004 ± 0.0006%, for 100 g betaine/d and 200 g betaine/d, respectively) tended to differ among treatments ($P = 0.1$ for both). Results showed however that DMI (25.4, 25.4, 25.4 ± 0.11 kg/d), milk yield (29.7, 29.3, and 30.0 ± 0.7 kg/d), and energy-corrected milk yield (28.5, 28.1, and 29.0 ± 0.8 kg/d) did not differ among treatments (0, 100, and 200g betaine/d per cow, respectively.) Overall, the result showed that betaine supplementation slightly affected the total-tract digestibility and milk fat of mid-lactation dairy cows whereas no major effect was observed in production measures tested.

Key Words: betaine, digestibility, milk component

M250 Physical characterization of palmitic acid supplements of varying enrichment. R. Shepardson*, E. Bazilevskaya, and K. Harvatine, *Penn State University, University Park, PA.*

Fatty acid (FA) supplements containing palmitic acid (PA) are often included in lactating cow diets to increase diet energy. Previous publications have shown that PA supplements with moderate enrichment (~85%) have a higher digestibility compared with those that are very highly enriched (≥98%). Saturated FA have the potential to form organized secondary structures, which may attribute to their change in digestibility. Differential scanning calorimetry (DSC) is a thermal technique used to analyze changes in heat flow as energy is absorbed or released from a sample during heating. In the current project, DSC was used to determine melting temperature (MT) and enthalpy, a measure of energy flow during melting. Our hypothesis was that MT and enthalpy would increase as the enrichment of PA supplements increased. Supplements containing approximately 55, 80, 95, 98, and 99% C16:0 were analyzed using a Q600 STD (TA Instruments). Briefly, a ~40-mg sample was heated at 5°C/min from 25°C to 110°C. Instrument software (TA Universal Analysis, TA Instruments) was used to determine MT and total enthalpy of melting (area under the curve). Treatment means were compared with a Protected LSD Test in a model that included the fixed effect of PA enrichment. Melting temperature for the 55, 80, 95, 98, and 99% categories was 60.9, 65.6, 66.5, 67.3, and 68.1°C, respectively. These supplements also had enthalpy values of 196.0, 199.8, 217.4, 221.7, and 218.8 mW, respectively. The MT of the 55% category was lower than all others, but there was no difference between 80 and 95% or 98 and 99% ($P < 0.0001$). Both the 55 and 80% decreased enthalpy compared with the 95, 98, and 99% ($P < 0.0001$), but was not different between the 3 most enriched supplements. This data indicates large differences in physical characteristics of fat supplements differing in PA enrichment, but only minimal differences between those exceeding 95% enrichment in PA. These physical characteristics may explain differences in digestibility, although more work is needed to understand this relationship.

Key Words: fatty acid supplement, melting point, enthalpy

M251 Performance and metabolism of multiparous Holstein dairy cows as affected by corn silage type and supplementation with monensin throughout the transition period. S. E. LaCount*, C. M. Ryan, M. E. Van Amburgh, and T. R. Overton, *Cornell University, Ithaca, NY.*

The objective of this study was to evaluate the effects of corn silage (CS) type and monensin supplementation throughout the transition period on metabolism and performance. Multiparous Holstein cows (n = 85) were enrolled 28 d before expected parturition and randomly assigned to treatments in 2 × 2 factorial arrangement with diets containing con-

ventional (CON) or brown midrib (BMR) CS, with monensin (M; 330 mg/d prepartum and 450 mg/d postpartum) or without M (NO; 0 mg/d). Diets were formulated to be identical except for CS type and small inclusion mix delivering M or NO. Prepartum diets, CON (41.6% NDF, 17.8% starch, 94.3 g/kg DM MP) and BMR (40.8% NDF, 17.3% starch, 95.9 g/kg DM MP), were fed from −21 d through calving. Postpartum diets, CON (30.3% NDF, 27.8% starch, 116.6 g/kg DM MP) and BMR (29.2% NDF, 27.7% starch, 118.1 g/kg DM MP), were fed from calving through 42 d in milk. Blood samples were taken 1×/wk prepartum, 2×/wk for wk 1 and 2, and 1×/wk through wk 6 postpartum. Repeated measures data were analyzed using PROC MIXED in SAS with main effects of treatments and time (T), and all interactions. Prepartum and postpartum data were analyzed separately. Prepartum dry matter intake (DMI) was higher for cows fed BMR than CON (14.7 vs. 14.0 kg/d; $P = 0.03$), whereas cows fed M had lower intake than cows fed NO (13.9 vs. 14.8 kg/d; $P < 0.01$). Postpartum DMI was not different. A 3-way interaction of CS, M, and T was observed for milk yield ($P = 0.02$) where BMR-M was highest and CON-NO lowest during wk 5 and 6 postpartum. A CS × T interaction was observed such that NEFA in wk −2 and −1 ($P < 0.02$) and BHBA in wk 5 and 6 postpartum ($P = 0.01$) were lower for cows fed BMR than those fed CON. Cows fed M had lower BHBA than cows fed NO both prepartum ($P = 0.04$) and postpartum ($P = 0.01$). Postpartum, cows fed BMR had lower NEFA than cows fed CON ($P = 0.01$). Cows fed BMR and cows fed M throughout the transition period exhibited improvements in performance and metabolism in the prepartum period, with a possible synergistic effect of CS and M supplementation on milk yield.

Key Words: transition cow, energy metabolism, monensin

M252 Evaluation of eight prototypes of rumen-protected lysine on performance of lactating Holstein cows. M. I. Rivelli*[1], M. J. Cecava[2], P. H. Doane[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*ADM Research Division, Decatur, IL.*

The objective of this study was to determine the short-term effects of targeted rumen-protected and postruminal amino acid supplementation to dairy cows on protein and amino acids in blood; and production of milk and milk components. Eight prototypes (treatment A to H) were tested in 4 different experiments. In each experiment, 2 prototypes were tested at the same time. Treatments were CON, cows were fed a control diet + ground corn without a Lys source; AJP cows were fed a control diet + a commercially available rumen-protected Lys source; and A-H cows were fed a control diet + a not commercially available rumen-protected Lys source. Treatments AJP and A-H were formulated to provide 112% of the cow's lysine requirements while cows in CON received 94% of the cow's lysine requirements. Treatments were delivered twice a day (12 h-intervals) via 28-mL gelatin and administered orally via balling gun. Cows were assigned to 1 of 4 treatments in a replicated 4 × 4 Latin Square Design with experimental periods 7 d in length. Total length of the experiment for 2 prototypes was 28 d. Periods (7d) were divided in washout phase (d 1, no treatment was delivered), adaptation phase (d 2 to 4), in which treatments were delivered in gelatin capsules, and phase for statistical inferences (d 5 to 7) in which treatments were also delivered in gelatin capsules. Data were analyzed using the MIXED procedure of SAS. Three treatment orthogonal contrasts were used. Contrast 1 (CONT1): CON compared with AJP, Contrast 2 (CONT2): AJP compared with A (C, E, and G in each round respectively); and Contrast 3 (CONT3): AJP compared with B (D, F, and H in each round respectively). There were no treatment differences for either contrasts (CONT1 or CONT2) for DMI, BW, or BCS ($P > 0.10$). Milk protein yield was higher for cows in B (1.21 ± 0.1 kg/d) than AJP (1.14 ± 0.06 kg/d, $P = 0.02$; CONT3).Cows in B (16.15 ± 0.5mg/dL) had lower MUN concentration compared with cows in AJP (17.2 ± 0.6mg/dL, $P = 0.05$, CONT3). However, there were no treatment differences for either CONT2 or CONT3, and MUN concentrations were lower for cows in both C and D than AJP ($P > 0.10$). In conclusion, cows in B performed similarly than cows in AJP and seem to have lower protein breakdown than cows in AJP.

Key Words: rumen-protected lysine, MUN, milk protein

M253 Effects of a novel rumen-undegradable protein source on nitrogen utilization in lactating Holstein cows. M. I. Rivelli*[1], M. J. Cecava[2], P. H. Doane[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*ADM Research, Decatur, IL.*

The objective of this study was to determine the effects of a novel RUP source (ADM) on nutrient digestion and performance by Holstein cows. A total of 18 Holstein cows [BW (653.0 ± 96.5 kg); DIM (123 ± 50 d)] were assigned to 1 of 3 treatments in a replicated 3 × 3 Latin Square Design with experimental periods 28 d in length. Periods were divided into an adaptation phase (d 1 to 21) and measurement phase (d 22 to 28). Treatments were CON, cows were fed a control diet (16% CP, 70% RDP, and 30% RUP); AMP, cows fed a diet with RUP source 1 (16% CP, 60% RDP, and 40% RUP); and ADM, cows fed a diet with RUP source 2 (16% CP, 60% RDP, and 40% RUP). Fecal grab samples were collected on d 25 to 27 of each period so that every 3 h in a 24-h period were represented (n = 8). Total mixed rations, orts, and fecal samples were composited by cow within each period and analyzed for DM, OM, CP, aNDF. Indigestible NDF was used as an internal marker. Spot urine samples were obtained approximately 6 h pre-feeding and 6 h post-feeding on d 20 of each period. Daily urinary volume and excretion of urea N, total N, and allantoin were estimated from urinary creatinine concentration assuming a creatinine excretion rate of 29 mg/kg of BW. Data were analyzed using the MIXED procedure of SAS. Contrast 1 (CONT1): CON compared with ADM and Contrast 2 (CONT2): AMP compared with ADM. Nitrogen intake and milk protein N was greater for cows in CON (639.4 ± 24.1 and 178.5 ± 5.0 g/d, respectively) than cows in AMP (576.2 ± 24.1 and 168.5 ± 5.0 g/d, respectively $P = 0.01$, CONT1). However, milk protein N as a percentage of N intake tended to be greater for cows in AMP than cows in CON (30.05 and 28.50 ± 1.12%, respectively, $P = 0.07$, CONT1). Dry matter intake was greater for cows in CON than cows in AMP (22.51 and 20.31 ± 0.90 kg/d, respectively, $P = 0.01$, CONT1). Crude protein intake was greater for cows in CON than cows in ADM (3.99 and 3.55 ± 0.15 kg/d, respectively, $P = 0.01$, CONT1). There were no treatment differences for CONT2 for DMI, N intake, protein N milk protein N as a proportion of N intake, DMI, or CP intake ($P > 0.10$). In conclusion, cows fed ADM novel RUP exhibited better N utilization than CON, thereby reducing environmental impact.

Key Words: rumen-undegradable protein, nitrogen utilization, milk protein

M254 Effects of eight rumen-protected lysine prototypes on plasma amino acids concentrations in lactating Holstein cows. M. I. Rivelli*[1], M. J. Cecava[2], P. H. Doane[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*ADM Research Division, Decatur, IL.*

The objective of this study was to determine the short-term effects of targeted rumen-protected and postruminal amino acid supplementation to dairy cows on protein and amino acids in blood; and production of milk and milk components. Eight prototypes (treatment A to H) were tested in 4 different experiments. In each experiment, 2 prototypes were

tested at the same time. Treatments were CON, cows were fed a control diet + ground corn without a Lys source; AJP cows were fed a control diet + a commercially available rumen-protected Lys source; and A-H cows were fed a control diet + a not commercially available rumen-protected Lys source. Treatments AJP and A-H were formulated to provide 112% of the cow's lysine requirements while cows in CON received 94% of the cow's lysine requirements. Treatments were delivered twice a day (12-h intervals) via 28 mL of gelatin and administered orally via balling gun. Cows were assigned to 1 of 4 treatments in a replicated 4 × 4 Latin square design with experimental periods 7 d in length. Total length of the experiment for 2 prototypes was 28 d. Periods (7 d) were divided in washout phase (d 1, no treatment was delivered), adaptation phase (d 2 to 4), in which treatments were delivered in gelatin capsules, and phase for statistical inferences (d 5 to 7) in which treatments were also delivered in gelatin capsules. Data were analyzed using the MIXED procedure of SAS. Contrast 1 (CONT1): CON compared with AJP, contrast 2 (CONT2): AJP compared with A (C, E, and G in each round respectively); and contrast 3 (CONT3): AJP compared with B (D, F, and H in each round respectively). Four blood samples were collected at 2-h intervals following the morning treatment delivery from each cow on the last 3 d of the covariate period and the last 3 d of each experimental period. Overall, there were a tendency for cows in AJP to have higher plasma lysine concentrations than cows in CON (93.56 and 85.86 ± 3.42 μmol/L, respectively, $0.05 < P \leq 0.11$, CONT1). There were no treatment differences for either contrasts (CONT2 or CONT3) for plasma lysine concentrations (93.56, 86.77, and 90.12 ± 3.42, respectively, $P > 0.10$). In conclusion, cows in A-H showed similar plasma lysine concentrations than cows in AJP.

Key Words: rumen-protected lysine, plasma lysine

M255 In vitro ruminal dry matter degradability and volatile fatty acid and gas production of DDGS with varying fat content. K. C. Krogstad[1], J. L. Anderson*[1], J. S. Osorio[1], and K. J. Herrick[2], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*POET Nutrition, Sioux Fall, SD.*

Our objective was to compare dry matter degradation (DMD), volatile fatty acid (VFA) production, and gas production among dried distillers grains with solubles (DDGS) with varying fat contents. We hypothesized that DDGS with greater fat content would decrease fermentation and impact VFA and gas production. Three 24-h in vitro trials were conducted on different days with the Ankom Technology in vitro bottle system using rumen fluid pooled from 3 cannulated lactating dairy cows. Six DDGS samples (DG1 to DG6) were tested from different sources that varied in fat content. A soybean meal (SBM) sample was included as a control. Samples were incubated in duplicate F57 fiber filter bags within each bottle and bottles were run in triplicate for each feed within a trial. Gas pressure was measured every 10 min by sensors in the bottle caps. Fluid samples were collected for VFA analysis at 24 h from each bottle. Data were analyzed using MIXED procedures of SAS 9.4 and means were compared using PDIFF. Results are shown in Table 1. The 24-h DMD was least in DG2 and DG3, which had the greatest fat contents. Total VFA, acetate, and butyrate production were similar among DDGS, which were all less than SBM. Propionate production did vary among DDGS and DG3 with the highest fat had the least propionate production. All DDGS had less gas production than SBM and it varied among DDGS but did not appear to correspond with fat. Overall, the fat content in DDGS affects DMD and propionate production. Difference sources of DDGS had different gas production, but it was not attributable to fat content.

Key Words: distillers grains, fat, digestibility

M256 Linearity of response of plasma sulfur amino acids in lactating dairy cows to abomasally infused DL-2-hydroxy-4-methylthiobutanoic acid. N. L. Whitehouse*[1], C. G. Schwab[1,2], and S. M. Fredin[2], [1]*University of New Hampshire, Durham, NH,* [2]*Schwab Consulting LLC, Boscobel, WI,* [3]*Adisseo, Alpharetta, GA.*

The plasma free AA dose-response method has been used to determine the relative bioavailability of AA in encapsulated rumen-protected AA supplements The objectives of this experiment were to determine which of the sulfur-containing plasma metabolites would respond in a positive linear fashion to increasing amounts of absorbed DL-2-hydroxy-4-methylthiobutanoic acid (HMTBa) and which expressions of metabolite concentrations would provide the greatest precision of measurement. Five mid-lactatation multiparous Holstein cows, equipped with ruminal cannulas and fed a Met-deficient diet, were assigned to a 5 × 5 Latin square with 7-d experimental periods. Treatments were daily abomasal infusions of 0, 6, 12, 18 and 24 g/d of HMTBa. Blood samples were taken from the tail vein the last 3 d of each period at 2, 4, 6 and 8 h after the morning feeding, cooled, and then centrifuged. One sample of plasma was deproteinized for AA analysis and one sample of plasma was retained for HMTBa analysis. Daily composite samples from the 4 sampling times for each cow were stored at −80°C for later analysis. Production and plasma data were analyzed using the PROC MIXED and PROC REG procedures of SAS 9.4. Statistical significance was declared at $P \leq 0.05$. Intake of DM and milk yield were not affected by treatments. Increasing amounts of HMTBa resulted in linear increases

Table 1 (Abstr. M255). Production parameters of dried distillers grains with solubles (DDGS) with varying fat contents compared with soybean meal (SBM) as control

Item	DG1	DG2	DG3	DG4	DG5	DG6	SBM	SEM
DM, %	92.1	93.2	92.9	91.9	91.5	92.0	90.4	
CP, % DM	30.9	30.0	28.7	31.2	29.9	32.0	22.2	-
Ether extract, % DM	7.62	9.52	10.48	5.63	5.79	4.97	0.85	-
24-h DMD, %	50.3^{b}	44.1^{d}	39.9^{e}	47.5^{c}	47.8^{c}	48.4^{bc}	61.4^{a}	0.84
Acetate, mmol/g	5.00^{b}	4.31^{b}	4.78^{b}	5.83^{b}	6.41^{b}	6.56^{b}	10.59^{a}	0.82
Propionate, mmol/g	5.76^{ab}	5.20^{ab}	4.89^{b}	5.90^{ab}	6.33^{a}	5.96^{ab}	7.05^{a}	0.49
Butyrate, mmol/g	1.26^{b}	1.13^{b}	1.06^{b}	1.34^{b}	1.35^{b}	1.42^{b}	2.31^{a}	0.15
Total VFA, mmol/g	12.7^{b}	11.0^{b}	11.2^{b}	13.8^{b}	14.8^{b}	14.7^{b}	21.7^{a}	1.58
Gas production, mL/g	64.5^{b}	57.7^{b}	54.5^{bc}	45.6^{c}	54.8^{bc}	65.9^{b}	83.6^{a}	10.44

$^{a–e}$Values with unlike superscripts differ by $P < 0.05$ using pdiff.

Table 1 (Abstr. M257). Regression equations in cows abomasallyinfused with incremental amounts of His

Iteration	Equation	SE	CI	CV	R^2
His, μ*M*	Y = 0.6437x + 55.73	0.103	0.2011	3.07	0.93
His, %TAA	Y = 0.0249x + 2.35	0.003	0.0053	1.92	0.97
His, %TAA-His	Y = 0.0263x + 2.41	0.003	0.0049	1.88	0.97
His+Car+3MHis, μ*M*	Y = 0.6674x + 79.68	0.117	0.2295	2.53	0.92
His+Car+3MHis, %TAA	Y = 0.0255x + 3.36	0.002	0.0042	1.36	0.97
His+Car+3MHis, %TAA − His-Car-3MHis	Y = 0.0276x + 3.48	0.002	0.0038	1.30	0.97

in milk protein concentrations ($P = 0.03$) and plasma concentrations of HMTBa, Met, cystathionine/allocystathionine, total S-AA plus HMTBa ($P < 0.001$) and total S-AA ($P = 0.009$). As indicated by lower CV and higher r^2 values, precision of the described linear relationships for plasma metabolites were improved by expressing their concentrations as percentages of total AA. There were no effects of treatments on other plasma AA. We conclude the plasma free AA dose-response method can be used in future experiments to determine the relative Met bioavailability of Met analogs fed to lactating dairy cows.

Key Words: amino acids, methionine, DL-2-hydroxy-4-methylthiobutanoic acid (HMTBa)

M257 Linear relationships between abomasal infusions of histidine and plasma histidine and histidine metabolites. N. L. Whitehouse*[1], Y. Zang[1], B. L. Basiel[1], A. F. Brito[1], and M. Miura[2], [1]*University of New Hampshire, Durham, NH,* [2]*Ajinomoto Co. Inc, Kawasaki-shi, Japan.*

There is limited research regarding the response of circulating His to post-ruminal His infusion. Histidine is unique in the sense that cows can rely on endogenous pools of His (anserine, carnosine, hemoglobin) to meet requirements. The objective of this study was to investigate whether abomasal infusion of incremental amounts of His would result in a linear response in plasma His concentration. Five multiparous lactating Holstein cows (149 ± 92 DIM) fitted with ruminal cannulas were used in a replicated 5 × 5 Latin squares with 7-d experimental periods. All the cows were fed a basal diet to provide adequate energy and metabolizable protein (NRC, 2001). Treatments were 0, 6, 12, 18, and 24 g/d of abomasally infused His. Blood samples were collected at 2, 4, 6, and 8 h after the morning feeding on the last 3 d of each period and composited by day. Plasma AA were quantified using a ultra performance liquid chromatography tandem mass spectrometer. Relationships between plasma His and His infused were described by the PROC REG procedure of SAS (Table 1). Incremental amounts of His infused increased plasma His and the sum of plasma His + carnosine + 3-methly His ($R^2 = 0.93$ and 0.92, respectively). However, plasma His expressed as the percentage of total AA or total AA minus plasma His improved the linear relationship ($R^2 = 0.97$ and 0.97, respectively). Data from this study showed a strong linear response between abomasal infusion of His and plasma His concentration, particularly when His was expressed as proportion of total AA (TAA) or TAA minus His metabolites.

Key Words: abomasal infusion, dairy cow, histidine

M258 Influence of rumen-protected amino acids supplementation pre- and postpartum on lactation performance by dairy cows. E. M. Paula*[1], L. F. Ferraretto[1], C. S. Ballard[2], C. J. Sniffen[3], I. Shinzato[4], and T. Takagi[4], [1]*University of Florida, Gainesville, FL,* [2]*The William H. Miner Agricultural Research Institute, Chazy, NY,* [3]*Fencrest LLC, Holderness, NH,* [4]*Ajinomoto Heartland Inc, Chicago, IL.*

The objective of this study was to evaluate the effect of rumen-protected lysine and methionine supplementation during the transition period on lactation performance by dairy cows. A meta-analysis was conducted with a data set comprised of 20 unpublished feeding trials; trials were designed as continuous lactation trials and evaluated the effects of lysine or lysine/methionine supplementation in dairy cows. For all comparisons, data were analyzed using Proc Mixed of SAS with treatments as fixed effects and trial as a random effect with treatments weighted according to the number of experimental units reported in each trial. Our first comparison evaluated the effects rumen-protected lysine and/or methionine supplementation in pre- and postpartum (PREPOS), or only postpartum (POS) compared with cows fed a basal diet (CON). Cows supplemented with rumen-protected amino acid PREPOS produced 0.9 and 1.0 kg/d more ($P < 0.01$) milk and ECM, respectively, than cows supplemented POS or CON. Milk fat yield was greater for cows supplemented PREPOS compared with CON ($P < 0.01$; 1.59 vs. 1.55 kg/d). Milk protein yield was greater for cows supplemented PREPOS and POS compared with CON ($P < 0.01$; 1.21 vs. 1.18 kg/d). Our second comparison evaluated the effects of supplementation pre- and postpartum of rumen-protected lysine and methionine (RPLM), or rumen-protected lysine in combination with methionine from dietary ingredients (RPLDM) compared with cows fed a basal diet (CONT). Cows supplemented RPLM produced 1.6 and 2.2 kg/d more ($P < 0.01$) milk and ECM than cows supplemented with RPLDM or CONT. Milk fat yield was greater for cows supplemented RPLM compared with RPLDM and CON ($P < 0.01$; 1.60 vs. 1.51 kg/d). Milk protein yield was greatest for cows supplemented with RPLM, intermediate for RPLDM, and lowest for CONT ($P < 0.01$; 1.22, 1.18, and 1.15 kg/d, respectively). These results indicate that supplementation with rumen-protected lysine and methionine throughout the entire transition period enhances lactation performance by dairy cows.

Key Words: lysine/methionine, transition period, milk production

M259 Methionine and choline supply alter transmethylation, transulfuration, and CDP-choline pathways to different extents in primary dairy cow hepatocytes. Z. Zhou*[1,2], Y. Zhou[1,3], and J. J. Loor[1], [1]*University of Illinois, Urbana-Champaign, Urbana, IL,* [2]*Clemson University, Clemson, SC,* [3]*Huazhong Agricultural University, Wuhan, Hubei, China.*

Insufficient peripartal supply of methionine (Met) and choline (Chol) could compromise hepatic metabolism in dairy cows. The objectives of this study were to isolate primary dairy cow hepatocytes to examine transmethylation, transsulfuration, and CDP-choline pathways in response to Met or Chol. Hepatocytes were isolated from mid-lactation

multiparous Holstein cows from liver biopsy and maintained in monolayer culture. Purity of isolated hepatocytes were determined using flow cytometry with cytokeratin 18 as marker for hepatocytes. Hepatocytes were treated with control (Met and Chol free), Met (40 μM) or Chol (80 mg/dL) for 24 h in triplicates before harvested for gene and protein abundance analyses using qPCR and Western blot. Data were analyzed with PROC MIXED of SAS 9.4 with fixed effect of treatment and random effect of wells. Flow cytometry assay revealed 91% of isolated hepatocytes are cytokeratin 18 positive. Compared with control and Chol, MAT1A and BHMT protein abundance were greater ($P < 0.05$) in Met treated hepatocytes. Similarly, mRNA and abundance of genes in transmethylation pathway (MAT1A, PEMT, SAHH, BHMT, SARDH) were greater ($P < 0.05$) in Met treated hepatocytes, suggesting enhanced transmethylation and phosphatidylcholine (PC) synthesis in resonse to Met supply. The abundance of genes associated with the CDP-choline pathway (CHKA, CHKB, PCYT1a, and CEPT1) were greater ($P < 0.05$) in hepatocytes treated with Chol compared with control or Met, suggesting enhanced PC synthesis via CDP-choline pathway. Protein abundance of cystathionine β-synthase (CBS) was greater ($P < 0.05$) in hepatocytes treated with Met compared with control or Chol. Additionally, mRNA abundance of genes in transsulfuration pathway (CBS, CSAD, GCLC, and GSR) were also greater ($P < 0.05$) in hepatocytes treated with Met compared with control or Chol, indicating enhanced transsulfuration pathway in response to Met supply. Overall, these findings suggest transmethylation and transsulfuration appear more responsive to Met supply, while the CDP-choline pathway is more responsive to Chol supply.

Key Words: one-carbon metabolism, methyl donor, lactation

M260 In vivo evaluation of a new rumen-protected methionine supplement. H. L. Diaz*[1], J. Albrecht[1], C. Soderholm[1], J. Linn[1], J. Firkins[2], P. Kononoff[3], and J. K. Bernard[4], [1]*Milk Specialties Global, Eden Prairie, MN,* [2]*The Ohio State University, Columbus, OH,* [3]*University of Nebraska, Lincoln, NE,* [4]*University of Georgia, Tifton, GA.*

The objective of this research was to assess a new rumen-protected Met supplement (EBMet) for rumen undegradability, intestinal and total-tract CP digestibility and blood plasma AA response. EBMet contains 34% DL Met coated with saturated FA. Statistical analysis was done using JMP and means compared using Student's *t*-test (Significance at $P < 0.05$). In study 1, EBMet samples were incubated in the rumen of 2 cannulated cows for 16 h. Rumen undegradable N (RUN, % of N) content of EBMet was determined to be 91.8% (SEM = 0.38). In study 2, using 2 steers fitted with rumen and duodenal cannulas, the RUP, intestinal digestibility of RUP (dRUP), and total-tract CP digestibility (TTCPDig %) of EBMet were determined with the mobile bag technique and Soypass as a reference. RUP was not different between EBMet and CON (91.3 vs. 102.7, SEM = 2.79) nor was dRUP (98.9 vs. 100.0, SEM = 0.36) or TTCPDig (99.0 vs 100.0, SEM = 0.30). Study 3, a completely randomized design, measured plasma AA in 8 lactating Holstein cows (DIM 126 ± 19, MY 42.4 kg/d, Parity = 2). Four cows were fed a diet with no Met supplementation (0Met) and 4 cows fed the same diet with 170 g/d of EBMet. Diets were fed for 5 d and blood from the coccygeal vein was collected at 1000, 1200, 1400, and 1600 h on d 5. Plasma Met (μg/mL) was 3.29 in EBMet fed cows and higher ($P < 0.05$) than the 2.78 of 0Met fed cows (SEM = 0.15). Met as % of Total AA (1.10 vs. 0.91, SEM = 0.03) and % of Essential AA (2.29 vs. 1.91, SEM = 0.08) was higher ($P < 0.05$) for EBMet than 0Met fed cows. In study 4 on a commercial farm in Minnesota, 8 Holstein cows (DIM 117 ± 56, MY 39.6 kg/d, Parity = 2.1) were fed a CON diet (no Met supplementation) for 7 d followed by 7 d of CON with EBMet (170 g/d). Blood from the coccygeal vein was collected on d 7 of each feeding period at 1000 and 1400 h. Cows fed EBMet had higher ($P < 0.05$) concentration of plasma Met (μg/mL) (3.27 vs. 2.81, SEM = 0.12), Met as % of Total AA (1.37 vs. 1.03, SEM = 0.04) and Met as % of Essential AA (3.25 vs. 2.35, SEM = 0.10) than CON fed cows. Data suggests EBMet is a highly metabolizable Met source as shown by RUP, dRUP, TTCPDig exceeding 90.0% and increased plasma Met concentrations in vivo.

Key Words: methionine, amino acid, blood plasma

M261 Predicting weekly calf starter intake by measuring calf starter intake once, twice, or three times a week. Y. Liang*, E. Davis, T. Batchelder, and M. Ballou, *Texas Tech University, Lubbock, TX.*

The objectives of these analyses were to investigate the relationships and the degrees of associations between measuring calf starter intake daily and using once, twice, or 3 times a week measurements to predict weekly calf starter intake. Data from 180 Holstein calves from 2 experiments that represented 9 different treatments were used in the analyses. All calves were fed 700 g of a 22% CP and 20% fat milk replacer daily and offered ad libitum access to either a pelleted (n = 80 calves) or a texturized (n = 100 calves) calf starter. All calves were weaned at 56 d of age. The quantities of calf starter refused from the previous day and the amount offered each day were recorded, so daily starter intake could be calculated. Average weekly starter intake was calculated from the daily measurements and used as the dependent variable in all subsequent analyses. Additionally, average weekly starter intake was estimated by measuring starter intake once, twice, or 3 times weekly and used as the independent variables. Data were collected for the first 8 weeks of life and simple linear regression models were analyzed by week using Proc Reg in SAS (v. 9.4). The median coefficients of determination were 0.923, 0.955, and 0.966 and median slope estimates were 0.900, 0.920, and 0.953 for the once, twice, or 3 times weekly calf starter intake measurements, respectively. The first week had the lowest degree of association 0.708, 0.801, and 0.899 and the lowest slope estimates 0.718, 0.874, and 0.880 for the once, twice, or 3 times weekly measurements, respectively. These data indicate that measuring calf starter intake once a week can be used to estimate a calf's weekly starter intake. Implications of these data are that experimental designs where collection of daily calf starter intakes are not feasible can estimate individual weekly calf starter intake by measuring it once weekly.

Key Words: calf, intake, starter

M262 Effect of incremental amounts of rumen-protected His on plasma and muscle His and His-dipeptides in lactating dairy cows fed a low-CP diet. Y. Zang*[1], L. H. P. Silva[2], M. G. Khan[1], A. F. Brito[1], and M. Miura[3], [1]*University of New Hampshire, Durham, NH,* [2]*Federal University of Viçosa, MG, Brazil,* [3]*Ajinomoto Co. Inc., Kawasaki-shi, Japan.*

Supplementation of rumen-protected (RP) AA is a practical approach to balance dietary Met and Lys for optimal milk production. Histidine may become limiting in low-CP dairy diets. However, dairy cows may tap on endogenous pools of His (e.g., anserine, carnosine) to meet requirements. We aimed to examine the effect of incremental amounts of RP-His on endogenous pools of His and AA metabolism in dairy cows. Eight multiparous Holsteins (130 ± 30 DIM) were used in a replicated 4 × 4 Latin squares design with 28-d experimental periods. Treatments included a basal diet composed (DM basis) of 50% corn silage, 15% haylage, and 35% concentrate supplemented with 0, 82, 164, and 246 g/d

RP-His (Ajinomoto Inc.) and 11 g/d RP-Met (Smartamine M). Dietary His represented 2.06, 2.28, 2.52, and 2.75% of MP supply, respectively. The basal diet averaged 15.1% CP and 34.8% NDF. Blood (weekly) and muscle (biweekly) samples were collected and quantified for AA. Data were analyzed individually by week using the MIXED procedure of SAS, evaluating treatment effects with linear and quadratic contrasts. Plasma His increased linearly ($P < 0.001$) with RP-His from wk 1 (30.3 to 57.2 μ*M*) to wk 4 (33.2 to 63.1 μ*M*). Plasma Arg, Leu, Lys, and Phe decreased linearly ($P = 0.05$) and plasma Ile showed a trend for a linear decrease in response to RP-His in wk 3. In contrast, treatments did not result in linear responses for all other EAA across the 4 wk. Plasma carnosine increased linearly ($P = 0.03$) with RP-His supplementation in wk 1, 3, and 4, but not in wk 2. No linear effect was observed for plasma 3-Methyl-His. Inclusion of RP-His showed a linear effect on muscle His in both wk 2 (20.1 to 32.5 μ*M*; $P < 0.001$) and 4 (20.3 to 35.5 μ*M*; $P < 0.001$). Muscle Lys increased linearly in wk 2 (22.1 to 36.2 μ*M*; $P = 0.04$), and muscle Thr tended to increase linearly in response to RP-His in wk 4. Muscle carnosine and anserine were not affected by treatments, but they showed quadratic trends in wk 2. Overall, RP-His was effective to increase His in plasma and muscle independent of the endogenous pools of His.

Key Words: dairy cow, endogenous pool, rumen-protected histidine

M263 *In sacco* evaluation of the effect of a source of slow release urea on dry matter, nitrogen and NDF digestibility. C. Moran[1], J. Keegan[1], S. Salomaa[2], A. Koontz*[3], and J. Apajalahti[2], [1]*Alltech SARL, Vire, France,* [2]*Alimetrics Ltd., Espoo, Finland,* [3]*Alltech Inc, Nicholasville, KY.*

The aim of the current study was to assess the effect of a non-protein nitrogen product, Optigen II, on dry matter and nitrogen digestibility using a rumen *in sacco* method. The Control (CON) diet consisted of soybean meal (SBM), compound feed and grass haylage, while the Optigen II (OPT) diet replaced one-third of the SBM with an isonitrogenous quantity of OPT thereby, reducing the SBM content from 15 to 10% of the total dietary dry matter (DM). This translates to a cow consuming 20 kg of DM being provided 150 g OPT. The components of each diet were weighed and combined (5 g DM in total) in 10 × 20 cm Dacron bags. The Dacron bags were then incubated in the rumens of 2 different fistulated cows and triplicate bags removed after 2, 5, 8, 16, 24 or 48 h. Fermentation was stopped by dipping the bags in cold water, after which the bags were cleaned and freeze-dried. After each incubation interval, the bags were weighed to determine the residual DM present followed by determination of total nitrogen concentration of the DM residue. NDF content was determined at 0 and 24 h. At each time point, DM, N, and NDF digestibility were compared between CON and OPT diets by means of a *t*-test. After 48 h incubation, about 70% of the diet DM had disappeared from the bags, with diet having no effect on the rate of DM digestibility. Approximately 80% of dietary N disappeared from the bags after 48 h, with no differences in digestibility observed between the 2 diets. NDF digestibility, analyzed at 0 and 24 h, did not differ between the diets, with 85 and 83% of the initial NDF disappearing from the CON and OPT diets, respectively. Overall, this *in sacco* study found no differences in terms of DM, N, and NDF digestibility between the CON and OPT treatments.

Key Words: controlled-release urea, rumen simulation, digestibility

M264 Effect of microalgae on rumen microbiota and feed digestibility using an in vitro fermentation model. J. Apajalahti[1], O. Siikanen[1], A. Koontz*[2], J. Keegan[3], and C. Moran[3], [1]*Alimetrics, Espoo, Finland,* [2]*Alltech Inc., Nicholasville, KY,* [3]*Alltech SARL, Vire, France.*

Unprotected long-chain polyunsaturated fatty acids, such as the n-3 fatty acids DHA and EPA are considered unsuitable for inclusion in ruminant feeding programs due to biohydrogenation by rumen microbes resulting in milk fat depression and inefficient transfer to milk. The aim of the current study was to investigate the effects of feeding an unextracted DHA-rich microalgae on the rumen microbiota and on feed digestibility in an in vitro batch culture rumen fermentation model. Five treatment levels (0, 2.5, 6.25, 15, or 25 mg) of a heterotrophically grown *Aurantiochytrium limacinum* (CCAP 4087/2, ALL-G-RICH, Alltech Inc.) biomass were added to vessels containing 0.25 g of 60:40 grass silage: commercial compound feed mix. Each treatment was replicated 15 times, with 5 replicate fermentation vessels of each treatment analyzed at 3 different time points (0, 4 and 8 h). Simulation vessels were flushed with CO_2 after which 9 mL of anaerobic buffer solution (38°C) was introduced under oxygen-free CO_2 flow. The simulation was initiated following the addition of 1 mL of freshly strained rumen fluid from a rumen fistulated dairy cow. Fermentation kinetics were followed by withdrawing vessels for quantifying volatile fatty acids (VFA), lactic acid and DHA after 0, 4 and 8 h post-inoculation. Gas production was measured as an indicator of microbial metabolic activity. After 8 h fermentation, bacterial yield was determined by quantitative RT-PCR. Each treatment was compared with the Control (no algae) group using 2-tailed *t*-tests. The addition of algae had no effect on gas, VFA, or lactic acid production and had no effect on the growth of bacteria. DHA was reduced in proportion to the starting concentration, with reductions of 21, 14, 10, and 1% observed for the 2.5-, 6.25-, 15-, and 25-mg treatments respectively. The unextracted algal biomass appeared to protect the DHA from biohydrogenation by ruminal microbes. As the biohydrogenation capacity is higher in vivo, these results warrant further investigation in live animals.

Key Words: *Aurantiochytrium limacinum* algae, DHA, rumen

M265 Effect of *Aurantiochytrium* sp. microalgae on rumen fermentation, microbial population and milk fatty acid profile. C. Moran[1], T. Rinttilä[2], J. Keegan[1], A. Koontz*[3], and J. Apajalahti[2], [1]*Alltech SARL, Vire, France,* [2]*Alimetrics, Espoo, Finland,* [3]*Alltech Inc, Nicholasville, KY.*

An increased consumption of n-3 fatty acids (omega-3 FA) has been associated with various health benefits. Altering the fatty acid composition of milk fat is a desirable means to increase human n-3 FA consumption. The aim of this study was to investigate the effect of dietary supplementation with a docosahexaenoic acid (DHA) rich microalgae on rumen fermentation and milk fatty acid profile. The trial was conducted using 2 rumen-fistulated dairy cows, fed for a 2-week pre-trial feeding period (Control, CON), 4 weeks on the treatment diet (CON supplemented with 140g/cow/day of *Aurantiochytrium limacinum*; CCAP 4087/2, ALL-G-RICH, Alltech Inc.), followed by a 1-wk washout period (CON). Rumen fluid samples were taken 4 times per week providing 28 samples per animal over the course of the experiment. Rumen fluid samples were taken before milking and analyzed by GC for short chain fatty acids (SCFA), and bacterial yield and specific microbial populations (lactobacilli, methanogens, *Veillonella* spp., *Selenomonas ruminantum*, *Fibrobacter succinogenes*, *Megasphaera elsdenii* and Clostridal Cluster XIVa) were measured by quantitative RT-PCR using appropriate prim-

ers. Milk samples (n = 28/animal) were taken on the same days as the rumen fluid samples. The addition of microalgae had no effect on SCFA or the bacterial density in the rumen fluid. Milk fat concentration was unaffected but DHA content increased 100-fold during the 4 weeks of supplementation. Numbers of lactobacilli and methane-producing bacteria were reduced, while the number of beneficial fiber-degrading bacteria increased with microalgae addition to the diet. These results indicate that the addition of microalgae under these conditions does not depress rumen fermentation or milk fat content but significantly increases milk DHA. The effect of microalgae supplementation on *Fibrobacter succinogenes* merits further investigation.

Key Words: *Aurantiochytrium limacinum* algae, polyunsaturated fatty acids, rumen microbiota

M266 Effect of physical form and nutrient content of calf starter on growth and development. D. Vagnoni*[1], C. Sousa[1], J. Aenlle[1], and M. Messman[2], [1]*California Polytechnic State University, San Luis Obispo, CA,* [2]*Cargill Animal Nutrition, Elk River, MN.*

One hundred twenty-eight Holstein heifer calves (37.7 ± 4.6 kg BW) were allotted to 4 diets to evaluate physical form and nutrient composition of starter feed on performance and development. Diets consisted of a typical texturized calf starter (Diet A), texturized calf starter with reduced grain and increased NDF (Diet B), standard texturized calf starter with increased sugar content (Diet C), or a completely pelleted, relatively high NDF diet (Diet D). Calves received colostrum at their dairy of origin (n = 7) before arriving at the commercial calf operation where the study was conducted. Upon arrival, calves were weighed and assigned to dietary treatments in an attempt to equalize initial BW and distribution of dairy of origin across treatments. Calves received 2 L of pasteurized dairy waste milk twice daily until 42 d of age. Milk offered was reduced to 1 L per day until d 49 when weaning was complete. Calves received ad libitum water and starter feed throughout the trial, at 70 d of age. Starter feed offered and refused was measured daily. Body weights were recorded every 7 d and blood samples were obtained for measurement of glucose and β-hydroxy butyrate at 28, 42, 56, and 70 d of age. Data for pre-weaning and post-weaning weight gain and feed intake were analyzed as a completely randomized design. Blood concentrations were analyzed using a mixed linear model with time as a repeated factor. Diet did not affect ($P = 0.28$) pre-weaning weight gain (overall average = 0.55 kg/d) but did ($P = 0.047$) affect post-weaning weight gain (overall average = 0.82 kg/d), with diets A and B performing better than diets C and D. Diet affected pre-weaning ($P = 0.038$) but not post-weaning ($P = 0.9$) starter intakes. Concentrations of glucose (overall average = 87 mg/dl) decreased ($P < 0.01$) and concentrations of β-hydroxybutyrate (overall average = 0.12 mM) increased ($P < 0.01$) as calves transitioned from milk to primarily solid feed intake, reflecting the development of ruminal fermentation. Results indicated that acceptable calf performance can be obtained with a variety of dietary carbohydrate formulations and that blood concentrations of glucose and β-hydroxybutyrate may be useful indicators of ruminal development and readiness for weaning.

Key Words: calf starter, rumen development

M267 Towards the compositional prediction of the ruminal microbial community using temporal modeling in healthy and milk depressed states. C. Martino*[1], G. Gogel[1], J. Gaffney[1], A. Lago[2], and M. Embree[1], [1]*Ascus Biosciences, San Diego, CA,* [2]*Dairy-Experts Inc., Tulare, CA.*

Sixteen ruminally cannulated cows, 8 Holsteins and 8 Jerseys, were used in a milk fat depression (MFD) model to characterize the temporal changes of rumen bacterial populations in cows shifting between a healthy, MFD, and recovery state. The experiment consisted of a 10-d covariate period (Cov) followed by a 10-d MFD induction (Ind), and an 18-d MFD recovery (Rec). Animals were fed a common TMR (16.3% CP, 37.3% NDF, 0.67 Mcal of NEI/lb) during the Cov and Rec. During the Ind, animals were fed a low-fiber, high-starch diet that caused a 0.6% and 1.5% mean decrease in milk fat in Jersey and Holstein cows, respectively. All animals were milked and fed twice a day in addition to daily rumen sampling. Bacterial populations were characterized via 16S rRNA gene amplicon sequencing of rumen samples. MFD induced substantial transformations in the rumen bacterial populations (Cov vs. Ind vs. Rec, $P = 0.001$) and increased α diversity during Ind ($P < 0.01$). The resulting operational taxonomic unit (OTU) table was centered-log ratio (clr) transformed and bi-clustered to reveal 2 unsupervised naturally underlying group fluctuations amplified during MFD induction. Of the 2 groupings, 4 of the most universally fluctuating bacterial classes showed significant linear correlation between relative abundance and milk fat percentage during Ind. The 4 classes were *Fibrobacterales* (group 1, $R^2 = 0.64$, $P = 0.0072$), *Clostridiales* (group 1, $R^2 = 0.57$, $P = 0.022$), *Bacteroidales* (group 2, $R^2 = -0.66$, $P = 0.0056$), and *Selemonadales* (group 2, $R^2 = -0.16$, $P = 0.55$). The 2 groups' respective combined abundunace plotted over time revealed an oscillatory nature and fit well to generative Lotka-Volterra models. The dynamics of the resulting model exhibited stable oscillatory behaviors ($\lambda = -0.44, 0.44$) with a cyclic periodicity of 6 d. Ordinary least squares regression on compositional balances were applied to the data set and results indicate that the composition of microbial communities can be accurately predicted ($R^2 = 0.81$ *MSE* = 4.0) from daily environmental and milk composition data.

Key Words: microbiome, milk fat depression, sequencing

M268 Influence of supplemental copper and selenium source on reproductive parameters, milk yield and composition in Normando dairy cattle. P. Rodriguez-Hernandez*, H. Laviano-Medina, J. Pardo-Guzman, C. F. -De Rubio, and R. Castaneda-Serrano, *Universidad del Tolima, Ibague, Tolima, Colombia.*

The objective of this experiment was to evaluate the effects of supplemental Cu and Se organic source on reproduction parameters, milk yield and composition in Normando dairy cattle. Multiparous Normando cows (n = 36), BW = 600 ± 32 kg were used from 30 d before calving until 120 d of lactation. Cows were blocked by expected calving date and randomly assigned to treatments. T0: without mineral supplementation, T1: inorganic mineral supplementation of Cu and Se, T2: organic mineral supplementation of Cu and Se. Sulphates were used as inorganic sources and B-traxim (Pancosma) and Selemax (Biorigin) as organic source of Cu and Se respectively. The mineral requirements were adjusted according to the NRC (2001). Cows were kept in a rotating pasture of *Pennisetum clandestinum* and supplemented during milking twice daily with treatments. Milk yield were recorded daily from 4 d until 120 d of lactation. Milk samples were taken for composition analysis of each cow every 30 d. Reproductive parameters were evaluated (Table 1). No differences were observed in the yield or composition of the milk ($P > 0.05$), However, there were significant differences with respect to open days and services per conception ($P < 0.05$) and a trend ($P = 0.09$) in the decrease of the days opened in T1 and T2. In conclusion, these data suggest that supplementation with organic and inorganic minerals did not influence on milk yield and composition. However, supplementation with organic minerals can improve the reproductive parameters in Normando dairy cows.

Table 1 (Abstr. M268). Effect of supplemental copper and selenium source on milk yield and composition and reproductive parameters in Normando dairy cattle

	Treatment[1]				
Item	T0	T1	T2	SEM	*P*-value
Milk yield, L/d	25.6	25.7	24.0	1.5	0.67
Protein, %	3.0	3.3	3.0	0.1	0.17
Fat, %	3.8	3.7	3.8	0.5	0.98
Total solids, %	12.8	13.1	11.1	0.7	0.17
SCC (1000/mL)	152.2	81.7	81.9	41.7	0.38
Days to first service	61.1	62.9	58.4	3.5	0.90
Open days	110.6	97.4	86.9	11.5	0.09
Services per conception	2.8[a]	2.6[a]	1.9[b]	0.4	0.01

[1]T0 = without mineral supplementation, T1 = inorganic mineral supplementation of Cu and Se, T2 = organic mineral supplementation of Cu and Se.

Key Words: mineral mixture, ruminant, trace mineral

M269 Genome sequencing of native rumen microorganisms from Holstein cows reveals diverse range of functional capabilities. J. Gaffney*, C. Martino, G. Gogul, M. Striluk, and M. Embree, *Ascus Biosciences, San Diego, CA.*

Traditionally, 16S data have been used to profile ruminal microbial communities and functionality has been inferred based on broad level taxonomic classifications. However, the accuracy of taxonomic calls is often lacking due to the poor resolution and lack of cultured representatives from the native rumen microbiome. The objective was to profile the metabolic capabilities of 20 native rumen microorganisms via in-depth analysis of their genomes coupled with metabolic modeling and flux balance analysis (FBA). For this study, 16 novel bacteria and 4 novel fungi were isolated from the rumen content of 3 healthy, mid-lactation Holsteins on TMR (16.3% CP, 37.3% NDF, 0.67 Mcal of NEI/lb). Strains were isolated using a diverse media panel and whole-genome sequenced (WGS) using Illumina Miseq and Oxford Nanopore sequencing platforms. Reads were assembled, annotated, and analyzed using metabolic modeling. All novel organisms shared less than 70% nucleotide homology to their closest neighbor in the NCBI database at the whole genome level, and were thus considered novel. Subsequent sequence analysis revealed the pivotal roles that these 20 microorganisms contribute to feed digestibility and milk production. For instance, isolates sequenced from the family *Lachnospiraceae* possessed a unique spectrum of genes associated with biohydrogenation and acetate production, which are commonly associated ruminal functions of *Lachnospiraceae*. The family *Lachnospiraceae* includes the genus *Butyrivibrio*, identified for xylan degradation and butyrate production in the rumen, and isolates from the genus *Butyrivibrio* displayed distinct metabolic profiles, with respect to amino acid metabolism and carbon source utilization. The *Neocallimastigaceae* isolates had unique polysaccharide metabolisms and docking mechanisms, suggesting that each fungal species may employ unique mechanisms to drive cellulolytic activity. These findings emphasize that functional capability can vary greatly between members of the same taxa. Looking beyond taxonomy can help us gain further insight into how bacterial communities affect ruminal health.

Key Words: rumen microbiome, whole genome, sequencing

M270 The effects of cut height and ensiling time on the fermentation profile of whole-plant corn silage. T. A. Damery*[1], R. T. Pate[1], M. Atkins[2], R. Myers[3], and F. C. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*University of Wisconsin-Madison, Marshfield, WI,* [3]*Bayer CropScience LP, Research Triangle Park, NC.*

Applying foliar fungicide and raising cut height at harvest may increase the nutritive value of whole-plant corn silage (WPCS). The objective of this study was to evaluate the effects of foliar fungicide application (prothioconazole and trifloxystrobin; Delaro, Bayer CropScience) and harvest cut height on the fermentation profile of WPCS ensiled for 0, 30, 60 and 90 d post-harvest. Fungicide treatments were randomly assigned, and replicated 3 times, to one of 16 0.21-ha plots as follows: control (CON), plants received no application; (V5), plants received one application at corn vegetative stage 5 (V5); (V5R1), plants received 2 applications at V5 and corn reproductive stage 1 (R1); (R1), plants received one application at R1. WPCS was harvested on August 30, 2017 at 34.0 ± 1.6% dry matter (DM). Each plot consisted of 16 rows; 8 were harvested at 30.5 cm (LC) and 8 were harvested 56 cm (HC). From each plot, 3 mini-silos containing 600 g of composited WPCS were immediately vacuum-sealed (Minipack MVS-20, Doug Care Equipment, Springville, CA). Mini-silos at 0d were immediately frozen. Remaining mini-silos were allowed to ferment at room temperature before freezing at 30, 60, and 90d. A VFA score of 0–10, 10 being the most desirable, was used to evaluate overall silage quality (Diary One, Ithaca, NY). At 90 d, V5 had a higher ($P = 0.02$) VFA score than CON (9.66 and 9.42; SEM = 0.07, respectively). At 60 d, fungicide treated corn had higher ($P = 0.03$) lactic acid concentrations than CON corn (8.02%, 8.92%, 8.52%, 8.33%; SEM = 0.26 for CON, V5, V5R1, and R1, respectively). At 60 d, fungicide treated corn had higher ($P = 0.0006$) ammonia-N concentrations than CON corn (4.00%, 5.38%, 5.38%, 5.13%; SEM = 0.33 for CON, V5, V5R1, and R1, respectively). At 90 d, HC corn had less ($P < 0.0001$) lactic acid (9.49% and 8.15%; SEM = 0.17, respectively) and a lower ($P = 0.0002$) VFA score (9.59 and 9.25; SEM = 0.05, respectively). In conclusion, raising cut height did not improve fermentation. However, fungicide application did improve fermentation of WPCS and has the potential to increase milk production when fed to dairy cattle.

Key Words: fungicide, whole-plant corn silage, lactic acid

M271 Comparison of residual feed intake, net energy, and economic models of feed efficiency in dairy cattle. D. J. Seymour*, F. Miglior, G. Ritchie, V. R. Osborne, J. P. Cant, and A. Cánovas, *University of Guelph, Guelph, ON, Canada.*

Feed efficiency is an economically important trait across livestock species and is of growing importance as a breeding goal in dairy cattle. One of the most widely used measures of feed efficiency is residual feed intake (RFI), which uses a regression method to rank animals based on predicted dry matter intake. The objective of this study was to compare RFI rankings to net energy- and economic-based rankings of feed efficiency in dairy cattle. 6,654 daily records from 93 primiparous lactating Holstein cows ranging from 0 to 150 d postpartum were selected for analysis from a larger data set based on completeness of records. RFI was calculated using a mixed model with week of lactation, milk energy content, metabolic BW, and BW change as covariates. Net energy efficiency was calculated as the energy content of milk as a proportion of dietary net energy for lactation (NEL) intake. Lastly, return over feed cost was calculated as the difference between the producer milk price based on component yields and the cost of TMR dry matter consumed. Animal ranks based on RFI and net energy were highly correlated (0.872), whereas return over feed cost ranking was moderately correlated with both RFI (0.668) and net energy (0.619). Both net energy ($R^2 = 0.95$, $P < 0.01$) and RFI ($R^2 = 0.75$, $P < 0.01$)

ranks were negatively associated with mean daily net energy balance, suggesting that more feed efficient animals meet the energy demands of lactation by mobilizing body energy reserves to supplement that obtained from the diet. Return over feed cost rank was poorly associated (R^2 = 0.29, $P < 0.01$) with net energy balance. This model does not account for the costs associated with persistent negative energy balance (e.g., metabolic disorders), and as such the maximal output may not reflect the optimal output. With the addition of body condition score data, as well as records over the course of an entire lactation, future work will be able to more accurately characterize net energy flow and identify animal characteristics regarding mobilization of body reserves and the relationship to feed efficiency.

Key Words: feed efficiency, net energy, residual feed intake (RFI)

M272 Production responses to rumen-protected choline and methionine supplemented during the transition period differ for primi- and multiparous cows. S. B. Potts, C. M. Scholte*, and R. A. Erdman, *University of Maryland, College Park, MD.*

Our objective was to examine performance responses to feeding rumen-protected choline (RPC), methionine (RPM), or both during the transition period. Fifty-four Holstein cows (25 primigravid, 29 multigravid) were used in a randomized block design experiment with a 2 × 2 factorial treatment structure. Cows were blocked by expected calving date and parity and assigned to 1 of 4 treatments: CON (no RPC or RPM); CHO (60 g/d RPC); MET (12 g/d RPM prepartum; 18 g/d RPM postpartum); or CHO + MET. Treatments were applied once daily as a top-dress from 3 wk before through 5 wk after calving. Dry matter intake and milk production were recorded daily and milk samples were obtained once weekly. Data were analyzed for primi- and multiparous cows separately using a repeated measures mixed model that included random effects of cow and block and fixed effects of CHO, MET, week, and their interactions; week served the repeated effect. Interactions were considered significant if $P < 0.10$. Overall DMI was unaffected by treatment although RPC decreased DMI for multiparous cows during wk 1 postpartum (CHO × Week: $P = 0.02$) and RPM increased DMI for primiparous cows on the day of calving (MET × Week: $P < 0.01$). RPC tended to enhance overall milk yield (CHO: $P = 0.07$) and 4% FCM (CHO: $P = 0.08$) by 3.7 and 2.7 kg/d, respectively, for primiparous cows. In contrast, treatment did not affect overall milk yield for multiparous cows. Overall energy balance (EB) was not affected by treatment; however, on the day of calving, RPC improved EB by 9.7 Mcal for multiparous cows (CHO × Week: $P < 0.01$), while RPM improved EB by 3.3 Mcal for primiparous cows (MET × Week: $P = 0.06$). RPM increased overall milk fat percentage by 0.28% (MET: $P = 0.03$) and milk protein percentage by 0.33 and 0.37% during wk 1 and 2 postpartum (MET × Week: $P = 0.10$) for multiparous cows, but not primiparous cows. These results suggest that primi- and multiparous cows may respond differently to RPC and RPM during the transition period. This variation in response could be mediated by differences in choline and methionine requirements.

Key Words: choline, methionine, transition cow

M273 Associations between ruminal and reticular pH during induction and recovery from subacute ruminal acidosis in dairy cows. E. Sandri[1], Y. Couture[2], R. Gervais[3], L. Fadul-Pacheco[4,5], J. Levesque[1], and D. Rico*[1], [1]*CRSAD, Deschambault, QC, Canada,* [2]*Université de Montreal, Saint-Hyacinthe, QC, Canada,* [3]*Université Laval, Quebec, QC, Canada,* [4]*Valacta, Ste-Anne-de-Bellevue, QC, Canada,* [5]*Université McGill, Ste-Anne-de-Bellevue, QC, Canada.*

Twelve ruminally cannulated cows (120 ± 52 DIM; 35.5 ± 8.9 kg of milk/d; mean ± SD) were randomly assigned to treatment in a Latin square design with 21-d periods. Treatments were (1) subacute ruminal acidosis (SARA) induction, (2) recovery, and (3) control. Using indwelling pH probes, pH was measured simultaneously in the reticulum and in the rumen ventral sac (every 5 min for 24 h) on d 0, 3, 7, 14, and 21 of each period. The SARA diet contained 29% starch, 24% NDF, and 2.8% fatty acids (FA) whereas the recovery and control diets contained 20% starch, 31% NDF, and 2.3% FA. Data were analyzed as repeated measures using a mixed model. An acidosis index (AI; area under pH 5.8/DMI) was calculated for each cow to classify them as tolerant (AI < mean AI - 0.5 × SD) or susceptible (AI > mean AI + 0.5 × SD) to SARA. The average and minimum ruminal pH in cows fed the SARA diet were lower than that of cows fed the control diet on d 3, 14 and 21 ($P < 0.05$). As compared with control, ruminal pH remained below 5.6 for a longer time when cows received the SARA diet on d 3 (145 vs. 378 min/d; $P < 0.05$) and d 7 (124 vs. 264; $P < 0.08$). In the reticulum, the pH duration below 5.6 was greater in cows fed the SARA diet on d 3 (31 vs. 218 min/d) and 14 (8.4 vs. 127 min/d) for control and SARA, respectively ($P < 0.05$). The association between the ruminal and reticular pH was low ($R^2 = 0.33$; concordance correlation coefficient = 0.52, confidence interval = 0.51–0.53). Dry matter intake, milk and milk fat yield were greater in SARA-susceptible than in SARA-tolerant cows ($P = 0.03$, 0.06 and 0.04, respectively), whereas milk fat concentration was lower in the SARA-susceptible group ($P < 0.05$). Furthermore, SARA-susceptible cows had lower mean and maximal pH ($P < 0.05$), but minimum pH or pH variance were not different. In addition, SARA-susceptible cows had greater values for time under pH 5.8 and 5.6 ($P < 0.001$). In conclusion, the reticular pH measurements may underestimate the incidence of SARA and would require adjustment before this technology can be used on commercial dairy farms. The production performance of dairy cows fed high-concentrate diets was related to their susceptibility to SARA, as higher producing cows were at increased risk.

Key Words: acidosis, reticulum, rumen

M274 Effect of rumen-bypass flaxseed supplementation for 8 weeks on milk production and milk fatty acid composition in Jersey cows. K. Swanson[1], S. Akers*[1], R. Wilson[1], M. Keller[1], L. Goddick[1], G. Cherian[1], R. Day[2], and G. Bobe[1], [1]*Oregon State University, Corvallis, OR,* [2]*N3Feed, Tualatin, OR.*

Current flaxseed processing methods have shown limited or varying success in improving the fatty acid profile of milk beyond what is achieved with feeding unprocessed flaxseed. In this study, a novel method to "rumen-protect" flaxseed is proposed, which encapsulates flaxseed with soybean protein using a proprietary method (12BT40; *N3*Feed LLC; Tualatin, OR; 23.8% CF, 31.7% CP). To determine the long-term efficacy, 12 Jersey cows (blocked by parity) in mid lactation were fed 2.5 kg of 12BT40 for 8 weeks as top dressing to their TMR (2.9% CF, 14.8% CP). Milk and serum samples were collected at the end of wk 0, 2, 4, 6, and 8 and analyzed for fatty acid profile and metabolic indicators. Data were analyzed using PROC MIXED in SAS version 9.4. Fixed effects were supplementation (control, bypass flaxseed), week of supplementation (2, 4, 6, and 8), their interaction, and parity (primiparous, multiparous) and baseline concentration as linear covariate; a first-order heterogeneous autoregressive variance-covariance structure was fitted for repeated measures within cows. Bypass flaxseed supplementation consistently increased throughout the supplementation period milk production (+4.42 ± 1.52 kg/d; $P = 0.02$) without significant changes in milk composition. In regards to milk fatty acid profile, bypass flaxseed supplementation consistently increased C18:3n-3 proportions

throughout the supplementation period (+1.91 ± 0.20 wt% FA; $P <$ 0.0001). In conclusion, feeding rumen-bypass flaxseed is a potential management tool to improve the nutritional value of milk and dairy products, while at the same time increasing milk yield.

Key Words: flaxseed, rumen protection, milk fatty acid composition

M275 The relationship between circulating ceramides, plasma fatty acids, and adipose tissue measures of inflammation and lipolysis. W. A. Myers*[1], J. E. Rico[1], D. E. Rico[2], Q. Zeng[3], J. de Souza[4], A. L. Lock[4], P. Y. Chouinard[2], G. A. Contreras[4], R. Gervais[2], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Université Laval, Quebec, QC, Canada,* [3]*West Virginia University, Morgantown, WV,* [4]*Michigan State University, East Lansing, MI.*

Saturated fatty acids (FA) stimulate the synthesis of ceramide which promotes insulin antagonism, lipolysis, and inflammation. We established that ceramide mediates insulin antagonism in bovine adipocytes, and feeding palmitic acid (C16:0) promotes ceramide accumulation in dairy cows. Our objective was to evaluate the relationship between circulating ceramide and (1) the plasma FA profile, and (2) measures of subcutaneous adipose tissue (AT) inflammation and lipolysis. In study 1, 11 cannulated mid-lactation Holstein cows were abomasally infused (280 g/d) C16:0 (PA; 85% C16:0), stearic acid (SA; 98% C18:0), or medium-chain triglycerides (MCT; C8:0/C10:0) for 7 d in a replicated Latin square design. In study 2, 16 early lactation Holstein cows received a corn silage-based diet supplemented with no added fat (soyhulls) or C16:0 (85% C16:0) at 1.5% of diet DM for 1–10 DIM. Blood and AT were collected post treatment. Lipids were measured using gas chromatography and mass spectrometry. Gene expression was assessed by qPCR. Data were analyzed under a mixed model, and correlation analyses were performed. In study 1, PA increased plasma C16:0 ($P <$ 0.05), relative to SA or MCT. MCT increased plasma C10:0 ($P < 0.01$), relative to PA. Select ceramides tended to be positively correlated with plasma C16:0 (e.g., C16:0-ceramide; $P \leq 0.10$). The majority of ceramides were inversely related to C8:0, C12:0, and *cis*-9 C18:1 (e.g., C8:0 vs C24:0-ceramide, $r = -0.61$; $P < 0.05$). In study 2, ceramides were positively correlated with hormone sensitive lipase activation (phosphorylation status; e.g., C18:1-ceramide, $r = 0.83$; $P < 0.01$) and body weight loss (e.g., C18:0-ceramide, $r = 0.49$; $P < 0.05$). Many plasma ceramides were positively correlated with AT CD44 expression (e.g., C22:0-ceramide, $r = 0.67$; $P < 0.01$), a macrophage receptor. Moreover, the ratio of C24:0- to C16:0-ceramide was positively correlated with AT macrophage trafficking measured by flow cytometry (i.e., $CD14^+$ cells, $r = 0.49$; $P = 0.05$). We conclude that elevations in circulating ceramides develop with a modified FA profile, lipolysis and inflammation.

Key Words: ceramide, inflammation, palmitic acid

M276 A lipidomic analysis of bovine liver during metabolic disease. S. S. Samii[1,2], Y. Zang[2], W. A. Myers*[1,2], E. Grilli[3], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV,* [3]*University of Bologna, Bologna, Italy.*

Steatosis develops with elevations in hepatic free fatty acid (FFA) uptake, triacylglycerol (TAG) esterification, and ketogenesis. We previously performed a plasma lipidomic analysis of peripartal dairy cows to reveal postpartum (pp) decreases in plasma phosphatidylcholines (PC; e.g., PC 32:3) as a feature of fatty liver, hyperlipidemia, and ketosis. Our objective was to identify specific hepatic lipids that are related to steatosis, lipolysis, and ketone production using lipidomics. Thirty multiparous Holstein cows were enrolled −28 d prepartum and fed diets formulated to meet or exceed requirements. Blood and liver samples were routinely collected. Untargeted lipidomics was performed using mass spectrometry. Univariate and multivariate analyses of auto-scaled lipidomic data were performed. Cows were separately categorized into low or high FFA area under the curve (FFA_{AUC}; d 1 – 14 pp; 4,915 ± 1,369 vs. 12,501 ± 2,761 [μmol/L × 14 d]; n = 18), β-hydroxybutyrate area under the curve (BHB_{AUC}; d 1 – 14 pp; 4,583 ± 459 vs. 7901 ± 1,206 [μmol/L × 14 d]; n = 18), or mean pp liver lipid content (d 5 and 14 pp; 5 ± 1 vs. 12 ± 2% of wet weight; n = 18). Lipidomics revealed 403 lipids. Random forest analysis determined that cows were distinguishable across time (class error = 0.10). PLS-DA score plots demonstrated good fitness and high predictability of model with R^2 and Q^2 values ≥0.75 and 0.74, respectively. Postpartum increases in monoalkyl-diacylglycerols (MADAG; e.g., 58:2; $P < 0.05$) were observed. In contrast, pp changes in hepatic phosphatidylethanolamine (PE) levels were inconsistent (e.g., PE 42:4 increased, whereas PE 38:2 decreased; $P < 0.05$). Interestingly, hepatic TAG saturation increased while PC saturation decreased as steatosis progressed ($P < 0.05$). Cows with high liver lipid experienced elevations in hepatic TAG 56:9 and 54:1 as well as phosphatidylserine (PS) 39:3 ($P < 0.05$). Cows with lower FFA_{AUC} had higher levels of PE 35:0 and phosphatidic acid (PA) 39:1 ($P < 0.05$). Cows with high BHB_{AUC} had greater postpartum levels of hepatic MADAG 64:4, PA 45:2, and PS 46:2 ($P < 0.05$). We conclude that dynamic remodeling of the bovine liver is related to metabolic disease.

Key Words: lipidome, peripartum, steatosis

M277 Metabolic profile of Holstein heifers fed carinata meal compared with canola meal and a control diet. K. Rodriguez-Hernandez*[1,2], J. L. Anderson[1], J. A. Clapper[3], and G. A. Perry[3], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Matamoros, Coahuila, México,* [3]*Animal Science Department, South Dakota State University, Brookings, SD.*

Carinata meal is a developing oilseed meal that contains glucosinolates which may impair thyroid gland function and consequently metabolism and reproduction. Our objective was to compare the metabolic profile and onset of puberty of dairy heifers fed diets containing carinata meal, canola meal, or a control diet. A 16-wk randomized block design experiment with 36 Holstein heifers (6.3 ± 0.1 mo of age, and 207 ± 3 kg of BW) was conducted. Heifers were blocked by age. Treatments were (1) carinata meal (CRM), (2) canola meal (CAN), and (3) a control diet (CON) with most of the protein provided from soybean meal. Test meals were solvent extracted and included at 10% of diet DM. Diets were isocaloric and isonitrogenous and contained similar ingredients, other than the test feeds. Heifers were limit-fed rations at 2.4% of BW on DM basis using a Calan gate system. Jugular blood samples were collected 4 h post-feeding on 2 d during wk 0, 4, 8, 12, and 16 for metabolite and thyroid hormones analyses. To determine onset of puberty, blood samples were taken every 3 or 4 d for progesterone analysis. Data were analyzed using MIXED procedures with repeated measures in SAS 9.4. Puberty data were analyzed as binomial data (cycling or not cycling) and using repeated measures by 10-d and 10-kg intervals of age and BW. Significance was declared at $P < 0.05$. Glucose (75.9, 75.8, and 77.1 mg/dL; SEM = 1.65 for CRM, CAN and CON, respectively), cholesterol (81.7, 81.2, and 79.0 mg/dL; SEM = 2.95), triglycerides (23.4, 23.3, and 21.3 mg/dL; SEM = 1.13), plasma urea nitrogen (18.4, 18.0, and 17.2 mg/dL; SEM = 0.41), triiodothyronine (135.4, 140.7, and 141.6 ng/dL; SEM = 5.73), and thyroxine (4.7, 4.3, and 4.6 μg/dL; SEM = 0.17) concentrations were similar ($P > 0.05$). Age (297, 290, 294 d; SEM = 3.1) at puberty was similar among treatments. Heifers fed CRM and

CON weighed less at puberty than heifers fed CAN (318 and 317 vs. 329 kg; SEM = 3.2; $P = 0.02$). Results demonstrate that heifers can be limit-fed diets with 10% CRM without negative effects on metabolic profile and onset of puberty, showing it is viable as a feed for heifers.

Key Words: carinata meal, metabolic profile, dairy heifer

M278 Effects of replacing dietary starch with digestible NDF at two agitation speeds on fiber digestibility and fermentation parameters in continuous culture fermentors. H. E. Johnson*, Y. Sun, G. E. Margaria, P. Piantoni, A. A. A. Jacobs, N. A. Nachtrieb, G. T. Sales, and G. F. Schroeder, *Cargill Animal Nutrition Innovation Campus, Elk River, MN.*

The objective of this study was to examine the interaction between partial replacement of dietary starch by digestible NDF (dNDF) and the speed of agitation on digestion and fermentation profile in vitro. Ten dual-flow continuous culture fermentors were utilized in a randomized complete block design experiment with treatments assigned in factorial arrangement: High starch diet (HS) with 29.9% starch and 13.5% dNFD (DM basis) vs. High dNDF diet (HF) with 22.0% starch and 18.6% dNDF, and agitation speeds of 75 vs. 125 rpm. Fermentors were blocked by period, with two 10-d periods (7-d adaptation and 3-d collection) being executed (n = 5). Liquid and solid dilution rates were set at 8 and 4%/h, respectively. Fermentors were fed once daily (0.023 mg DM/mL of buffer infused/d) and pH controlled to stay within a range of 5.5 to 7.0. No interactions were observed between agitation speed and dietary dNDF level for NDF and DM digestibilities or VFA concentration. Compared with 125 rpm, 75 rpm increased average pH (6.53 vs. 6.40; $P = 0.01$) and valeric acid concentration (4.73 vs. 3.21 m*M*; $P \leq 0.05$) and tended to increase isovaleric acid concentration (3.50 vs. 2.43 m*M*; $P = 0.09$), without affecting any other parameters. Partial replacement of starch by dNDF increased NDF digestibility (43.2 vs. 35.5%; $P = 0.03$) and tended to decrease DM digestibility (64.0 vs. 61.7%; $P = 0.10$). Concentrations of ammonia (1.55 vs. 1.38 mg/dL), total VFA (60.2 vs. 47.1 m*M*) and butyric acid (12.8 vs. 9.89 m*M*) were higher in HF compared with HS (all $P < 0.05$). HF also tended to increase acetic (24.2 vs. 19.6 m*M*), propionic (13.7 vs. 10.5 m*M*), and isovaleric (3.32 vs. 2.56 m*M*) concentration (all $P < 0.10$). Although HF increased total VFA, no differences in average pH (6.44 vs. 6.48; $P = 0.27$) or time below 5.7 (130 vs. 158 min/d; $P = 0.49$) were observed, suggesting a slower rate of fermentation with HF compared with HS. In conclusion, speed of agitation did not affect most of the fermentation parameters evaluated and substitution of starch for dNDF increased NDF degradation and ammonia concentration.

Key Words: rumen, fiber digestibility, in vitro

M279 Inhibition of serine palmitoyltransferase prevents palmitic acid-induced ceramide synthesis in bovine primary hepatocytes. J. W. McFadden*[1], J. E. Rico[1], S. J. Erb[2], and H. M. White[2], [1]*Cornell University, Ithaca, NY,* [2]*University of Wisconsin, Madison, WI.*

Ceramide is synthesized by the condensation of palmitoyl-CoA with serine in a reaction controlled by serine palmitoyltransferase (SPT). The enhanced hepatocyte uptake of saturated fatty acids contributes to the activation of SPT and de novo ceramide synthesis. In dairy cattle, hepatic ceramide accrual has been linked to metabolic disease, and palmitic acid feeding increases hepatic and circulating ceramide. Therefore, we hypothesized that palmitic acid increases hepatocyte ceramide concentrations by activating SPT. To test our hypothesis, primary hepatocytes isolated from 4 neonatal Holstein calves were maintained as monolayer cultures for 24 h. At 24 h, media was refreshed with 1% fatty acid-free BSA, and cells were unsupplemented (control) or exposed to 1 m*M* palmitic acid in the absence or presence of 10 μ*M* myriocin (SPT inhibitor) for 18 h before collection in PBS. Ceramide, monohexosylceramides (GlcCer), and lactosylceramides (LacCer) were extracted then quantified using mass spectrometry. For normalization purposes, cellular protein was quantified using the bicinchoninic acid assay. Data were analyzed using a mixed model (fixed effect of treatment and random effect of calf). Reflective of bovine liver and plasma, C24:0, C22:0, and C16:0 ceramide represented 30, 21, and 20% of total ceramide in unsupplemented cells, respectively. A similar profile was observed for GlcCer, whereas C16:0 LacCer represented the most abundant LacCer. Palmitic acid tended to increase total ceramide and GlcCer by 53 and 63%, respectively ($P \leq 0.10$), relative to control. Specifically, palmitic acid significantly increased C16:0, C18:0, and C22:0 ceramide ($P < 0.05$), and tended to increase C24:0 ceramide ($P = 0.07$). Moreover, palmitic acid increased C16:0 and C16:1 GlcCer ($P < 0.01$). Notably, the inhibition of SPT by myriocin prevented palmitic acid-induced de novo ceramide synthesis. For example, myriocin completely prevented the ability of palmitic acid to increase C16:0 and C24:0 ceramide ($P < 0.05$). We conclude that palmitic acid induces de novo ceramide synthesis in bovine primary hepatocytes.

Key Words: ceramide, hepatocyte, palmitic acid

M280 The effect of feeding zeolite A during the prepartum period on serum mineral concentrations in multiparous Holstein cows. A. L. Kerwin*[1], C. M. Ryan[1], B. M. Leno[1], M. Jakobsen[2], P. Theilgaard[3], and T. R. Overton[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Protekta Inc., Lucknow, ON, Canada,* [3]*Vitfoss, Graasten, Denmarke.*

The objective was to determine the effect of feeding zeolite A during the prepartum period on peripartum serum mineral status. Holstein cows (n = 55) entering second or greater lactation were assigned randomly to one of 2 dietary treatments starting 21 d before expected calving; control (CON): 40% corn silage, 33% wheat straw, and 27% concentrate or experimental (EXP): CON plus zeolite A (X-Zelit, Protekta Inc./Vitfoss) at 3.3% of DM, targeting 500 g/d as-fed. Blood was collected 1×/wk from enrollment until 7 d before expected calving, daily through 7 DIM with 2 samples within the 24 h of calving, and 3x/wk from wk 2 to 4. Data were analyzed in SAS v. 9.4 with repeated-measures ANOVA using the MIXED procedure. The difference in prevalence of subclinical hypocalcemia (SCH; serum Ca <8.5 mg/dL) between treatments by day and chronic SCH (cSCH; all serum samples between 0 and 3 DIM with Ca <8.5 mg/dL) was tested with Fisher's exact test. There was a treatment by day effect on serum Ca (prepartum: $P = 0.06$, postpartum: $P < 0.001$), P (prepartum: $P = 0.04$, postpartum: < 0.001) and Mg (prepartum: $P < 0.01$, postpartum: $P < 0.001$). Cows fed EXP had higher prepartum serum Ca (9.88 ± 0.10 vs. 9.26 ± 0.10 mg/dL, $P < 0.001$), lower P (3.20 ± 0.13 vs 6.30 ± 0.12 mg/dL, $P < 0.001$) and lower Mg (1.70 ± 0.03 vs. 1.84 ± 0.03 mEq/L, $P < 0.01$). Cows fed EXP had higher postpartum serum Ca (9.33 ± 0.09 vs. 8.60 ± 0.09 mg/dL, $P < 0.001$) and lower P (3.70 ± 0.13 vs 4.32 ± 0.12 mg/dL, $P < 0.001$). The prevalence of hypocalcemia was higher in cows fed CON at −3, −1, 0.25, 0.75, 2, 3, and 15 DIM and tended to be higher at 6 DIM. The largest difference in hypocalcemia prevalence was at 0.75 DIM; 90% of cows fed CON vs. 31% of cows fed EXP. No cows fed EXP had cSCH vs. 35% of CON cows and half of the EXP fed cows had no SCH through 3 DIM compared with 3% of CON cows ($P < 0.001$). Cows fed EXP had higher serum Ca and lower serum P and Mg with differences being most

pronounced during the immediate peripartum period. Feeding zeolite A during the prepartum period resulted in markedly improved Ca status during the periparturient period.

Key Words: hypocalcemia, zeolite A, mineral status

M281 The effect of feeding zeolite A during the prepartum period on peripartum performance in multiparous Holstein cows. A. L. Kerwin*[1], C. M. Ryan[1], B. M. Leno[1], M. Jakobsen[2], P. Theilgaard[3], and T. R. Overton[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Protekta Inc., Lucknow, ON, Canada,* [3]*Vitfoss, Graasten, Denmark.*

The objective was to determine the effect of feeding zeolite A during the prepartum period on performance. Holstein cows (n = 55) entering second or greater lactation were assigned randomly to 1 of 2 dietary treatments starting 21 d before expected calving; Control (CON): 40% corn silage, 33% wheat straw, and 27% concentrate or Experiment (EXP): CON plus zeolite A (X-Zelit, Protekta Inc., ON, Canada/Vitfoss, Graasten, Denmark) at 3.3% of DM, targeting 500 g/d as-fed. Intake, rumination (HR Tags, SCR Dairy, Netanya, Israel), and milk production were recorded daily. Colostrum and milk samples (1×/wk) were collected for composition and multiple peripartum blood samples were analyzed for oxidative stress. Data were analyzed in SAS with PROC MIXED and repeated-measures analysis was conducted for measures with multiple time points. Survival analysis for time to pregnancy by 150 DIM was analyzed with PROC LIFETEST. Cows fed EXP tended to have lower prepartum DMI (14.0 ± 0.2 kg/d vs. 14.6 ± 0.2 kg/d, $P = 0.07$) but not when expressed as a percent of BW ($P = 0.44$). Prepartum rumination was higher in cows fed CON (521 ± 6 min/d vs. 500 ± 7 min/d, $P < 0.01$). There was no difference in postpartum DMI ($P = 0.51$) or rumination ($P = 0.61$). Oxidative status, expressed as reactive oxygen and nitrogen species/the antioxidant potential, was not different between treatments pre- or postpartum ($P \geq 0.54$). There was no difference in colostrum quantity (CON: 7.3 ± 0.7 kg vs. EXP: 5.8 ± 0.8 kg, $P = 0.16$) or IgG concentration (CON: 7629 ± 455 g/L vs. EXP: 8342 ± 484 g/L, $P = 0.29$). There was a tendency for cows fed EXP to have higher percentage of milk protein (3.30 ± 0.05% vs. 3.19 ± 0.04%, $P = 0.09$), total solids (13.32 ± 0.11% vs. 13.03 ± 0.11%, $P = 0.07$) and there was a treatment by week effect for percent fat ($P = 0.05$) such that EXP fed cows had higher milk fat content, particularly in wk 3 and 4. There were no effects of treatment on milk yield (wk 1–4: $P = 0.60$; wk 1–9: $P = 0.37$), ECM ($P = 0.50$), or ECM/DMI ($P = 0.94$). Median time to pregnancy for EXP cows was 70 d and 89 d for CON cows ($P = 0.17$). Overall, postpartum performance was similar between both treatment groups.

Key Words: hypocalcemia, zeolite A, periparturient performance

M282 Impact of a direct-fed microbial blend on performance of early lactation dairy cows. D. M. Paulus Compart* and T. P. Karnezos, *PMI Nutritional Additives, Shoreview, MN.*

Ninety-six multiparous early lactation (66 ± 23 DIM) Holstein cows were used in a randomized complete block design with 2 treatments, control (C) and additive (AD). Cows were blocked by DIM and milk yield then divided between 12 pens. Cows were fed a TMR 1 time daily with pens on the AD treatment being offered a direct-fed microbial blend (PMI Nutritional Additives, Shoreview, MN) and cows on the C treatment being offered a placebo (PMI Nutritional Additives) mixed into the TMR daily at a feed rate of 113 g per cow per day. Total mixed ration samples were collected daily and composited by week for nutrient analysis. Feed refusals were collected and assessed daily for dry matter (DM). Water intake for each pen was determined daily and averaged by week. Cows were milked 3 times daily and total milk yield was averaged weekly for each cow. Individual cow milk samples were collected once weekly at 3 consecutive milkings and composited for determination of fat, protein, solids nonfat (SNF), MUN, lactose, and SCC content. Somatic cell score was calculated for each cow based on SCC. Energy-corrected milk, DMI, and ECM:DMI were determined for each pen. Cow BW was determined daily and averaged by week. No significant treatment effects were found for any measures. All parameters except for SCS differed significantly by week ($P < 0.03$) as cow DIM increased. Block had a significant effect on milk yield, ECM, percent and kg of milk fat and protein, lactose, SNF, and ECM: DMI. A block by week effect ($P = 0.03$) was observed for water intake and MUN ($P = 0.05$). Additionally, a treatment by week effect ($P < 0.0001$) was observed for MUN with AD cows having lower MUN during wk 5 relative to C cows (10.5 and 12.5 mg/dL respectively). In summary, feeding AD to multiparous early lactation cows did not impact cow performance, but may affect MUN at various points during lactation.

Key Words: direct-fed microbial

M283 Mycotoxin mitigation on commercial farms when feeding Equalize Dairy. K. M. Hultquist* and D. P. Casper, *Furst-McNess Company, Freeport, IL.*

The 2016 corn crop was heavily contaminated with mycotoxins; thus, the objective was to evaluate 2 commercially available products—Competitor (COMP) and Equalize Dairy (EqD; both from Furst-McNess Company) to mitigate mycotoxins on 2 large commercial dairy farms. Both products are combinations of technologies that have multiple mechanisms of action. The experiment was a completely random design (CRD) using approximately 5,300 lactating cows with 13 pens fed COMP and 8 pens fed EqD for Dairy WP and approximately 2,800 lactating cows with 3 pens fed COMP and 5 pens fed EqD for Dairy WD for 8 wk during February and March 2017. The COMP and EqD were incorporated at 30 g/cow/d in the total mixed ration (TMR). Cows were housed in a freestall facility and milked 3 × /d with all milk weights electronically recorded and averaged by cow and then by pen on a weekly basis. Amounts fed and orts were recorded for each pen daily. Corn based TMR ingredients contained varying fumonisin, zearalenone, and vomitoxin concentrations. All data were subjected to least squares ANOVA for a CRD via the PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) with pen as the experimental unit and week as a repeated measure. Milk production data were covariately adjusted using the week before trial initiation when all cows were fed the COMP. Milk production tended to be greater for cows fed EqD on Dairy WP (35.6 and 38.3 kg/d for COMP and EqD, respectively), but was similar for cows fed both products for Dairy WD (42.4 and 42.1 kg/d) and was similar overall (39.0 and 40.2 kg/d). Milk fat percentages were similar on Dairy WP (4.44 and 4.32%), but were greater for cows fed EqD on Dairy WD (3.70 and 3.85%) than cows fed COMP. Somatic cell counts were significantly lower for cows fed EqD on Dairy WP and tended to be lower for cows fed EqD on Dairy WD compared with cows fed COMP. Dry matter intakes were similar on Dairy WP but were increased for cows fed EqD on Dairy WD (26.0 and 28.7 kg/d) and were greater overall for cows fed EqD compared with COMP (26.2 and 28.1 kg/d). In conclusion, feeding Equalize Dairy demonstrated a potential to improve lactational performance and feed intake when dairy cows were fed mycotoxin contaminated feeds.

Key Words: dairy cow, milk yield, mycotoxin

M284 Milk enterolactone concentration in response to sucrose and flaxseed oil supplementation to dairy cows fed flaxseed meal. C. P. Ghedini, A. Fonseca de Brito*, K. Krieger, and G. Tempera, *Department of Biological Sciences, University of New Hampshire, Durham, NH.*

Flaxseed meal (FM) is the richest source of secoisolariciresinol diglucoside (SDG), which is converted by microbes to enterolactone (EL), a metabolite with potential human health benefits. Flaxseed oil and sucrose may favor ruminal microbes involved in SDG metabolism. The objective of this study was to evaluate the effects of feeding sucrose and flaxseed oil on production and milk EL concentration in diets containing FM. Sixteen multiparous Holstein cows (94 ± 37.6 DIM and 680 ± 79.1 kg of BW) were assigned to treatment sequences in a replicated 4 × 4 Latin square with 18 d for diet adaptation and 7 d for data and sample collection. Diets were fed as TMR and included (DM basis): (1) 8% soybean meal + 23% ground corn (-control, -CRTL); (2) 15% FM + 10.7% ground corn + 5% sucrose (SUCR); (3) 15% FM + 15.4% ground corn + 3% flaxseed oil (FXO); (4) 15% FM + 10.2% ground corn + 5% sucrose + 3% flaxseed oil (SUCR+FXO). All diets contained 40% corn silage and 20% grass-legume haylage, had similar energy and RDP/RUP content and averaged 17.2% CP and 32.1% NDF. Cows fed diets containing FM had lower DMI (22.7 kg/d) than those fed the –CRTL diet (24.6 kg/d; $P < 0.001$). Within the FM diets, FXO and SUCR+FO had the lowest DMI (22.3 kg/d). Milk yield was greatest in cows fed –CRTL (36.2 kg/d) and lowest in those fed SUCR+FXO (33.4 kg/d; $P = 0.05$). Cows fed FXO and SUCR+FXO had lower ECM (31 kg/d) than those fed -CRTL (36.3 kg/d) and SUCR (36.4 kg/d; $P < 0.001$). Milk concentration of EL was lowest in cows fed -CRTL (76.8 n*M*), but no difference was observed in diets containing FM [293, 332, and 338 n*M* with feeding SUCR, FXO and SUCR+FXO, respectively ($P < 0.01$)]. Milk fat concentration and yield were lowest in diets with flaxseed oil and averaged: 3.66% and 1.33 kg/d; 3.69% and 1.32 kg/d; 2.96% and 1.03 kg/d; and 2.83% and 0.93 kg/d in cows fed -CRTL, SUCR, FXO, and SUCR+FXO, respectively ($P < 0.001$). Milk protein concentration and yield did not differ and averaged 2.95% and 1.01 kg/d, respectively. In conclusion, there were no synergistic effects of sucrose and flaxseed oil on the conversion of SDG to EL in the rumen.

Key Words: dairy cow, enterolactone, flaxseed meal

M285 Meta-analysis of the use of canola meal in diets for dairy cows. D. C. Moura, K. C. Alessi*, J. R. Assis, R. N. Torres, S. R. Soares, A. B. Donadia, H. M. Silva, L. Menegazzo, M. L. Marques, I. Martins, and A. S. Oliveira, *Dairy Cattle Research Lab, Universidade Federal de Mato Grosso, Sinop, Sinop, Mato Grosso, Brazil.*

Data from 37 peer-reviewed papers were summarized to examine the effect of canola meal (CM) in diet on the performance of dairy cows. The effects were compared by raw mean differences (RMD) between CM diet and control treatment means and weighted by inverse variance using random-effect models. Control treatments (28.6 ± 6.9 kg milk/d) were soybean meal (SBM; 57%), dried destillers grains and solubles (DDGS; 21%), corn gluten meal (7%), cottonseed meal (5%), sunflower meal (2%) and other protein sources (9%). Heterogeneity level was analyzed by I^2 statistic (low ≤25%; moderate = 26 to 50%; and high >50%). In overall, use of CM as protein source increased DM intake (RMD = 0.22 ± 0.12 kg DM/d; $P < 0.01$; n = 79; $I^2 = 9.1\%$) and crude protein (CP) intake (RMD = 0.14 ± 0.07 kg CP/d; $P < 0.01$; n = 33; $I^2 = 21.1\%$), but it did not affect organic matter total-tract digestibility ($P = 0.50$; n = 12; $I^2 = 29.2\%$). In overall, use of CM increased milk yield (RMD = 0.69 ± 0.35 kg/d; $P < 0.01$; n = 88; $I^2 = 74.9\%$), but its effect depends on protein sources comparation: replacement of CM versus SBM did not affect milk yield (RMD = 0.23 ± 0.66 kg/d; $P = 0.50$; n = 50), but milk yield increased with CM compare with DDG (RMD = 2.03 ± 1.67 kg/d; $P < 0.01$; n = 18) and other protein sources (RMD = 0.82 ± 0.43 kg/d; $P < 0.01$; n = 20). In overall, CM use did not affect milk protein content ($P = 0.08$; n = 60; $I^2 = 19.5\%$) and milk fat content ($P = 0.20$; n = 60; $I^2 = 16.9\%$), but CM increased milk protein yield (RMD = 0.02 ± 0.01 kg/d; $P < 0.01$; n = 60; $I^2 = 0\%$). Use of CM reduced milk urea nitrogen (N) (RMD = −0.98 ± 0.31 mg/dL; $P < 0.01$; n = 22; $I^2 = 32.2\%$) and increased N intake milk efficiency (RMD = 0.22% N milk/N intake ± 0.07 mg/dL; $P \leq 0.05$; n = 34; $I^2 = 0\%$), both with low heterogeneity. We concluded that CM is similar protein source to SBM and it is more effective than DDG and other sources (cottonseed meal, corn gluten meal and sunflower meal) to lactating dairy cows.

Key Words: canola meal, effect size, heterogeneity

M286 Effect of prepartal maternal diets supplemented with essential fatty acids and their preweaned calves on performance of the newborn calf. A. Jolazadeh[1], T. Mohammadabadi[1], M. Dehghan-Banadaky*[2], M. Chaji[1], and M. Garcia[3], [1]*Department of Animal Science, Khuzestan Ramin Agricultural and Natural Resources University, Mollasani, Ahvaz, Iran,* [2]*Department of Animal Science, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran,* [3]*Department of Animal Science and Industry, Kansas State University, Manhattan, KS.*

The objective was to evaluate the effect of supplementing Ca-salts of fish oil or Ca-salts of soybean oil during the last 3 wk of gestation and during the preweaning period of calves on growth performance of Holstein calves. During the last 3 wk of pregnancy, Holstein cattle (n = 120) were fed no fat supplement (CON), supplemented with Ca-salts of soybean oil (CSO, 140 g/cow/daily) or supplemented with Ca-salts of fish oil (CFO, 140 g/cow/daily). Eighty-four Holstein calves were used (n = 14 calves per treatment) in a completely randomized design, with dietary treatments in a 3 × 2 factorial arrangement (3 dam diets and 2 calf starters; n = 6). Newborn calves were fed a starter diet with either no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2; contained 85% fat (1% C14:0, 28% C16:0, 3% C16:1, 5% C18:0, 26% C18:1, 30% C18:2, 3% C18:3, 4% others) and 9% Ca produced by Persiafat, Kimiya Danesh Alvand Co., Tehran, Iran). Total intake of starter during all the experimental period was not affected by type of calf starter (CS) or dam diets. Calves born from cattle fed CFO and CSO prepartum tended ($P = 0.07$) to have greater overall ADG compared with calves born from cattle fed no fat supplement prepartum (648, 643 versus 609 g/d, respectively). Calves fed the FC-2 CS had consistently greater ADG and feed efficiency (FE; kg of BW gain/kg of DMI) compared with calves fed the FC-0 CS (640 and 0.537 vs. 580 and 0.469, respectively). Calves born from dams fed fat tended ($P = 0.09$) to have greater weaning weight (WW) than those born from dams fed CON, but calves fed FC-2 CS had greater WW and final weight during the experimental period. Result indicated that feeding moderate amounts of polyunsaturated long-chain fatty acids during the last weeks of uterine life and preweaning time could improve ADG and FE on newborn calf.

Key Words: dairy calf, fatty acid, performance

M287 Effect of camelina meal and camelina expeller on rumen microbial fermentation and nutrient flow in a continuous culture system. H. Salas*, L. Castillejos, M. Lopez-Suarez, and A. Ferret, *Animal Nutrition and Welfare Service (SNIBA), Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

Camelina meal and camelina expeller could become an alternative to currently available sources of protein due to their great protein content. The objective of this study was to compare the effect of camelina meal and camelina expeller on rumen fermentation and nutrient flow in an in vitro system. Treatments were 4 diets in which the main protein sources were: soybean meal-44 (SM), 00-rapeseed meal (RM), camelina meal (CM) and camelina expeller (CE) at 12.7%, 16.6%, 16.9% and 18.1% of inclusion (DM basis), respectively. Diets were formulated with a 90:10 concentrate:forage ratio, and to be isocaloric (2.8 Mcal ME/kg DM) and isonitrogenous (13.5% CP, on DM basis). Eight 1,320-mL dual-flow continuous culture fermentors were used in 2 replicated periods with 5 d for adaptation and 3 d for sampling. Temperature (39°C), pH (6.2), and liquid (0.10/h) and solid (0.05/h) dilution rates were maintained constant. Diets (95 g DM/d) were fed in 3 equal portions during the day. Effluent samples were collected from a composite of the 3 sampling days, and bacteria were isolated from fermentor flasks on the last day of each period. A randomized block design was used. Differences were analyzed using the MIXED procedure of SAS. The model contained treatment as fixed effect and period as random effect. The CE diet tended to present a higher ($P < 0.06$) OM true digestibility (57.4%) than SM (48.0%). Total VFA was higher ($P < 0.009$) in CE and CM than in SM (138.8 m*M* and 135.6 m*M* vs 119.6 m*M*; respectively) and butyrate proportion was lower ($P < 0.02$) in CM than in SM (12.1 mol/100 mol vs 16.4 mol/100 mol; respectively). Dietary nitrogen flow tended ($P < 0.06$) to be lower in CE (1.13 g/d) than in CM (1.61 g/d) and CP degradation tended ($P < 0.07$) to be higher in CE than in CM. Although the efficiency of microbial protein synthesis was not affected by treatment, CE showed a higher digestibility than SM and higher CP degradation than CM, whereas CM showed similar results to the reference proteins (SM and RM) but increasing the total VFA concentration.

Key Words: protein source, camelina co-product, rumen microbial fermentation

M288 Repeated inoculation of young calves failed to modulate rumen microbiota consistently but lowered diarrhea. L. Wang[1], L. Ma[2,3], X. Zhang[2], J. Xu[2,3], Z. Yu[1], and D. Bu*[2,4], [1]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [2]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China,* [4]*Hunan Co-Innovation Center of Safety Animal Production, Changsha, Hunan, China.*

The complex rumen microbiota exhibits some host specificity. The undeveloped simple rumen microbiota is hypothetically more amendable. The objective of this study was to investigate if the rumen microbial assemblage of young calves can be shaped by oral inoculation with rumen fluid of adult cows. Fresh rumen fluid collected from 4 donor cattle served as the inoculum for 4 groups (n = 5) of newborn calves. Each calf was drenched at d 3, d 7, d 21, d 42, and d 50 with 100, 200, 300, 400, or 500 mL inoculum, respectively. Two of the 4 calf groups received fresh rumen fluid, while the other 2 received autoclaved rumen fluid. The fresh rumen fluid did not affect ($P > 0.05$) feed intake, body growth, heart girth, or feed/BW ratio. However, fresh rumen fluid significantly ($P < 0.01$) lowered instance of diarrhea. At the age of 77 d, rumen fluid samples were collected from all the calves and subjected to analysis for fermentation characteristics and to microbiota characterization. Fermentation characteristics were not affected. Amplicon sequencing of the V4-V5 region of the 16S rRNA genes using MiSeq followed with sequences analysis using Qiime showed that donor adult cattle as expected had a more diverse microbiota than the calves, which had a rumen microbiota distinct from that of the donors. Two *Succinivibrionaceae* OTUs, 2 *Prevotella* OTUs, and one *Succiniclasticum* OTU were predominant (relative abundance > 2%) in the donors, but only one *Succinivibrionaceae* OTU was found in the calves. On the other hand, 5 other *Prevotella* OTUs were predominant (>3%) in the calves, but none of them was a major OTU in the donors. No correlation was observed in relative abundance of major OTUs or genera between the donor and the calves. Principal coordinates analysis of weighted UniFrac distance showed no significant difference in microbiota profiles among the 4 calf groups. None of the calf rumen microbiota showed any clustering with their donor's. Repeated oral inoculation with rumen fluid probably has little effect on the development of rumen microbiota, and the rumen microbiota seems to develop following a program determined by the host and other factors.

Key Words: calf, oral inoculation, rumen microbiota

M289 Effects of selenium source on, performance and antioxidant status in lactating dairy cows during oxidative stress-inducing conditions. L. Sun[1], S. Gao[1], K. Wang[1], M. V. Sanz Fernandez[4], L. H. Baumgard[5], and D. Bu*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China,* [3]*Hunan Co-Innovation Center of Safety Animal Production, Changsha, Hunan, China,* [4]*Comparative Physiology Group, SGIT-INIA, Madrid, Spain,* [5]*Department of Animal Science, Iowa State University, Ames, IA.*

In the current study, we utilized heat stress (HS) as an oxidative stress model to examine the effects of hydroxy-selenomethionine (HMSeBA). A novel dietary organic selenium (Se) source was compared with sodium selenite (SS) on performance, antioxidant status, and Se concentrations in serum and milk of dairy cows. Eight mid-lactation Holstein dairy cows (141 ± 27 DIM, 35.3 ± 2.8 kg of milk/d, parity 2 or 3) were individually housed in environmental chambers and randomly assigned to 1 of 2 treatments: SS supplementation (0.3 mg Se/kg DM; n = 4) or HMSeBA supplementation (0.3 mg Se/kg DM; n = 4). The trial was divided into 3 continuous periods: covariate period (9 d), thermal neutral (TN) period (28 d), and a heat stress (HS) period (9 d). During the covariate and TN periods, all cows were housed under TN conditions (20°C, 55% humidity). During HS, all cows were exposed to cyclical HS conditions (32–36°C, 40% humidity). All cows were fed SS during the covariate period, and dietary treatments were implemented during the TN and HS periods. The statistical model used to analyze the effect of treatment on the parameter was: $Yijkl = \mu + Ti + Pj + TPij + bXjk + C(TP)ijk + Dl + DTil + DPjl + DTPijl + \varepsilon ijkl$. Compared with SS controls, cows fed with HMSeBA had higher total Se concentrations in serum (25.5%, $P < 0.05$) and milk (71.4%, $P < 0.0001$ = and total Se milk:serum concentration ratio (35.7%, $P < 0.01$). During HS, supplementing HMSeBA tended to increase milk yield (9.3%, $P < 0.1$) and decreased milk fat content during HS (18.1%, $P < 0.05$). There were no significant treatment effects on blood biochemistry, except for a lower alanine aminotransferase activity in the HMSeBA treatment (24.4%, $P < 0.05$). Glutathione peroxidase and superoxide dismutase activity did not differ between treatments, but cows supplemented with HMSeBA had higher total antioxidant capacity (20%, $P < 0.05$), and lower malondialdehyde (15.1%, $P < 0.05$), hydrogen peroxide (22.5%, $P < 0.05$), and nitric oxide (29.6%, $P < 0,05$) concentrations in serum. These results

demonstrate that HMSeBA dietary supplementation effectively reduces HS-induced oxidative stress.

Key Words: dairy cow, hydroxy-selenomethionine, sodium selenite

M290 Effects of rumen-protected methionine supplementation on dairy cows during early postpartum. T. C. Michelotti[1], H. A. Pacheco[1], F. Lopes[2], and R. de Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Adisseo South America, São Paulo, SP, Brazil.*

Rumen-protected methionine (RPM) could be an important tool to optimize nutrition and health in transition dairy cows. Therefore, the objective of this study was to investigate the effects of RPM during early lactation on productive and metabolic parameters of dairy cows. The trial was conducted in a commercial herd in Paraná State, Southern Brazil, from January to February 2017. A total of 42 cows were blocked by lactation order and calving date. The treatment group received 12 g daily of methionine (Smartamine®, Adisseo) and 38 g of corn meal, while the control group received 50 g/cow/d of corn meal. The supplementation was carried out for 29 ± 8 d postpartum, beginning at calving. Total mixed ration (TMR) samples were collected weekly throughout the experiment period. Six blood samples were collected on d 0, 1, 3, 5, 7, and 14 postpartum. Data were analyzed using the MIXED procedure of SAS with a model containing the effects of calving order, treatment, time, and treatment*time interaction as fixed effects and cow within treatment as a random effect. Milk yield in the first 30 d of lactation did not differ (34.9 kg/d in the control and 33.6 kg/d in the RPM group; SEM = 1.6 kg/d, $P = 0.53$) between experimental groups. Animals supplemented with RPM produced milk with higher fat content (4.07 vs. 3.59% ± 0.11; $P < 0.01$) and higher fat to protein ratio (1.14 vs. 1.05 ± 0.03; $P < 0.05$) than control cows. There was a trend of the methionine supplemented cows to produce milk with higher protein (3.58 vs. 3.45% ± 0.05; $P = 0.07$) and casein (2.82 vs. 2.72% ± 0.05; $P = 0.10$) contents and higher milk energy output (26.7 vs. 24.4 Mcal/d ± 1.1; $P = 0.13$) than control cows. Analysis of metabolites on serum blood (NEFA, BHB, bilirubin, Ca, albumin, glucose, total protein, cholesterol, triglycerides, urea, and aspartate aminotransferase) and urine (creatinine, urea, and uric acid) did not demonstrate ($P > 0.10$) any beneficial effects of RPM, possibly as a result of supplementation only in the postpartum period. In summary, methionine supplementation in the immediate postpartum period showed positive results restricted to milk composition.

Key Words: amino acid, milk composition, transition period

M291 Characterization of sphingomyelin in bovine lipoproteins during the peripartum. A. N. Davis*[1,2], J. E. Rico[1,2], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV.*

Lipoprotein sphingomyelin (SM) is a choline-containing phospholipid. Hepatic steatosis and hyperlipidemia promotes long-chain SM accumulation in liver. Following the hepatic secretion of SM within lipoproteins, circulating SM may be hydrolyzed to increase the insulin antagonist ceramide. Our objective was to characterize changes in bovine lipoprotein SM during the peripartum, and compare lipoprotein SM levels with insulin sensitivity. Twenty-five pregnant, multiparous Holstein dairy cows were enrolled 28 d before expected parturition and fed a diet formulated to meet or exceed nutrient requirements. The hyperinsulinemic-euglycemic clamp procedure was performed to assess insulin action. Blood and liver were collected at d −12 and 10, relative to parturition. Blood was also collected at calving. To isolate TAG-rich, low-density, and high-density lipoproteins (VLDL, LDL, and HDL, respectively), we used fast protein liquid chromatography. Lipoprotein SM were measured using mass spectrometry. Data were analyzed using a mixed model with repeated measures including fixed effect of time and random effect of cow. VLDL total SM and dihydro-SM levels decreased by ~75% from d −12 to parturition ($P < 0.01$) and remained low through d 10 postpartum. In contrast, LDL total SM decreased 22% from d −12 to 0 then increased 72% by d 10 ($P < 0.01$). A less pronounced but similar pattern was observed for HDL total SM. Across all lipoprotein classes, C22:0, C22:1, C20:0, and C16:0 SM were the most abundant SM species. All 8 SM and 3 dihydro-SM quantified in VLDL decreased postpartum ($P < 0.01$); however, species differences were observed for LDL and HDL SM. For example, postpartum elevations in LDL C16:0, C20:0, and C24:0 SM were observed, whereas LDL C24:1 SM decreased ($P < 0.01$). Interestingly, VLDL total SM was inversely related to liver lipid content ($r = −0.59$; $P < 0.01$) and basal fatty acid levels ($r = −0.44$; $P < 0.01$). Prepartum and postpartum VLDL total SM were negatively correlated with insulin sensitivity (clamp index; ~$r = −0.50$; $P < 0.05$), a response not observed for LDL or HDL total SM. Our results confirm dynamic remodeling of bovine lipoprotein SM during the peripartum. Moreover, changes in VLDL SM relate to steatosis, hyperlipidemia, and insulin sensitivity.

Key Words: lipoprotein, peripartum, sphingomyelin

M292 Application of fast protein liquid chromatography to characterize bovine lipoproteins during the periparturient period. A. N. Davis[1,2], J. E. Rico*[1,2], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV.*

The isolation of bovine lipoproteins often involves ultracentrifugation and gel filtration chromatography; however, large sample volumes, lengthy run times, and high centrifugal forces and salt concentrations that may cause dissociation of lipoproteins are often applied. Alternatively, the utilization of size exclusion chromatography by fast protein liquid chromatography (FPLC) avoids these method inputs. Our objective was to utilize FPLC to characterize the lipid and protein composition of bovine lipoproteins during the periparturient period. Blood samples were collected from 25 peripartal Holstein dairy cows before feeding at d −12, 0, and 10, relative to parturition. Serum lipoproteins were isolated using FPLC and a size exclusion column. Measurement of total triacylglycerol (TAG), phospholipid (PL), cholesterol, and protein was performed using colorimetry. Data were analyzed as repeated measures using a mixed model (fixed effect of fraction and random effect of cow). Our approach revealed 4 distinct fractions: TAG-rich (VLDL), low-density (LDL), and large (buoyant) and small (dense) high-density lipoprotein (HDL) subclasses. VLDL primarily contained TAG (56, 31, and 35% of total components at d −12, 0, and 10, respectively). VLDL TAG levels were greater at d −12 ($P < 0.01$). LDL primarily contained PL (54, 52, and 55% of total components at d −12, 0, and 10, respectively) and cholesterol (42, 45, and 42% of total components at d −12, 0, and 10, respectively). LDL PL levels were lowest at parturition ($P < 0.01$). LDL cholesterol followed a similar pattern ($P < 0.01$). Buoyant HDL contained equal levels of PL and cholesterol (36 and 45% of total components, respectively), and buoyant HDL PL and cholesterol levels were lowest at parturition ($P < 0.01$). Protein levels were greatest in dense HDL (73% of total components; $P < 0.01$). Protein levels within buoyant HDL were greatest at d 10 ($P < 0.05$), whereas dense HDL protein levels were not modified by time. Because the observed lipoprotein composition is in agreement with previous work using

alternative methods, we conclude that the use of FPLC is a means to isolate bovine lipoproteins from peripartal cows.

Key Words: chromatography, lipoprotein, peripartum

M293 Effect of washing method, grinding size, and the determination of an undegraded fraction on in situ effective ruminal disappearance and disappearance rate of starch in mature corn grain. T. Fernandes[1,2], C. L. S. Ávila[1], M. N. Pereira[1], and L. F. Ferraretto*[2], [1]*Federal University of Lavras, Lavras, MG, Brazil,* [2]*University of Florida, Gainesville, FL.*

Our objectives were to determine (1) the best method to estimate the fast degradable fraction (A); (2) a time point to measure the undegraded fraction (C); and (3) the viability of using less time points to estimate starch fractional disappearance rate (kd) of mature corn ground through 4 grinding sizes (1, 2, 4, and 6 mm). Fraction A was determined by rinsing in a bucket or washing machine, rumen immersion followed by bucket or washing machine, warm water immersion for 30-min followed by bucket or washing machine. Ruminal in situ incubations were performed at 48, 72, 96, and 120-h to determine fraction C, and at 0 (A; washing machine), 3, 6, 12, 18, 24, and 48-h to determine kinetics of starch disappearance. Models were used with either 2 or 3-pools and kd determined by the slope of the 'ln'. Effective ruminal disappearance (ERD) was calculated as: A + B [kd / (kd + kp)], kp = 7.47% h^{-1}. Data were analyzed using PROC MIXED with the fixed effect of method (either washing or model), grinding size, and their interaction and the random effect of run or cow. Correlation between estimates calculated using all time points or combinations of 2 and 3 time points were determined using PROC CORR. Fraction A was affected by grinding size ($P < 0.01$; 23.5, 15.7, 10.4 and 8.5%, for 1, 2, 4, and 6-mm); but not ($P > 0.10$) washing method. Samples ground at 6-mm had greater ($P < 0.05$) fraction C than other grinding sizes at 48 (14.7 vs. 2.45%), 72 (9.1 vs. 1.7%) or 96-h (2.9 vs. 0.3%); but not ($P > 0.10$; 0.1%) at 120-h. Models differed ($P < 0.01$) by 1.8%-unit for fraction B. As grinding size increased from 1 to 6-mm, greater fraction B (66.0, 73.8, 74.9, and 79.4%), but reduced kd (5.4, 5.1, 4.9, and 3.4% h^{-1}) and ERD (59.1, 53.7, 48.8, and 40.5%) were observed ($P < 0.05$). Based on correlation analysis the 2-pools model, and the incubation times of 0, 3, and 48-h were suitable ($P < 0.001$, r = 0.998) to evaluate starch in mature corn. Ruminal in situ incubations of 0, 3, and 48-h for starch disappearance kinetics using a 2-pool model are suggested for mature corn. Grinding size affects starch disappearance kinetics but not fraction A method.

Key Words: effective ruminal disappearance, disappearance rate, starch

M294 Relationships among chewing activity and lactation performance by dairy cows and dietary nutrient composition. L. M. Krentz[1], L. F. Ferraretto*[2], and R. D. Shaver[3], [1]*Vita Plus Corporation, Madison, WI,* [2]*University of Florida, Gainesville, FL,* [3]*University of Wisconsin, Madison, WI.*

A meta-analysis was performed with a data set of 431 treatment means from 117 trials reported in 107 published peer-review journal reports from 1988 to 2015. The study had 3 steps: (1) examine relationships between chewing activity and selected dietary treatments; (2) determine effects of chewing activity on lactation performance by dairy cows; and (3) develop predictive equations for rumination time (min/d) and milk fat content and yield. For steps 1 and 2, data were analyzed using PROC MIXED of SAS with fixed effects of treatment and random effect of trial. A manual deletion stepwise backward elimination of multiple regressions was performed to generate predictive equations for step 3. Intake of DM was unrelated to eating, rumination and chewing times (min/d; $P > 0.90$); but negatively related to rumination and chewing times (min/kg of DMI; $P = 0.001$). Eating, rumination and chewing times were positively related to dietary NDF and forage NDF (FNDF) concentrations. Greater consumption of NDF and FNDF both, when expressed as kg/d or percentage of BW, elicited ($P = 0.001$) greater eating, rumination and chewing times. Greater eating time reduced ($P < 0.05$) milk, milk protein, 3.5% FCM and ECM yields; but stimulated greater ($P = 0.001$) milk fat content. Greater rumination time was associated ($P < 0.01$) with enhanced milk fat content and yield, tended ($P = 0.07$) to reduce milk protein content, and was unrelated ($P > 0.10$) to other lactation performance parameters. Greater chewing time was related ($P = 0.001$) to greater milk fat content but reduced ($P < 0.05$) milk yield and milk protein content. Best-fit model to predict rumination time included feed efficiency (kg milk/kg DMI), FNDF (% of DM) and DMI (kg/d). Prediction of milk fat content was best achieved by NDF intake (kg/d) and dietary NDF and FNDF concentrations. Chewing activity was affected by dietary concentrations or intake of NDF and FNDF. Chewing activity influenced lactation performance.

Key Words: rumination, chewing activity, milk fat

M295 Evaluating the impact of Bovamine on performance, nutrient digestibility, and digestive function in lactating dairy cows. M. Conklin*[1], S. Polukis[1], A. Barnard[1], K. Nestor[2], L. Kung Jr.[1], and T. Gressley[1], [1]*University of Delaware, Newark, DE,* [2]*Chr. Hansen Animal Health and Nutrition, Hørsholm, Denmark.*

Two experiments evaluated the effect of a direct-fed microbial (Bovamine, Chr. Hansen, Hørsholm, Denmark) on performance and digestion of dairy cows. In Experiment 1 (Exp1), 30 multiparous cows (40–160 DIM) were assigned to one of 2 treatments fed for 10 weeks, DFM (3×10^9 cfu/d Bovamine) or CON (control, no Bovamine). In Experiment 2 (Exp2), 6 ruminally cannulated cows (123 ± 129 DIM) were assigned to a crossover design with 6-wk periods with the same treatments as Exp1 except that cows were fed a 23.8% starch diet during wk 1–5 of each period and then abruptly switched to a 31.1% starch diet for wk 6. For both experiments, intake and milk yield were measured daily and milk samples were collected weekly. In Exp1, fecal grab samples were collected every 6 h on d 7 of wk 1, 2, 4, 6, 8, and 10, fecal consistency was scored, and fecal starch measured in composited daily samples. Composites from a subset of 7 cows per treatment were used to measure apparent total-tract nutrient digestibility. In Exp2, rumen pH was continuously recorded during wk 5–6. Rumen in situ digestibility was measured on wk5d7, wk6d1, and wk6d7. On those dates, rumen fluid and feces were collected every 6 h for rumen VFA, fecal pH, and fecal starch (composited by cow within day). In Exp1, treatment did not affect intake, milk yield or composition, fecal score or fecal starch. DFM tended to increase starch digestibility compared with CON (98.74 vs. 98.46%, $P = 0.051$), but digestibility of other nutrients was unaffected. In Exp2, intake, milk yield, and milk composition were unaffected when evaluated over the entire study. However, during the abrupt switch to the high starch diet, milk fat yield was increased for DFM compared with CON (1.39 vs. 1.28 kg/d, $P = 0.002$) and milk fat tended to increase (3.59 vs. 3.42%, $P = 0.09$). Treatment did not affect rumen pH, rumen VFA, or in situ digestibility. Contrary to Exp1, DFM increased fecal starch compared with CON during the high starch feeding (2.49 vs. 2.03%, $P = 0.02$). Overall, Bovamine modestly improved digestion as demonstrated by increased starch digestibility in Exp1 and increased milk fat during the high starch challenge in Exp2.

M296 The effects of rumen degradable starch and fiber on differential gene expression of the rumen epithelium. L. Beckett*, A. Bedford, C. Gleason, D. Liebe, T. T. Yohe, K. M. Daniels, and R. R. White, *Virginia Tech, Blacksburg, VA.*

Absorbative and metabolic capacity of the rumen epithelium adapt to different supplies of substrate; however, the nature of this adaptation is poorly characterized. This study sought to determine the effect of different levels of rumen degradable starch and fiber on gene expression in the rumen epithelium. Six yearling, ruminally cannulated Holstein heifers (BW 329 ± 11 kg) were used in a partially replicated Latin Square experiment with 4 treatment diets consisting of high and low rumen degradable starch and NDF arranged factorially. Animals underwent a 3-d diet adaptation period and 14-d diet treatment period. In situ digestibility was assessed by incubating nylon bags of each treatment diet in the rumen from d 10 to 14. Rumen papillae biopsy samples were collected from the ventral sac on d 18 and stored in RNALater at −80°C until RNA isolation. Real-time quantitative reverse transcription PCR was used to measure relative gene abundance. Mixed effect models with fixed effects of starch and fiber and random effects of heifer and period were used for all statistical analyses. In situ results demonstrated the diets had different rates of ruminal starch digestion (4.4%/h vs 7.1%/h; $P = 0.05$), and different ruminal degradability of NDF (29.9% of dietary NDF vs 38.3%; $P = 0.003$). There was a significant fiber effect on HSP70 ($P = 0.02$) and MCT4 ($P = 0.04$), and a tendency for a fiber effect on MCT1 ($P = 0.09$). When high ruminally degradable fiber diets were fed, MCT1 and MCT4 expression were reduced, which suggests a negative effect on short-chain fatty acid (SCFA) absorption. Also, AKT1 had a significant starch by fiber interaction ($P = 0.049$). AKT1 regulates different aspects of metabolism suggesting ruminal nutrient availabilities likely affect basic metabolic function within the rumen epithelium. Reduced expression of SCFA transporters and AKT1 suggests different nutrient degradabilities could impair normal absorbative and metabolic function within the rumen epithelium.

Key Words: nutrient degradability, gene expression, rumen epithelium

M297 Effect of palmitic acid-enriched supplements with different levels of fatty acid peroxidation on short-term feed intake and production responses of lactating dairy cows. A. Pineda* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the short-term effects of feeding palmitic acid (PA)-enriched supplements with different levels of peroxidation on feed intake and production responses of dairy cows. Eight Holstein cows (147 ± 40 DIM, 44.6 ± 4.84 kg/d milk yield) were assigned randomly to treatment sequence in a replicated 4 × 4 Latin square design balanced for carryover effects. Cows received a common basal diet which contained (% DM) 29.1% NDF, 17.3% CP, and 28.6% starch. Fatty acid (FA) treatments contained different combinations of a commercially available PA-enriched supplement (PA-NORM) and a PA-enriched supplement with a high level of peroxidation (PA-HIGH) as follows: (1) control (CON) supplemented with 1.5% DM PA-NORM; (2) low peroxidation (LPO) supplemented with 1.0% DM PA-NORM and 0.5% DM PA-HIGH; (3) medium peroxidation (MPO) supplemented with 0.5% DM PA-NORM and 1.0% DM PA-HIGH; and (4) high peroxidation (HPO) supplemented with 1.5% DM PA-HIGH. Each 14-d period consisted of a 7-d washout during which all cows received CON followed by 7-d of feeding all treatment diets with the final 3 d used for data and sample collection. The statistical model included the random effect of cow and the fixed effects of FA treatment, period, and their interactions. The major FA in PA-NORM and PA-HIGH were C16:0 (82.2 and 93.8%), C18:0 (5.23 and 3.49%), and *cis*-9 18:1 (9.41 and 1.30%), respectively. Results in the text are presented in the following order: CON, LPO, MPO, and HPO. Fatty acid treatments did not affect DMI (29.4, 29.6, 30.7, and 30.5 kg/d; $P = 0.13$) or yields of milk (44.1, 44.2, 44.5, and 45.0 kg/d; $P = 0.76$), milk fat (1.65, 1.61, 1.62, and 1.68 kg/d; $P = 0.13$), or milk protein (1.44, 1.42, 1.44, and 1.47 kg/d; $P = 0.69$). There was no effect ($P > 0.20$) of treatment on BW, BCS, or yields of de novo, mixed, and preformed milk FA. Our results demonstrate that short-term feeding of PA-enriched supplements with high levels of peroxidation does not negatively affect DMI or production responses of mid lactation dairy cows.

Key Words: lipid peroxidation, fatty acid, milk production

M298 Effects of dietary undigested and physically effective neutral detergent fiber on ruminal pH, volatile fatty acids, and ruminal digesta characteristics of lactating dairy cows. W. A. Smith*[1], K. Ishida[2], J. W. Darrah[1], H. M. Dann[1], C. S. Ballard[1], M. D. Miller[1], and R. J. Grant[1], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*ZEN-NOH National Federation of Agricultural Cooperative Associations, Japan.*

The objective of this study was to evaluate the effect of feeding different dietary concentrations of 240-h undigested neutral detergent fiber (uNDF240) and physically effective neutral detergent fiber (peNDF) on ruminal pH, volatile fatty acid (VFA) concentrations and ruminal digesta characteristics. Sixteen Holstein cows, 8 ruminally cannulated, averaging 123 (SD = 9) days in milk (DIM) were used in a replicated

Table 1 (Abstr. M298).

Item	LULP	LUHP	HULP	HUHP	SE	*P*-value
Daily mean pH	6.11[b]	6.17[ab]	6.22[ab]	6.24[a]	0.05	0.03
Daily standard deviation pH	0.29	0.29	0.24	0.28	0.03	0.10
Area under curve[1] < 5.8	52.0	49.6	33.5	30.0	15.0	0.29
Total VFA, m*M*	122.8[a]	120.6[ab]	118.3[ab]	112.3[b]	4.1	0.05
Acetate (A), %	63.4	63.8	63.9	64.1	0.94	0.18
Propionate (P), %	22.7[a]	22.5[a]	21.5[b]	21.6[b]	0.83	<0.01
A:P	2.83[c]	2.89[bc]	3.04[a]	3.01[ab]	0.15	<0.01
Ammonia-N, mg/dL	4.38	5.04	4.72	4.93	0.61	0.45
Ruminal digesta volume, L	110	111	116	111	4	0.42
Ruminal digesta mass, kg	95	95	100	97	4	0.34

[a–c]Within a row, values with different superscripts differ ($P \leq 0.05$).

[1]Area < 5.8 = ruminal pH units below 5.8 by hour.

4 × 4 Latin square design with 4-wk periods. Cows were fed diets formulated to be isoenergetic and isonitrogenous but varying in uNDF240 and peNDF by changing forage to concentrate ratio and particle length of hay. Treatments were 1) 8.6% uNDF240 and 18.4% peNDF (LULP), 2) 8.6% uNDF240 and 19.9% peNDF (LUHP), 3) 10.5% uNDF240 and 16.2% peNDF (HULP), and 4) 10.5% uNDF240 and 19.0% peNDF (HUHP). Cows were housed in individual tie stalls and fed TMR once daily. Indwelling pH loggers recorded ruminal pH of cannulated cows on d 23–26 and ruminal fluid samples were collected every 4 h on d 26 and analyzed for VFA and NH_3-N concentrations. Ruminal evacuations occurred on d 27 and 28 to determine ruminal digesta characteristics. All data were analyzed as a replicated Latin square design using ANOVA and the MIXED procedure of SAS (version 9.4). Models included the fixed effects of diet, period, replicate, and time (as appropriate) and the random effect of cow within replicate. Greater forage particle size with low uNDF240 diets resulted in ruminal pH, VFA concentration, and ruminal digesta characteristics not different to higher uNDF240 diets with smaller particle size (Table 1).

Key Words: ruminal digesta, particle size, undigested fiber

M299 Immunoactivation is related to low plasma arginine and branched-chain amino acid concentrations in lactating dairy cows. J. A. D. R. N. Appuhamy, E. A. Horst, S. K. Kvidera, H. J. P. Wickramasinghe*, and L. H. Baumgard, *Iowa State University, Ames, IA.*

Immune system activation increases nutrient and oxygen demand and could alter availability of amino acids (AA) for milk production. Given that the plasma free AA pool is small, changes in the concentrations could reflect changes in uptake or supply of AA by different organs. Plasma concentration of 3-methylhistidine (3-MH) is a good indicator for protein mobilization from muscle. Immune challenge can be modeled by administrating lipopolysaccharides (LPS) eliciting a well-characterized and robust immune response. Study objectives were to examine the impact of immune system activation by LPS on plasma free amino acid and 3-MH concentrations in lactating dairy cows. Twelve lactating Holstein cows were jugular catheterized and assigned to 1 of 2 bolus treatments: control (CON; 5 mL of saline, n = 6) and LPS (1.5 μg/kg of BW; *Escherichia coli* 055: B5; n = 6). Blood samples were obtained from the jugular vein immediately before, and 360 min after administrating saline or LPS. Protein was precipitated from plasma and individual amino acids and 3-MH in the filtrate were detected in an ion trap LC-MS system. Immunoactivation was successfully induced within 360 min as indicated by increased circulating levels of haptoglobin, serum amyloid A, and LPS-binding protein (all > 50%). The immunocativation was related to 80% and 7% decreases in milk yield and milk protein percentage, respectively and a 20% increase in milk urea nitrogen. Plasma concentration of arginine in LPS cows declined from baseline and was 60% less than that of CON cows (P = 0.07) at 360 min after LPS administration. Plasma concentrations of total branched-chain amino acids (BCAA, P = 0.07), and isoleucine alone (P = 0.07) were lower in LPS cows than CON cows throughout the LPS-challenge. Plasma concentration of the other essential amino acids, non-essential amino acids, or 3-MH did not change in response to the immunoactivation. The data indicate an acutely activated immune system could be associated with a lack of arginine and BCAA supply, while the contribution of AA mobilization from muscle is marginal.

Key Words: amino acid, dairy cow, immunoactivation

M300 Effects of two endomicrobial supplement combinations on Holstein heifers milk composition and yield. J. Embree*[1], J. Wong[1], M. Striluk[1], J. Gaffney[1], G. Gogel[1], C. Martino[1], T. TerHune[2], and M. Embree[1], [1]*Ascus Biosciences, San Diego, CA,* [2]*HMS Veterinary Development, Tulare, CA.*

This study evaluated the response to 2 ruminally injected endomicrobial supplement (EMS1 and EMS2) combinations on milk composition and yield in lactating Holstein cows. The 38-d study (7-d baseline, 28-d treatment, and 10-d post-treatment period) involved 24 Holstein cows randomly allocated to 3 treatments. Animals were fed a common TMR (16.3% CP, 37.3% NDF, 0.67 Mcal of NEI/lb). Throughout the treatment period, the EMS and control treatments were directly administered to the rumen via daily injection behind the last rib in the paralumbar fossa during morning feedings. Group 1 (G1) received EMS1 containing *Clostridium butyricum* sp. nov. and *Pichia kudriavzevii* sp. nov. injected at a total of 1×10^9 and 1×10^9 cells/d; Group 2 (G2) received EMS2 containing *C. butyricum* sp. nov., *P. kudriavzevii* sp. nov., and *Ruminococcus s*p. nov. injected at a total of 1×10^9, 1×10^9, and 1×10^8 cells/d; and Group 3 (G3) the control, received a basal medium suspension. Cows were milked twice daily, and milk production measurements were collected daily. Rumen tube samplings of each cow were collected on d 1, 8, 16, 24, 28, 35, and 38 to determine colonization patterns of the administered microbes via Illumina sequencing of the ITS and 16S rRNA V1-V3 hypervariable regions. All statistical comparisons of treatment main effect and 2-way interactions with treatment main effect were performed using the R package "nlme" and lme function for linear mixed models. Treatment by week interactions were observed to be significantly different for milk production (G2 vs. G3 × wk2, P = 0.0185; G2 vs. G3 × wk3, P = 0.0754), milk protein yield (G1 vs. G2 × wk2, P = 0.0302), energy-corrected milk yield (G1 vs. G3 × wk2, P = 0.0942; G2 vs. G3 × wk2, P = 0.0303), and milk protein % (G1 vs. G2 × wk5+2d, P = 0.0001; G1 vs. G3 × wk5+2d, P = 0.0009). Colonization of EMS1 and EMS2 were confirmed via integration of sequencing results with rumen content cell count data, performed using a fluorescent-activated cell sorter. These data indicate that either effective combination of EMS can have a positive effect on milk production and performance of Holstein cows.

Key Words: endomicrobial supplement, microbiome, Holstein

M301 Supplementation of Red Propolis for dairy calves: effects on performance, health and metabolism. G. S. Slanzon, A. F. Toledo, A. P. Silva, M. D. Silva, M. G. Coelho, and C. M. M. Bittar*, *Depart. Of Animal Sciences, College of Agriculture Luiz de Queiroz (ESALQ), University of Sao Paulo, Piracicaba, SP, Brazil.*

Red Brazilian Propolis (RBP) is a new type of propolis with distinct chemical compounds, especially isoflavones, which can enhance the immune system and act against several diseases. The objective of this study was to evaluate the effects of RBP supplementation trough milk on animal performance, metabolism, and health. Thirty-two newborn calves were individually housed, with free access to water and concentrate, and fed 6 L/d of milk. The animals were blocked according to birth weight and distributed in 2 treatments: (1) Control; (2) daily supplementation of milk with 4mL/d of RBP ethanoic extract (30%; 300 mg of RBP/mL). Feed intake and fecal scores were monitored daily; weight was measured weekly. Blood samples were collected weekly for metabolites determination. Data were analyzed as repeated measures using a mixed model. Supplementation of RBP in milk had no effect on average feed intake, weight gain, and final weight (P > 0.05), however there was a significant age effect (P < 0.05). Supplementation of RBP had a positive effect on fecal score (P < 0.05), reducing the feces fluidity and clinical

signs of diarrheic animals. No effects of supplementation were observed for blood metabolites or hematocrit. Nevertheless, there was an age effect for those parameters, associated with rumen development, and an interaction of supplementation and age for glucose (higher at wk 5 and 7) and BHB (higher at wk 5 and lower at wk 7). RBP supplementation had no effect on performance but decrease diarrhea severity. Results are shown in Table 1.

Table 1 (Abstr. M301). Performance and blood parameters of calves supplemented or not with Red Brazilian Propolis (RBP)

	Treatment (Trt)			*P*-value		
Item	Control	RBP	SEM	Trt	Age	Trt × Age
Feed intake, g/d	259.6	245.8	36.62	0.79	<0.0001	0.50
Initial BW, kg	35.8	35.1	1.21	0.89	—	—
Final BW, kg	72.5	73.6	1.64	0.89	—	—
BW gain, g/d	654.5	694.2	23.47	0.17	<0.0001	0.27
Fecal score	1.8	1.6	0.07	0.02	<0.0001	0.76
Hematocrit, %	22.5	23.2	0.64	0.36	0.006	0.93
Glucose, mg/dL	125.5	127.9	2.37	0.15	0.002	0.01
Urea, mg/dL	17.3	17.5	0.58	0.75	0.009	0.71
Lactate, mg/dL	14.0	14.3	0.72	0.81	<0.0001	0.69
Total protein, g/dL	6.9	6.9	0.14	0.85	<0.0001	0.35
BHB, mmol/L	0.1	0.1	0.01	0.83	0.002	0.04

Key Words: blood metabolite, diarrhea, milk-fed calves

M302 Investigating a novel source of nutritional selenium for ruminant animals. K. Nedelkov[1], C. M. M. R. Martins[2], X. Chen[3], A. Melgar[4], M. T. Harper*[4], S. Räisänen[4], J. Oh[4], E. H. Wall[5], and A. N. Hristov[4], [1]*Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria,* [2]*School of Veterinary Medicine and Animal Science, University of Sao Paulo, Pirassununga, Brazil,* [3]*College of Pastoral Agriculture Science and Technology, Lanzhou University, Gansu, China,* [4]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [5]*Pancosma, Geneva, Switzerland.*

The objective of the present study was to investigate the effectiveness of a novel selenium (Se) product (P-Se) as a source of bioavailable Se in sheep. Six Dorset ewes (93 kg BW; SD = 6.2 kg), housed in individual pens, were used in a 3 × 3 Latin square design experiment. The study had a 2-wk background, a 2-wk low-Se period, and 3 experimental periods of 3 wk each with 2-wk washout periods between them. The basal diet consisted of 1.5 kg chopped grass hay and 0.2 kg ground corn grain containing 1.5% trace mineralized salt without Se. Treatments were (1) unsupplemented control (0.065 mg feed Se/kg of dietary DM), (2) basal diet supplemented with P-Se (Pancosma, Switzerland) providing 0.25 mg Se/kg dietary DM; and (3) basal diet supplemented with sodium selenite providing 0.27 mg Se/kg dietary DM. Data were analyzed using the MIXED procedure of SAS with sheep and sheep within group as random effect. Plasma and fecal Se data were analyzed as repeated measures with AR(1) covariance structure. Compared with the control, Se concentration in blood plasma was increased ($P < 0.001$) by both Se treatments, but concentration was higher ($P < 0.001$) for P-Se (80.9 µg/L) compared with sodium selenite (72.4 µg/L). Plasma glutathione peroxidase activity was greater ($P < 0.01$) for the Se-supplemented diets compared with the control, but was not different between the 2 Se treatments. Absolute and background-corrected Se concentrations in feces were greater ($P < 0.001$) for the Se-supplemented diets than the control, and were less (or tended to be less; $P = 0.14$) for P-Se (441 or 422 µg/kg fecal DM) than for sodium selenite (526 or 511 µg/kg DM, respectively). Concentration of Se in urine was higher ($P = 0.006$) for P-Se (24.4 µg/L) compared with sodium selenite (12.2 µg/L) and the control (6.28 µg/L). Background-corrected urinary Se concentration, however, was increased by both dietary Se sources ($P = 0.003$) compared with the control and was higher in sheep supplemented with P-Se compared with sodium selenite. In this study, Se from P-Se had similar or greater bioavailability than Se from sodium selenite.

Key Words: selenium, bioavailability, sheep

M303 Effects of exogenous amylase on in vitro ruminal digestion kinetics of whole-crop corn silages harvested in late maturity stage. A. S. Silva[1], T. R. Tomich[2], M. S. Pedreira[1], F. S. Machado[2], M. M. Campos[2], C. S. Cortinhas*[3], T. S. Acedo[3], J. P. P. Rodrigues[4], and L. G. R. Pereira[2], [1]*State University of Southwestern Bahia, Itapetinga, BA, Brazil,* [2]*Embrapa Dairy Cattle, Juiz e Fora, MG, Brazil,* [3]*DSM Produtos Nutricionais Brasil SA, São Paulo, SP, Brazil,* [4]*Federal University of São João Del Rey, São João Del Rey, MG, Brazil.*

Our aim was to evaluate the effects of exogenous amylase on the in vitro (bath culture, 96 h of incubation period) ruminal digestion kinetics of whole-crop corn silage harvested at late maturity stage. Two corn hybrids were evaluated: RB9004 – dent type harvested at 110 (375 of DM; 65.2 of CP; 475 of NDF; 383 of NFC g/kg in DM basis) and RB9308 – flint type harvested at 105 d after sowing (348 of DM; 72.6 of CP; 503 of NDF; 337 of NFC g/kg in DM basis). The in vitro tests were performed using rumen fluid from 2 nonlactating rumen fistulated cows fed diets composed of 53:47 forage:concentrate ratio (180 g/kg CP and 300 g/kg NDF), and mineral premix containing the treatments: control (without amylase) or amylase (0.7 g/kg DM of Ronozyme Rumistar; DSM Nutritional Products Brazil). The 2 cows were changed over between the 2 treatments and the rumen fluid were collected after 14 d of adaptation. The experimental design was split plot on random complete blocks, where the blocks were the rounds (2), addition or not of amylase were the plots and the hybrids (2) were the subplots. Digestion parameters were obtained by bicompartimental logistic model describing the digestion kinetics of NFC and fibrous carbohydrate (FC) fractions. Amylase increases (10%) gas production of NFC fraction in both hybrids (160.5 vs. 145.5 mL, $P = 0.002$). The gas production of FC fraction increased in dent hybrid ($P = 0.016$) with no difference in flint hybrid. In both hybrids, amylase increased in 12.5% gas production rate of NFC fraction (2.43 vs. 2.16%/h, $P < 0.001$) and in 16% the gas production rate of FC fraction (10.3 vs. 8.9%/h, $P < 0.001$). Amylase decreased the total gas production in dent hybrid (252 vs. 275 mL), and increased in flint hybrid (265 vs. 236 mL). The in vitro dry matter digestibility (IVDMD) increased 6% in amylase treatment in both hybrids (616 vs. 582 g/kg of DM; $P = 0.002$). Exogenous amylase improves ruminal digestion kinetics and increase IVDMD of whole-crop corn silages harvested at late maturity stage.

Key Words: digestibility, gas production, maturity

M304 Effects of exogenous amylase and essential oils in cross-breed dairy cows diets: Energy use, methane production, and blood parameters. L. D. R. Freire[1], T. R. Tomich[2], A. L. Ferreira[2], F. S. Machado[2], M. M. Campos[2], C. S. Cortinhas*[3], T. S. Acedo[3], L. F. M. Tamassia[3], M. S. Pedreira[1], and L. G. R. Pereira[2], [1]*State University of Southwestern Bahia, Itapetinga, BA, Brazil,* [2]*Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil,* [3]*DSM Produtos Nutricionais Brasil SA, São Paulo, SP, Brazil.*

We aimed to evaluate the effect of exogenous amylase and essential oils (EO) fed to cross-breed dairy cows. Thirty-nine lactating Holstein × Gyr cows (75 ± 34 DIM, 502 ± 57 BW) were distributed into 3 treatments and evaluated for 49 d. Treatments were: monensin (15.8 mg/kg of DM); monensin + amylase (15.8 mg/kg of DM monensin and 658 mg/kg of DM Ronozyme RumiStar, DSM Nutritional Products Brazil SA) and EO + amylase (52.7 mg/kg of DM CRINA Ruminants and 658 mg/kg of DM Ronozyme RumiStar; DSM Nutritional Products Brazil SA). Diet composition was 467 g/kg DM of concentrate, 480 g/kg DM maize silage and 53 g/kg DM Tifton hay. Chemical diet composition was: 192 g/kg CP, 300 g/kg NDF, and 249 g/kg starch. Four open-circuit respirometry chambers were used for the energy evaluation, CH_4 production (g day^{-1}), yield (g kg DM^{-1}, $NDFI^{-1}$ and $NDFD^{-1}$) and intensity (g kg ECM^{-1}). Blood sampling was performed 4 h after morning feeding. The experimental design was completely randomized. Treatment effects were tested using ANOVA (SAS), and means were compared by LSM at $P < 0.05$. Energy partitioning, energy efficiency, and the CH_4 total production (g/d) were similar for all treatments. The CH_4 yield per unit of neutral detergent fiber intake increased (7.1%) with the amylase addition into a diet with monensin, and reduced (14.5%) when monensin was replaced by EO (65.1, 70.1 and 59.9 g/kg NDFI for treatments monensin, monensin+amylase, EO+amylase, respectively; $P = 0.017$). The CH_4 yield in grams per unit of neutral detergent fiber digestible was increased for the treatment monensin+amylase in comparison to monensin and EO+ monensin treatments (132, 113 and 114 g/kg NDFD, respectively; $P = 0.028$). The replacement of monensin by EO reduced the nonesterified fatty acids blood concentration (NEFA, 0.40 *vs* 0.55 mmol/L) and did not change the values of glucose, urea, triglycerides and D-3-hydroxybutyrate. The EO as alternative to monensin for lactating dairy cows did not affect CH_4 production or intensity, had no effect on energy efficiency use and decreased NEFA concentrations.

Key Words: bioenergetic, enzyme, greenhouse gas

M305 Validating intrinsic markers and optimizing spot sampling frequency to estimate fecal outputs. D. L. Morris*, L. R. Rebelo, P. A. Dieter, and C. Lee, *Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH.*

Improving accuracy of intrinsic markers and optimizing sampling procedures to estimate fecal output and nutrient digestibility is critical. This experiment was designed to determine the accuracy of indigestible neutral detergent fiber (iNDF) or acid-insoluble ash (AIA) as markers to estimate fecal outputs and nutrient digestibility compared with total collection (TC), and to determine the optimal number of spot samples (SP) to account for diurnal variation in marker and nutrient outputs. The experiment used 12 Holstein cows in a randomized complete block design fed a diet with corn silage or alfalfa silage as the sole forage. During the final 3 d of 21 d periods, total fecal output was collected and 12 spot samples were collected to represent every 2 h in a 24-h cycle. Data were analyzed with a model that included the fixed effect of diet, method or time point, and 2-way interaction and the random effect of cow and cow within block. Estimated fecal outputs and digestibility when using iNDF as a marker were not different from TC, whereas AIA overestimated ($P < 0.01$) fecal output by 44–61% and underestimated ($P < 0.01$) DM, OM, and N digestibility by 16–32%. Data from individual SP events were aggregated to represent a sampling frequency of 12 (SP12), 6 (SP6), 4 (SP4) or 2 (SP2) evenly spaced events starting at feeding time. Compared with TC, SP12 did not differ in fecal content of iNDF, OM and N, but fecal AIA content was overestimated ($P < 0.01$). Comparisons among sampling frequencies with iNDF indicated that, for the corn silage diet, SP6 did not differ from SP12 in fecal iNDF, and N content, whereas SP4 and SP2 over or underestimated ($P < 0.01$) fecal iNDF and N content compared with SP12. However, fecal iNDF, and N contents did not differ among SP12, SP6, SP4, and SP2 for the alfalfa silage diet. This study confirmed that iNDF was a better fecal marker than AIA to estimate fecal outputs and digestibility, and a spot sampling frequency of at least 6 evenly spaced events starting at feeding time was required for accurate estimation of fecal outputs.

Key Words: indigestible NDF, acid-insoluble ash, spot sampling frequency

M306 Frequency of diet delivery to dairy cows: Effect on methane emissions from stored manure. F. Hassanat*, C. Cherif, and C. Benchaar, *Sherbrooke Research and Development Center, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

The objective of this study was to evaluate the effect of frequency of diet delivery to dairy cows on CH_4 emissions from stored manure. For this purpose, 12 lactating cows were used in a randomized block design and offered a TMR (56:44, forage:concentrate ratio) once/d (0930 h), twice/d (0930 and 2130 h), or 4 times/d (0930, 1300, 1630, and 2130 h). Manure (feces and urine) was collected from each cow on 2 consecutive days, mixed (1:1) with an inoculum from a bioreactor and stored (20°C) under anaerobic conditions in glass bottles (4 replicates/cow) for 17 weeks. Quantity of gas produced was measured 4 times/week and samples taken to determine gas composition. Data were analyzed using the MIXED procedure of SAS and significance was declared at $P \leq 0.05$ using the Tukey test for multiple comparisons. Frequency of diet delivery did not affect the amount of manure excreted, which averaged 76 kg/d and 6.9 kg of volatile solids (VS)/d. Methane emission was unaffected ($P = 0.18$) by frequency of diet delivery (155, 164 and 125 L CH_4/kg VS; SEM = 15.9, for cows offered the diet once/d, twice/d, and 4 times/d; respectively). Volatile solids loss over the storage period averaged 27% and was not affected by frequency of diet delivery. In conclusion, under the conditions of this experiment, frequency of diet delivery to dairy cows had no effect on CH_4 emissions from stored manure.

Key Words: frequency of diet delivery, manure, CH_4 emission

M307 Frequency of diet delivery to dairy cows: Effect on enteric methane emissions. C. Cherif*[1,2], F. Hassanat[1], R. Gervais[2], and C. Benchaar[1], [1]*Sherbrooke Research and Development Center-Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [2]*Département des Sciences Animales, Université Laval, Québec, QC, Canada.*

The objective of this study was to examine the effect of frequency of diet delivery to dairy cows on enteric CH_4 emissions. Twelve lactating multiparous Holstein cows (DIM = 85 ± 23.7; milk yield = 50 ± 3.7 kg) were used in a replicated 3 × 3 Latin square design (35-d period; 14-d adaptation). Cows were offered (ad libitum, 5% orts; on an as-fed basis) a TMR (56:44, forage:concentrate ratio) once/d (0930 h), twice/d (0930 and 2130 h), or 4 times/d (0930, 1300, 1630, and 2130 h). Production of CH_4 was determined (5 consecutive days) using respiration chambers, while DMI, milk performance, and nutrient apparent-total-tract digestibility were determined over 7 consecutive days. Data were analyzed using the MIXED procedure of SAS and significance was declared at $P \leq 0.05$ using the Tukey test for multiple comparisons. Frequency of diet delivery had no effect on DMI (26 kg/d), ECM yield (46 kg/d), and DM apparent-total-tract digestibility (71%). Daily enteric CH_4 emission averaged 534 g/d and was not affected by diet delivery frequency. However, CH_4 emission expressed on DMI basis or as a proportion of gross energy intake (GEI) was lower ($P = 0.01$) when diet was offered

once/d (19.3 g/kg, 5.75%; respectively) compared with twice/d (20.3 g/kg, 6.06%; respectively) and 4 times/d (20.4 g/kg, 6.06%; respectively). Methane emission expressed on ECM yield basis averaged 11.7 g/kg and was not affected by diet delivery frequency. Results of this study show that increasing frequency of diet delivery increases enteric CH_4 losses (g/kg DMI; % GEI) without affecting DMI and milk production.

Key Words: frequency of diet delivery, methane, dairy cow

M308 A field study on prevention of subclinical hypocalcemia in dairy cows supplemented synthetic aluminums silicate or anionic salts in late pregnancy. T. Per*[1] and J. Morten[2], [1]*Vitfoss, Graasten, Denmark,* [2]*Protekta Inc, Ontario, Canada.*

The objective was to compare Ca serum concentration and frequency of severe [Ca <2.0 mmol/L] and moderate [Ca <2.125 mmol/L] subclinical hypocalcemia (SCH) in cows supplemented according to the dietary cation-anion differences (DCAD) principles or with synthetic zeolite. The trials were performed in 2 large commercial farms in US and blood samples were taken within the first 24 h after calving. The test consisted in comparing Ca serum concentration when the farms were using DCAD management for preventing hypocalcemia –in Farm 1 at 1.47 mEq/100 g DM (full DCAD) and in Farm 2 at 0.62 mEq/100 g DM (partial DCAD) - with the Ca serum concentration levels when synthetic aluminum silicate was supplemented by using the commercial product X-Zelit (Protekta Inc./Vitfoss, Graasten, DK). The differences in the plasma calcium concentrations between treatments were tested within farms with ANOVA using the lm procedure in the R package. Fisher's exact test was used to assess differences in incidences of SCH. The plasma calcium concentration was significantly ($P < 0.001$) higher in the X-Zelit group compared with the DCAD group on both farms (Table 1). The frequency of animals having severe SCH was 57 and 65 percent in farm 1 and 2, using full and partial DCAD approaches, respectively. During this period, the frequencies of moderate SCH were 83 and 88 percent in farms 1 and 2. During the treatment with X-Zelit the frequency of severe SCH were reduced by 5 and 0 percent in farm 1 and 2, respectively, while the reduction of incidence in moderate SCH were 20 and 13 percent respectively in farm 1 and 2, in both cases significant ($P < 0.001$). Results suggest a lower SCH frequency using X-Zelit compared to low or neutral DCAD feeding.

Table 1 (Abstr. M308). Least squares means ± error of mean of blood parameters in multiparous cows

	DCAD		X-Zelit	
Farm	n	Plasma Ca, mmol/L	n	Plasma Ca, mmol/L
1	54	1.98 ± 0.02	40	2.26 ± 0.02
2	76	1.92 ± 0.02	84	2.26 ± 0.01

Key Words: subclinical hypocalcemia, X-Zelit, DCAD

M309 In vitro fermentation parameters and gas production in high producing dairy cow diets with yeast and lactic acid bacteria as probiotics. H. F. Monteiro*[1], A. Faccenda[2,1], A. L. J. Lelis[1], A. S. Avila[3,1], V. L. N. Brandao[1], X. Dai[1], L. G. Silva[1], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, State University of Maringa, Maringa, PR, Brazil,* [3]*Department of Animal Sciences, State University of Western Parana, Marechal Candido Rondon, PR, Brazil.*

The objective of this study was to investigate the ruminal fermentation parameters and gas production of different probiotics in a high NFC dairy cow diet using 2 in vitro systems. We hypothesized that lactic acid bacteria would improve ruminal fermentation due to O_2 scavenging and competition against other starch fermenting bacteria (e.g., *S. bovis*). The diet was formulated according to the NRC (2001) for a cow producing 45 kg of milk/d and consisted of 46% NFC. Each experiment had four 48-h incubations. An AnkomRF gas production system was used in exp. 1 (n = 64) to evaluate final pH, total gas production (GP), kinetics of gas production, OM digestibility, VFA production, and NH_3-N concentration. A batch culture system was used in exp. 2 (n = 128) to evaluate pH, true OM digestibility, CO_2 and CH_4 production. Probiotic product inclusion rate and composition are described in Table 1. Mixed linear models in SAS were used to analyze the data. Kinetics of gas production was analyzed through nonlinear regression. In exp. 1, diets did not affect fermentation rate, final pH, OM digestibility, NH_3-N concentration, gas pool size, and GP at 24 and 48 h of fermentation; however, VFA production was reduced for P6 and P7, acetate and acetate:propionate ratio were lower for P5, and BCVFA tended to be lower for P6. In exp. 2, final pH, true OM digestibility, CO_2 and CH_4 concentration, as well as CH4 production, did not change among treatments. However, total CO_2 production had a trend to decrease with P7 yet CO_2 per g of digested OM decreased with P7. Based on these findings, yeast and lactic acid bacteria did not change in vitro fermentation when included in similar concentrations; however, *L. plantarum* at 0.15% and 0.20% of inclusion reduced fermentation.

Table 1 (Abstr. M309).

Item	Inclusion rate (% of DM)	Composition
Control	0.00	No Probiotic
Product 1 (P1)	0.10	*Saccharomyces cerevisiae*
P2	0.10	*Saccharomyces cerevisiae*
P3	0.01	*Lactobacillus acidophilus*
Propionibacterium freudenreichii		
P4	0.05	*Lactobacillus plantarum*
P5	0.10	*Lactobacillus plantarum*
P6	0.15	*Lactobacillus plantarum*
P7	0.20	*Lactobacillus plantarum*

Key Words: *Lactobacillus plantarum,* non-fiber carbohydrate, pH

M310 Pre- and post weaning performance and health of dairy calves fed milk replacers formulated with 5 or 10% levels of spray-dried bovine plasma. D. M. Ziegler*[1], H. Chester-Jones[1], R. M. Blome[2], and D. R. Wood[2], [1]*University of Minnesota, Waseca, MN,* [2]*Animix LLC, Juneau,WI,USA.*

One-hundred eleven (2 to 5 d old) individually fed Holstein heifer calves (40.0 ± 0.61 kg) from 3 commercial dairies were randomly assigned to 1 of 3 milk replacer treatments (MR) formulated with 5% (50.1 kg/t) or 10% (100.2 kg/t) spray-dried bovine plasma (BP) replacing 16 and 32% of milk protein. All MR were balanced for 2.4% Lys, 0.8% Met, and 1.6% Thr, with no additional synthetic amino acids added to evaluate pre- (d 1–42) and post-weaning (d 43–56) calf performance and health. The study was conducted between July and October 2017. Treatments included (1) all-milk-protein, non-medicated MR 24% CP:20% fat fed at 0.34 kg in 2.38 L of water 2× daily from d 1 to d 35 and 1× daily from d 36 to weaning at d 42, (CON); (2) MR 24% CP:20% fat, non-medicated fed as in CON formulated with 5% BP, (MRBP5); (3) MR 24% CP:20% fat, non-medicated fed as in CON formulated with 10% BP, (MRBP10);

Calf starter (CS; 18% CP as fed) and water were offered free choice from d 1 to 56. There were no differences in pre- (d 1–42), post weaning (d 43–56), and overall (d 1–56) gains averaging 0.60, 1.08, 0.72 kg/d respectively ($P > 0.05$). Pre- (d 1–42) and post weaning (d 43–56) CS starter intake was similar across treatments averaging 14.5 and 30.0 kg total intake ($P > 0.05$). There were no differences in gain/feed or daily fecal scores with treatment costs being similar across treatments ($P > 0.05$). There were no differences in the number of days fecal scores were 3 or higher (1 = normal, 4 = watery, d 1–42) averaging 4.17, 3.73 and 3.77 d, respectively ($P = 0.05$). Under conditions of this study, calves fed a non-medicated MR formulated with 5 or 10% BP and Lys, Met, and Thr levels balanced across treatments did not affect performance of calves compared with an all milk protein non-medicated MR.

Key Words: calf performance, milk replacer, bovine plasma

M311 Effects of feeding wood kraft pulp on preventing subacute ruminal acidosis in cattle. S. Sato*[1], Y. Kim[1], S. Kushibiki[2], and K. Kizaki[1], [1]*Cooperative Department of Veterinary Medicine, Iwate University, Morioka, Iwate, Japan,* [2]*National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan.*

Wood kraft pulp (KP) is a highly nutritious feed material with potential for mitigating subacute ruminal acidosis (SARA) due to its high digestibility and mild fermentation properties. This study identified the effects of KP feeding on changes in ruminal pH, fermentation, and epithelial transcriptomic dynamics during the 2 weeks of a SARA challenge. Five fistulated Holstein bulls (BW 215 ± 20 kg) were used. Following a 7-d high-forage adaptation, the SARA challenge was conducted for 14 d (d 1–14) and was repeated twice. During the challenge, the control group (n = 5) was fed the SARA challenge diet, and the KP group (n = 5) was fed the same diet, except that half of the flaked corn was replaced with KP (1 kg/day). Ruminal pH was measured continuously. Ruminal volatile fatty acid (VFA) and NH_3-N, and blood metabolites were determined. The rumen epithelium was biopsied on d 14. The mRNA expression profiles were examined by one-color microarray analysis. Differentially expressed genes were analyzed using an Ingenuity pathway analysis. The KP group showed significantly higher levels of acetic acid and blood urea nitrogen, and lower level of blood 3-hydroxybutyrate than did those in the control group. However, no significant differences were observed in ruminal pH, total VFA concentration, or other blood metabolites between the 2 groups. The *in silico* analysis revealed that the most activated canonical pathway involved PPAR signaling (z-score = 2.83, $P = 8.32 \times 10^{-6}$), and the immune modulatory Toll-like receptor (z-score = −2.12, $P = 8.51 \times 10^{-4}$) and NF-κB (z-score = −2.45, $P = 1.58 \times 10^{-4}$) signaling pathways were significantly inhibited. Furthermore, the genes involved in the immune function (CRK, IgG, MAPK, and NFKB1) and energy metabolism (APOB, INSIG1, and PPARA) were identified as significant upstream regulators based on their expression patterns. The inhibition of immune modulatory and inflammatory signaling pathways in the rumen epithelium suggests milder fermentable properties of the KP diet than those of the flaked corn diet.

Key Words: cattle, SARA, wood kraft pulp

M312 Evidence of intraflock variability in the feed efficiency of lactating Lacaune dairy ewes. E. González-García*[1], J. P. Dos Santos[2], and P. Hassoun[1], [1]*INRA SELMET (Systèmes d'*Èlevage Méditerranéens et Tropicaux), *Montpellier, France,* [2]*Faculty of Veterinary, Universidade Federal do Pará (UFPA), Castanhal, PA, Brazil.*

The intraflock variability of feed efficiency (FE) in dairy ewes was examined through monitoring residual feed intake (RFI). Primiparous lactating ewes (n = 43; 57.7 ± 0.91 kg BW at lambing), representative of a French Lacaune dairy flock, were allocated in an equilibrated 2 × 2 factorial design experiment, lasting 63 d during the mid-lactation and combining 2 litter sizes (singletons, SING or twins, TWIN) and 2 daily milking frequencies (once, ONE or twice, TWO). Ewes were individually fed a diet based on ryegrass silage, local hay, and supplements. Individual DMI was recorded daily and used to evaluate differences in RFI at 35, 42, 49, 56, 63, 70, 77, 84, 91, and 98 d after lambing. Total (BW) and metabolic BW ($BW^{0.75}$), BCS, milk yield and plasma NEFA concentration were monitored weekly. Differences in DMI were mainly due to the lactation stage and litter size and was, on average, 11% higher in ewes with TWIN compared with SING which was consistent with milk yield (i.e., TWIN > SING). The RFI was affected by litter size and the milking frequency × lactation stage interaction. Ewes with SING showed higher FE (–0.13 ± 0.020 vs. 0.08 ± 0.015 kg DM/ewe/d of RFI in SING vs. TWIN, respectively), whereas there were no differences in BW or BCS. Milking frequency did not affect DMI but milk yields were higher in TWO, which was related to a higher FE in this group (0.04 ± 0.017 vs. –0.10 ± 0.018 kg DM/ewe/d of RFI in ONE vs. TWO, respectively). The RFI was affected ($P < 0.0001$) by the ewe, thus allowing a ranking among individuals to be established. High (n = 22) or low (n = 21) FE ewes averaged –0.17 ± 0.09 or 0.18 ± 0.09 kg DM/d RFI, respectively. The RFI were not correlated with the individual milk production potential but were negatively correlated with NEFA, indicator of body reserves (BR) mobilization (i.e., low RFI ewes had higher NEFA). Despite the absence of differences in BW, $BW^{0.75}$ or BCS, high-efficiency ewes (i.e., with lower RFI and higher NEFA) mobilized almost 2-fold their BR compared with low-efficiency ewes. The observed inter-individual differences in RFI suggest considering these promising results for getting new insights in further studies looking for genetic improvements in FE.

Key Words: feed efficiency and adaptive capacity, residual feed intake, intraflock variability

M313 Weaning age affects rumen fermentation and bacterial communities of Hu lambs. H. Mao*[1,2], Y. Xu[1], C. Wang[1], and Z. Yu[2], [1]*College of Animal Science and Technology, Zhejiang A and F University, Lin'an, China,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Early weaning is desired in dairy production as it lowers production cost. The objective of this study was to evaluate how weaning age affect the development of ruminal microbiota using lambs as a model. Ten 5-d-old male Hu lambs weighing 3.6 ± 0.37 kg each were randomly assigned to 2 weaning age groups: early weaning (EW) at d 30 or normal weaning (NW) at d 45, with 5 lambs in group. All lambs had colostrum within 4 h after birth, and they were subjected to a 5-d adaptation to a milk replacer (MR; 18.4% CP, 13% CF). Starter (16% CP, 10.9 MJ/kg ME) and Chinese wild rye were fed ad libitum from d 10 on. To stimulate intake of solid feed, MR was given at 780 mL/d (based on consumption in the adaptation period) at d 11 and was then reduced at a rate of 30 mL/d until reaching 210 mL/d, which was the rate given until the weaning age. The lambs were then slaughtered to obtain the rumen content samples for analysis for volatile fatty acids (VFA) using gas chromatography and rumen microbiota using metagenomics. Data were analyzed by one-way ANOVA using GLM of SAS. The results showed that the early weaning had no effect on total VFA concentration (1.58 vs 2.50 mg/g; $P > 0.05$) but decreased the acetate (53.0 vs 69.2 m*M*/100 m*M*; $P < 0.05$) while increasing propionate (45.0 VS 29.5 m*M*/100 m*M*;

$P < 0.05$) molar proportion. The major phyla of the rumen microbiota included *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Fibrobacteres* irrespective of the weaning age. The EW group had a greater relative abundance of *Bacteroidetes* and *Proteobacteria* than the NW group ($P < 0.05$), but a lower relative abundance of *Actinobacteria* ($P < 0.05$). At the genus level, *Prevotella* was the most predominant. Compared with the NW group, the early weaning significantly increased the relative abundance of *Prevotella, Bacteroides*, *Succinivibrio*, *Clostridium* IV, *Dialister*, and *Fibrobacter*($P < 0.05$) while decreasing that of *Olsenella*, *Bifidobacterium*, *Ruminococcus* and *Selenomonas*. Early weaning could increase the propionate molar proportion and change the rumen bacterial communities, which may have beneficial effect on the rumen development of Hu lambs.

Key Words: early weaning, lamb, rumen bacteria

M314 Feed processing temperature affects lactational performance. A. Van De Kerckhove[1], A. Delaquis[2], F. Mueller[3], T. Steen[4], J. Guyader[5], and A. Park*[6], [1]*Federated Co-operatives Limited, Saskatoon, SK, Canada,* [2]*La Coop Fédérée, Montréal, QC, Canada,* [3]*Kalmbach Feeds Inc., Upper Sandusky, OH,* [4]*Tennesse Farmers Cooperative, LaVergne, TN,* [5]*Neovia, Château-Thierry, France,* [6]*Cooperative Research Farms, Richmond, VA.*

We evaluated the effects of increasing pelleting temperatures on milk yield and composition of post peak lactating dairy cows. Ninety-four cows at 65 d in milk were housed and randomly assigned to a treatment sequence in a 4 × 4 replicated Latin square pen design (2 pens per square) with 28 d periods including 21 d of washout and 7 d of data collection. Complete treatment supplements were as follows: control (meal form), P66 (pellet at 66°C), P74 (pellet at 74°C), and P82 (pellet at 82°C). Cows were milked 3 times per day with daily individual cow milk yield averaged by week. Milk composition was assessed weekly utilizing a 2 d average. Body weight and condition scores were taken at the beginning and completion of each period. Data were analyzed using the PROC GLIMMIX procedure (SAS) with differences noted at $P < 0.05$ and trends at $P < 0.15$. Orthogonal contrasts of linear and quadratic were used to further describe the relationships between pelleting temperatures. Pelleting temperature only resulted in a slight linear decrease in milk fat percentage ($P < 0.13$) and a tendency for a quadratic ($P < 0.06$) response in milk efficiency being highest for the P74 treatment. Estimate statements were utilized to assess feed form influence aside from milkfat percentage and milk efficiency. Changing the feed form to a pellet increased milk yield (2.54 kg/d; $P < 0.01$), 3.5% FCM (2.02 kg / d; $P < 0.04$), and ECM yield (2.13 kg/d; $P < 0.01$). The higher milk yield with the pellet also led to a greater milk fat (0.06 kg/d; $P < 0.05$) and milk protein yield (0.08 kg/d; $P < 0.01$). Pelleting elevated production efficiencies (FCM - $P < 0.02$, ECM; $P < 0.01$, and gross protein; $P < 0.01$). Lower MUN levels occurred for a pellet versus a meal (0.49 mg/dL; $P < 0.03$). Less change in BCS also took place for a pellet over meal treatments (0.03; $P < 0.12$). Pelleting temperature had little to no effect upon lactational performance while pelleting the feed appears to enhance lactational performance.

Key Words: lactation, feed form, temperature

M315 Non-linear relationship study between altered carbohydrate traits in hull-less barley (*Hordeum vulgare* L.) and predicted truly absorbed nutrient supply to dairy cattle. B. Sun, B. Refat, M. Sun, and P. Yu*, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.*

The objective of this study was to investigate the nonlinear relationship between altered carbohydrate (CHO) traits and predicted truly absorbed nutrient supply to dairy cattle in hull-less (hulless) barley. Four cultivars were developed at the Crop Development Centre, University of Saskatchewan, with differences in carbohydrates traits on the basis of amylose (1–40% DM) and β-glucan (5–10% DM) content. All cultivars were planted and grown in Saskatchewan (Canada) and then harvested in 3 consecutive years. The digestion in the rumen and intestine was determined used in situ dairy cows with a randomized complete block design (RCBD) with samples year as a random block effect. The rumen degradation was carried out with in situ nylon bag technique. The intestinal digestibility was carried out with 3-step in vitro technique with 16 h of pre-incubation. The truly absorbed nutrient supply to dairy cattle was evaluated using the Dutch protein evaluation system (called DVE/OEB in Dutch). The data from RCBD experiment design were analyzed with Proc Mixed of SAS. Results showed that OM fermented in the rumen (FOM), undigested inorganic matter (UASH), total protein supplied to the small intestine (TPSI), metabolizable protein (MP), microbial protein synthesized in the rumen based on available N (N_MCP) and truly absorbed microbial protein in the small intestine ($AMCP^{DVE}$) had cubical relation with amylose to amylopectin ratio (A:AP) and the β-glucan level. Digested (DOM) and undigested organic matter (UOM), undigested dry matter (UDM) and endogenous protein in the small intestine (ENDP) showed quadratic relation with the ratios of A:AP and the β-glucan levels. Truly absorbed protein in the small intestine ($ABCP^{DVE}$) and degraded protein balance (OEB^{DVE}) were quadratically related to the A:AP and cubically related to the β-glucan level. In conclusion, the predicted truly absorbed protein supply to dairy cows from the hulless barleys had curve-linear relationship with the altered carbohydrate traits. Further study on dairy milk production performance is needed.

Key Words: alteration of carbohydrate traits, hull-less barley, truly absorbed protein supply

M316 Comparison of near-infrared (NIR) and Fourier transform mid-infrared (ATR-FT/MIR) spectroscopy in prediction of intestinal protein digestibility in dairy cows. H. Shi, N. Liu, Y. Lei, L. L. Prates, B. Refat, and P. Yu*, *Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.*

The purpose of this study was to investigate the potential of using the near-infrared (NIR) and Fourier transform mid-infrared (ATR-FT/MIR) spectroscopy as potential techniques for the determination of intestinal CP digestibility (IPD) of wheat. Forty-eight wheat samples collected from western Canada were analyzed for CP content and in vitro IPD. For CP, the best NIR model was obtained with the 1400–2500 nm spectral range of standard normal variate and detrending preprocessed spectra, which showed an excellent prediction performance ($R^2 = 0.98$); the best MIR model was developed using the 1800–700 cm^{-1} region of multiplicative scattering corrected spectra, which also gave an excellent prediction performance ($R^2 = 0.96$). Regarding to IPD, the best model obtained by NIR technique (based on the 1100–2500 nm region of first derivative treated spectra) showed approximate quantitative predictive ability ($R^2 = 0.68$), and the best model generated by MIR technique (using the 1700–900 cm^{-1} region of first derivative preprocessed spectra) obtained similar prediction performance ($R^2 = 0.67$). The amide I and II bands played important roles in the development of PLS models for CP and IPD and the amide I region might be more important than amide II region. Results from this study demonstrated the potential of using IR spectroscopy for the prediction of nutrient digestibility while more

efforts are required to improve the performance of NIR and ATR-FT/MIR spectroscopy in predicting the IPD.

Key Words: molecular spectroscopy, near- and mid-infrared wavelength selection, spectral preprocess

M317 Optimizing microbial protein synthesis to increase milk production: A meta-analysis approach. J. Guyader*, P. Derbois, and C. Gerard, *Neovia, Talhouët, Saint-Nolff, France.*

Rumen microbial protein synthesis (MPS) represents up to 75% of the nitrogen reaching the duodenum. However, the effect of increasing MPS on milk production has not been numerically assessed. The aim of this meta-analysis was to assess the relationship between rumen MPS and milk production of lactating dairy cows. A database was built from 35 publications (47 experiments) reporting rumen MPS and milk production measured on the same groups of animals. The primary objective of these experiments was to test the impact of nutritional strategies (carbohydrate, nitrogen, plant extracts, enzymes, forage) on MPS. When available, diet composition, intake, rumen fermentation parameters, total-tract nutrient digestibility and milk composition were also registered. Milk production and MPS averaged 28.5 kg/d (10.8 to 44.1 kg/d) and 1.70 kg/d (0.39 to 2.94 kg/d), respectively. For both parameters, the within-experiment variation was calculated: experiments presenting the 25% lowest variations were considered as having no variation in the targeted variable, and were excluded from further analyses. In total, 35 experiments reported a variation in MPS between treatments (>90 g/d) among which 28 also reported a variation in milk production (>0.75 kg/d). Changing carbohydrate source was the nutritional strategy with the highest probability (75%) of increasing MPS and milk production. To quantity the effect of a variation in MPS on milk production, a linear regression model was tested with MPS and experiment as fixed effects. One experiment was excluded to ensure normality of MPS and milk production. A significant relationship was shown ($P < 0.001$): milk production (kg/d) = 25.5 + 4.21 × MPS (kg/d), with 30 publications (34 experiments, 96 cows in total), residual standard error = 0.94 and adjusted $R^2 = 0.98$. Therefore, in the conditions of our meta-design, a 10% rise in MPS would increase milk production by 0.72 kg/d. Further work is required to determine the potential factors interfering this relationship.

Key Words: meta-analysis, milk production, rumen microbial protein

M318 Mycotoxin mitigation when feeding Equalize Dairy. K. M. Hultquist* and D. P. Casper, *Furst-McNess Company, Freeport, IL.*

The 2016 corn crop was heavily contaminated with mycotoxins; thus, the objective was to evaluate 2 commercially available products—Competitor (COMP) and Equalize Dairy (EqD; both from Furst-McNess Company)—to mitigate mycotoxins. Both products are combinations of technologies that have multiple mechanisms of action. The experiment was a completely random design (CRD) using 157 lactating dairy cows (80 COMP and 77 EqD) initially balanced for parity, days in milk, and milk production and then added on a rolling basis with a 1 wk covariate and 8 wk experimental period during June and July 2017. The COMP and EqD were incorporated at 45 and 30 g/cow/d, respectively, when cows received 4.54 kg/d pellets when milked via 2 Lely Astronaut A4 robotic milking systems housed in a freestall facility. Corn based ingredients used in the partial mixed ration and robot pellets contained varying vomitoxin, zearalenone, fumonisin, and sterigmatocystin concentrations. All data were subjected to least squares ANOVA for a CRD via the PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) as a repeated-measure ANOVA. Milk production (34.5 and 38.1 kg/d for COMP and EqD, respectively), 4% FCM (32.8 vs. 35.7 kg/d), and ECM (32.3 and 35.0 kg/d) were greater for cows fed EqD compared with cows fed COMP. Milk fat percentages (3.70 and 3.66%) were similar for cows fed both treatments, but milk protein percentages (2.95 and 2.92%) were reduced for cows fed EqD compared with cows fed COMP. Milk conductivity measurements, as an indirect evaluation of somatic cell count, were lower in all 4 quarters for cows fed EqD compared with cows fed COMP. Body weights were similar for cows fed both treatments, while pellet dry matter intakes (4.6 and 4.9 kg/d) were greater for cows fed EqD compared with cows fed COMP. Total-tract nutrient digestibility of P (54.3 and 98.5%) was greater for cows fed EqD, while K (90.6 and 86.2%) digestibility was lower, but other nutrient digestibilities were similar. In conclusion, feeding Equalize Dairy demonstrated an improvement in lactational performance and feed intake when dairy cows were fed mycotoxin contaminated feeds compared with cows fed the COMP product.

Key Words: dairy cow, milk yield, mycotoxin

M319 Biohydrogenation patterns in digestive contents of lambs fed babassu or buriti oils. N. A. F. Machado[1], M. O. M. Parente[1], R. J. B. Bessa[2], H. N. Parente[1], S. P. Alves[2], G. S. Oliveira*[1], A. de Moura Zanine[1], D. Ferreira de Jesus[1], L. R. Bezerra[3], D. de Oliveira Maia[1], and L. F. Anjos[1], [1]*Universidade Federal do Maranhão, Chapadinha, Maranhão, Brazil,* [2]*Universidade de Lisboa, Lisboa, Portugal,* [3]*Universidade Federal de Campina Grande, Patos, Paraíba, Brazil.*

Brazil has a great variability of palm tree species with potential for vegetable oil production. Among them, babassu (*Attalea speciosa* Mart) and buriti (*Mauritia flexuosa* L.F.) are important species of the Amazon region. More than 75% of the fatty acids (FA) in babassu oil are saturated, especially in lauric acid (49%), while the buriti oil is primarily composed of oleic FA (76%). The rumen microorganisms are the main responsible for transform the unsaturated FA from feed in saturated and *trans* FA in ruminant products. The objective of this study was to evaluate the effects of adding babassu or buriti oil in the diet (DM basis) on biohydrogenation patterns in digestive contents of lambs. Twenty-one lambs (initial BW 18.9 ± 3.8 kg) were penned individually for 50 d and were distributed in a complete randomized block design. Animals were fed 1 of 3 diets: CONT (control diet containing 70% concentrate; compound by ground corn, soybean meal, mineral supplement and limestone) and 30% Tifton 85 hay; BAO (CONT plus 4% of babassu oil); or BUO (CONT plus 4% of buriti oil with ether extract content of 2.7, 5.9 and 6.0%, respectively). At the end of trial, lambs were slaughtered and samples of abomasal digesta contents were collected from each lamb. Samples were frozen, freeze-dried, milled and stored at −20°C until analysis by gas chromatography. Orthogonal contrasts were performed (CONT vs. diets containing oils and; BAO vs. BUO). BAO and BUO increased ($P \leq 0.05$) ruminal biohydrogenation rate (BHR) of oleic acid (C18:1 *cis*-9) compared with the CONT diet (83.63, 93.25 and 89.64% for CONT, BAO and BUO, respectively). However, the addition of oils reduced ($P \leq 0.05$) the BHR of linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3). Additionally, BAO reduced ($P \leq 0.05$) BHR of C18: n-3 compared with BUO (23.58 and 74.64% for BAO and BUO, respectively). BUO increased ($P \leq 0.05$) the biohydrogenation completeness compared with BAO, but did not differ from CONT (93.92; 77.29 and 97.26% for CONT, BAO and BUO, respectively) because of higher concentration of unsaturated FA. The addition of babassu oil reduces the biohydrogenation completeness of fatty acids, increasing in this way the concentration of intermediate FA (*trans*) of the biohydrogenation process.

Key Words: lipolysis, *trans* FA, unsaturated FA

M320 Assessment of nutrient digestibility in goats fed diets with increasing levels of babassu mesocarp flour. A. R. Dutra, M. O. M. Parente, H. N. Parente, M. A. M. Filho, G. Silva de Oliveira*, H. C. Lima, M. N. Sousa, N. A. F. Machado, R. M. Gomes, and L. M. Freitas, *Universidade Federal do Maranhão, Chapadinha, Maranhão, Brazil.*

The babassu mesocarp flour (BMF) is produced from the babassu (*Attalea speciosa* Mart ex), a palm tree native found between Cerrado and Amazon forest in Brazil. It is a by-product of the industrialization of the babassu coconut for the production of edible oil, babassu meal and others. It is important due the significant starch content of the mesocarp. However, epicarp and endocarp contamination can occur during mesocarp extraction, resulting in a BMF with high fiber content and low starch content. Twenty-eight crossbred male goats (25.8 ± 4.2 kg BW) were used in a randomized complete block design to evaluate the effect of increasing levels of BMF on feed intake, digestibility of nutrients and ruminal pH. Goats were confined individually and fed an isonitrogenous diet (12.6 ± 0.1 CP, DM basis) containing 70% of concentrate (compound by ground corn, soybean meal, wheat bran, mineral supplement, limestone and BMF, in diets with this coproduct) and 30% of Tifton 85 hay for 21 d, with 15 d for diet adaptation, 5 d for data collection of orts and total feces, and 1 d for ruminal content collection (0, 3, 6, 9 and 12 h after feeding). A representative sample of ruminal content from each animal was collected via esophageal tube. The first portion of rumen fluid was discarded, and the second portion, after being squeezed through 2 layers of cheesecloth, was used for determining ruminal pH. Increasing levels of BMF were 0, 10, 20 and 30% (DM basis), corresponding to experimental diets 0BMF, 10BMF, 20BMF and 30BMF, respectively. Orthogonal polynomials for diet responses were determined by linear and quadratic effects ($P = 0.05$). No significant differences were detected for DM (812 ± 224 g/d), CP (106 ± 29 g/d) and OM (770 ± 212 g/d) intake. Also, no differences were observed for DM (79.0 ± 4.1%), CP (76.5 ± 4.8%) and OM (80.0 ± 3.9%) digestibility. However, the NDF intake (245.2, 267.9, 447.6 and 420.6 g/d) increased linearly with increasing levels of BMF in the diet, while NDF digestibility (50.1, 61.3, 63.7 and 60.5% for 0BMF, 10BMF, 20BMF, and 30BMF, respectively) and ruminal pH (6.3, 6.4, 6.5 and 6.5 for 0BMF, 10BMF, 20BMF, and 30BMF) had a quadratic effect. The BMF is an alternative for energy source in goats feed and can be added at levels up to 30%

Key Words: by-product, energy, fiber

M321 Secretion of galectins-1, -3, and -9 in goat blood during the periparturient period. K. Ekwemalor*, S. Adjei-Fremah, E. Asiamah, E. Eluka-Okoludoh, B. Osei, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Parturition is a very dynamic period with a significant change in the physiology and immunology of goats. Periparturient immunosuppression can hinder the ability of goats to thwart infectious diseases. Galectins constitute an evolutionarily conserved family of β-galactoside-binding proteins that are secreted. They are involved in the regulation of homeostasis, innate and adaptive immune responses to infectious challenge. The objective of this study was to determine the secretion of galectins in goat blood during the periparturient period. Five BoerXSpanish goats (average body weight of 156kg and body condition score of 4) from the North Carolina Agricultural and Technical State University Small Ruminant Research Unit were used. Blood samples were collected from the jugular vein at 14 and 7 d before and 7 and 14 d after parturition. Plasma was collected from whole blood in tubes containing EDTA. Total plasma protein concentration was determined using the BCA assay (Thermo Scientific Pierce, Rockford, IL). The secretion of galectins-1, -3, and -9 was detected using goat specific enzyme-linked Immunosorbent assays (ABclonal Biotechnology, Woburn, MA). Data were analyzed using PROC GLM model in SAS 9.4 version ($P < 0.05$). There was no significant difference in total plasma protein concentration ($P > 0.05$) during the periparturient period. The average concentration of galectins-1, -3, and -9 was 150, 14, and 3 ng/μL of goat plasma respectively. A postpartum decrease in galectin-1 and an increase of galectins-3 and -9 was observed ($P < 0.05$). Our results indicate that

galectins-1, -3, and -9 are secreted in goat blood. The difference in the pattern of secretion may indicate distinct roles in homeostasis during the periparturient period in goats and needs further study.

Key Words: galectin, goat, periparturient period

M322 Spatial modeling of population membership in indigenous Eastern Adriatic sheep breeds using codominant marker genotypes. D. Salamon and A. Dzidic*, *Faculty of Agriculture, University of Zagreb, Zagreb, Croatia.*

Number of biological populations and their spatial boundaries for 13 sheep breeds of the Eastern Adriatic coast was inferred using Geneland 4.0.8. package for R. Random unrelated genotypes of 22 to 51 sheep from each breed were analyzed, where 51 samples of Istrian sheep were collected in Croatia and Slovenia. Clustering was performed under spatial and non-spatial models for the 28 genotyped microsatellite markers, with and without the assumption of correlated allele frequencies. Stochastic inference was tested for 4 different groups of models (10 repetitions each, 10^6 iterations of the Markov chain Monte Carlo, 200 burn-in runs). Models with one to 18 clusters of genotypes were tested, accounting for putative null-alleles and treating the double missing genotype as genuine missing data. Explicit spatial coordinates of the samples were clumped, covering area of approximately 160,000 km^2 and were treated with the parameter of uncertainty of 10. Maximal number of nuclei in non-spatial models was set to the number of samples (317), and 3 times as much in spatial models. Non-spatial models showed poor chain mixing implying problems with convergence that can arise due to departure from modeling assumptions. Spatial model without assumed frequencies correlation recognized only 3 clusters. The expected low differentiation of the clusters due to larger variability within the breeds than among them, and spatial information found to be relevant in other sheep clustering studies, adduced toward a posteriori preference of the spatial model with correlated allele frequencies. A map of population membership was created for the best run of modal k = 14. Samples clustered according to the breed origin with lower resolution for the southern populations. The estimated spatial scale parameter (b = 63) and the low a parameter (a = 3.9) confirm the admixture structured in space and the value of explicit spatial data in indigenous sheep diversity assessment. Possible isolation by distance pattern in the sample may adduce toward recommendation of clustering using tessellation polygons.

Key Words: indigenous sheep, landscape genetics, spatial clustering

M323 Effect of a blend of artificial sweetener and capsicum on productive performance and blood profile in lambs. X. Chen[1], K. Nedelkov[2], J. Oh*[3], M. Harper[3], E. Wall[4], and A. Hristov[3], [1]*Lanzhou University, Lanzhou, Gansu, China,* [2]*Trakia University, Stara Zagora, Bulgaria,* [3]*The Pennsylvania State University, University Park, PA,* [4]*Pancosma, Geneva, Switzerland.*

Data suggest that supplementation of growing animals with capsicum oleoresin or with SUCRAM (Suc) improves growth efficiency; however, responses to the 2 additives fed in combination have not been described. The objective of this study was to investigate the effects of a blend of rumen-protected capsicum oleoresin (RPC) and Suc on the growth performance, blood chemistry and cell counts, markers of fat mobilization, and hormones in growing lambs. Thirty-six lambs (Dorset and Suffolk × Hampshire) were used in a 7-wk randomized complete block design experiment. Lambs were housed in 9 pens (4 lambs/pen) and pens were blocked based on lambs' average body weight (47.4 kg; SD = 2.62). Pens within a block were randomly assigned to one of the following treatments (3 pens/treatment): (1) control (no additive), (2) 86 mg/head/d RPCSuc (RPCSucL; Pancosma, Switzerland), and (3) 171 mg/head/d of RPCSuc (RPCSucH). CapsSuc was mixed with the concentrate portion of the diet; hay was offered ad libitum. Body weight of the lambs was recorded weekly and blood samples were collected from the jugular vein once, 2 h after feeding, at the end of the experiment. Average daily gain was not affected by treatment ($P \geq 0.22$; 238 g/d; SEM = 12.1). RPCSuc tended to linearly decrease ($P = 0.06$) dry matter intake (1.60, 1.60 and 1.52 kg/d for control, RPCSucL, and RPCSucH, respectively; SEM = 0.021) and therefore tended to linearly decrease ($P = 0.10$) feed:gain (7.07, 6.77 and 6.22 kg/kg for control, RPCSucL, and RPCSucH, respectively; SEM = 0.294). Treatment had no effects ($P \geq 0.22$) on blood cell counts and concentrations of nonesterified fatty acids, β-hydroxybutyrate, and glucose. Blood serum insulin concentration was quadratically decreased ($P < 0.01$) by RPCSuc. RPCSuc tended to quadratically increase ($P = 0.06$) cortisol and had no effect on haptoglobin concentration in blood serum. In this experiment, dietary supplementation of a blend of artificial sweetener and capsicum had a modest effect on productive performance of lambs, and it appeared to have a regulatory effect on insulin secretion.

Key Words: capsicum, artificial sweetener, lamb

M324 Effect of algae supplementation on milk fatty acid profile in lactating dairy goats. P. Wang*[1], Y. Xue[2], A. Koontz[2], X. Zhang[1], and J. Luo[1], [1]*Alltech-NWAFU Animal Science Research Alliance, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China,* [2]*Alltech China, Chaoyang District, Beijing, China.*

The objective was to evaluate the effect of algae supplementation on milk fatty acid profile in lactating dairy goats. Eight multiparous Xinong Saanen dairy goats in late lactation (3.9 ± 0.4 Parities; 208.5 ± 2.7 DIM; 61.5 ± 6.2 kg BW) were randomly assigned to a replicated 4 × 4 Latin square design with 4 periods of 18 d. Goats were individually fed the basal diet with no algae (control), or basal diet supplemented with 10 (Alg-10), 20 (Alg-20) or 40 (Alg-40) g/goat of algae powder (Alltech Inc., Beijing, China) daily. Basal diet (forage: concentrate = 60: 40, DM basis) consisted of corn silage, alfalfa hay and concentrate was offered at 2 feedings (0830 and 1530) for 2 wk of acclimation before 4 experimental periods of 18 d. One hundred grams of concentrate was mixed with algae, and half of the mixture was offered before each feeding to guarantee all algae was consumed by goats during the experimental period. Milk fatty acid composition was analyzed on d 15 of each 18 d-period. Data were run by GLM procedure (SAS 9.2) and treatment means were compared by LSD test ($P < 0.05$). Fat- and protein-corrected milk (FPCM) yield was not affected by treatment. Further analysis on milk fatty acid composition showed that both MUFA and PUFA were changed with algae supplementation ($P < 0.05$). Higher concentration of C18:1n-9 *trans* and C20:1n-9 *trans* in milk for goats fed Alg-20 and Alg-40 diet was observed than those for goats fed control and Alg-10 diet ($P < 0.05$). Compared with control diet, experimental diets supplemented with algae induced increased concentration of C18:3n-3 and C18:3n-6 in milk. Moreover, increased responses in milk DHA and EPA were observed for treatment supplemented algae ($P < 0.05$). In conclusion, algae supplementation in lactating goat can change milk fatty acid composition, and indicated the potential to enhance some of the nutritionally healthy fatty acids in dairy goat milk.

Key Words: goat, algae, milk fatty acid profile

M325 Cooperative Real Education in Agriculture Management at the University of New Hampshire. A. B. Conroy, P. S. Erickson, K. M. Aragona*, and E. Hatungimana, *University of New Hampshire, Durham, NH.*

The 2-semester CREAM (Cooperative Real Education in Agriculture Management) course is an experiential and capstone experience for students in any major. Students participate in 4 h of classroom activities/week, including a business meeting and committee meetings with students serving on each committee for 6 wk. Weekly 2-h education sessions allow students to choose activities, lectures, and speakers related to running a dairy farm. The hands-on components include managing a herd of 25 dairy cows daily, including milking, feeding, heifer care, sire selection, designing rations, and monitoring the health and reproduction weekly. Students elect class officers to facilitate this flipped course. The president runs the business meetings and leads the students in reviewing committee reports and work, helping lead decisions on educational programming as well as cow management. Two faculty, a grad student, and farm manager advise the class, with veto power if student decisions are not in the best interest of the herd or academic and hands-on skill development. The course has no exams or term papers, but students peer grade each other 2×/semester (60% of grade) and must complete a self-evaluation based on set criteria, which provides feedback on learning outcomes. Students are graded by faculty (40% of grade) on a mini-lecture related to dairy cattle management, and taking on and following through with individual responsibilities. These include chores, maintaining the website, writing for or editing the newsletter, running open houses, and leading tours, planning a dairy-related class trip, planning the annual banquet, or recruiting students for the following year. Students are also encouraged to show initiative in solving issues related to herd management. Half of the students return for 6 wk the next year to train the new students, thereby demonstrating their competency in all areas. Challenges in running the course include making sure students take on responsibility and ownership in their education and the herd, encouraging their initiative and creativity, while ensuring a healthy and productive herd in a public university setting. Working together as a team and communicating all decisions and activities with each other and the farm manager have also been essential to success in the course.

Key Words: undergraduate, teaching, experiential

M326 Teaching final-year veterinary students about dairy cattle welfare. T. F. Duffield, L. J. Levison*, and D. B. Haley, *University of Guelph, Guelph, ON, Canada.*

A 1-wk elective rotation for final-year veterinary students began in 2016 at the Ontario Veterinary College (OVC), University of Guelph, to teach current scientific knowledge and practical elements of dairy cattle welfare. The rotation was developed as a response to a veterinary curriculum mapping exercise that identified gaps in welfare training, and from a private funding opportunity from Saputo Inc. to train veterinary students about dairy cattle welfare. The main objectives were to train students to (1) recall existing dairy welfare standards and recognize deficits; (2) carry out practical on-farm dairy cattle welfare assessments; (3) communicate with clients and colleagues about animal welfare. The rotation runs twice per academic calendar with a maximum of 12 student participants per offering; 6 spaces held for OVC students and 6 spaces for students from other American Veterinary Medical Association accredited schools. Learning is a balance of seminar style/interactive teaching, on-farm, student exercises, and open discussions around key and current dairy welfare issues. Students are evaluated through participation, presentations on key dairy welfare issues and farm reports prepared after conducting a proAction animal care assessment. Central components of the rotation are: defining welfare; identifying and prioritizing welfare issues affecting dairy cattle; Canada's proAction animal care assessment; cull cow decision-making, disbudding procedures; management of down cows and euthanasia decisions; students training in lesion scoring, lameness, and body condition; farm tours with a welfare focus. To date rotation effectiveness has been evaluated with student feedback. Students from the 5 veterinary colleges in Canada and veterinary students studying in Illinois, and Michigan have been involved in the elective. *Financial support of Saputo Inc. to create this elective is gratefully acknowledged.*

Key Words: welfare, veterinary

16 Efficiency of ceramic microfiltration removal of whey protein from sweet whey. B. Carter*[1], D. Barbano[2], and M. A. Drake[1], [1]*North Caroline State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Microfiltration (MF) is a commonly used technology in the dairy industry for removing bacteria, fractionating casein and serum proteins, and defatting of whey. In the production of whey protein isolate (WPI), MF or anion exchange is required to achieve protein concentrations above 90% of total solids. When using MF, an MF retentate co-product is produced called whey protein phospholipid concentrate (WPPC). WPI is sold for a higher price than WPPC; thus, improving whey protein passage during microfiltration will increase WPI protein yield and profit. A 3×, 3-stage spiral wound MF process at 50°C removes about 70% of serum protein (SP) from skim milk. In contrast, ceramic MF has been shown to remove 95 to 98% of serum proteins from skim milk in a 3×, 3-stage process at 50°C. The objective of this study was to determine if 95% removal of whey proteins is achievable with separated sweet whey as the starting material under the same conditions as reported for skim milk. Sweet whey was produced from a standard Cheddar cheese make and 375 kg of pasteurized, fat-separated whey was subjected to ceramic MF. The experiment was repeated 3 times with a 0.1 μm ceramic uniform transmembrane pressure (UTP) Membralox MF membrane. Retentate and permeate were collected, weighed, and sampled at each stage and analyzed for total protein by Kjeldahl, as well as protein profile using a reversed phase C4 UPLC column with a photodiode array detector. Peak areas were calculated from standard curves of individual proteins to quantify concentration of individual whey proteins in the retentate and permeate. Results were compared with previous published research from skim milk. Approximately 85% of the total nitrogen was removed from whey in 3 stages of MF of whey. Protein removal in permeate from sweet whey was 10% lower than values reported from ceramic MF of skim milk, but higher than the 70% reported for spiral wound MF membranes on skim milk. This research demonstrates that a high percentage removal of whey proteins can be achieved with 0.1 μ ceramic UTP MF Membralox membranes.

Key Words: microfiltration, whey protein, ceramic membranes

17 Concentration of acid whey from Greek-style yogurt using a combination of reverse osmosis and forward osmosis. P. Menchik* and C. Moraru, *Cornell University, Ithaca, NY.*

Acid whey from Greek-style yogurt (GAW) is a byproduct of interest in the dairy industry, due to the boom in its production and its potential negative environmental impact. Concentration of GAW solids can facilitate their use, storage, and transportation. This study focuses on achieving a high concentration factor of GAW using a combination of reverse osmosis (RO) and forward osmosis (FO), to avoid challenges and reduce costs associated with its thermal concentration. GAW from a NY State plant was first concentrated at 5 to 13°C using a RO rig (Osmonics, WI) with a spiral-wound aromatic polyamide membrane (Dow, NY), then by FO at 4 to 10°C using a FO unit (Ederna, France) with a modified spiral-wound cellulose triacetate membrane, and potassium lactate 60° Brix as draw solution. Experiments were conducted at least in triplicate, using different batches of GAW. The water fluxes were determined gravimetrically, and total specific energy for each process was calculated. The electrical energy required for pumping was calculated using the measured current and voltage in the system. For RO, the cooling energy was also determined. For FO, the energy required for regenerating the osmotic agent was estimated based on the specifications of the evaporator (Evaled, Italy). Fresh GAW, ranging in concentration from 5.9 to 6.8° Brix, was concentrated by RO to 19.1 ± 2.0° Brix. Part of this concentrate was then concentrated by FO to 38.1 ± 4.7° Brix. The water flux for RO dropped from 28.8 ± 0.5 L/m^{2}h to 14.7 ± 1.9 L/m^{2}h, and for FO from 3.7 ± 0.5 L/m^{2}h to 2.5 ± 0.2 L/m^{2}h. The total specific energy consumption was 0.29 ± 0.04 kWh/kg water for RO and 0.65 ± 0.06 kWh/kg water for FO. For FO, about half of the required energy pertains to the draw solution regeneration. This work will provide guidance on how to combine RO and FO to produce high quality GAW concentrates in a cost-effective manner. The energy calculations provided here serve as an initial estimate, and scaling to commercial scale can bring them down due to large volumes, continuous operation, and more efficient processing.

Key Words: Greek-style yogurt acid whey, reverse osmosis, forward osmosis

18 Feasibility of front-face fluorescence spectroscopy as a tool to understand protein leak during dairy ultrafiltration. Y. B. Ma* and J. K. Amamcharla, *Food Science Institute, Animal Sciences and Industry, Kansas State University, Manhattan, KS.*

Ultrafiltration (UF) is widely used in the dairy industry to separate proteins. However, certain undesirable conditions can cause proteins to leak through the membrane into the permeate. The protein leak can cause economic losses and quality deteriorations in permeate powders during storage. The objective of this study was to evaluate front-face fluorescence spectroscopy (FFFS) to understand and detect the presence of true protein in permeates. For this purpose, 12 whey/milk permeate powders were collected from 2 commercial suppliers. Total protein, true protein, and nonprotein nitrogen of the samples were analyzed. The average nonprotein nitrogen of permeate was 2.4 ± 0.18%, but the true protein varied between 0.09 and1.31%. Based on the true protein to total protein ratio, the samples were grouped as high-true-protein (HTP) and low-true-protein (LTP) permeates. The mean true protein of HTP and LTP were 0.77 and 0.22%, respectively and were significantly different ($P < 0.05$). The emission-excitation matrix (EEM) of one HTP and one LTP permeates (reconstituted to 5% solution) were collected by a spectrofluorometer at 25°C. The EEM was obtained by recording the emission (λ_{Em}) spectra from 300 to 500 nm corresponding excitation wavelengths (λ_{Ex}) ranging from 220 to 360 nm. Three fluorophores identified were tryptophan, Maillard browning products (MBP), and riboflavin. While the MBP and riboflavin showed similar intensities, HTP permeate indicated high fluorescence intensities in tryptophan region compared with the LTP permeate which was selected to characterize the protein leaks. Tryptophan emission spectra (λ_{Ex} at 280 nm; λ_{Em} 300–500 nm) and excitation spectra (λ_{Em} at 340 nm; λ_{Ex} 200–300 nm) were collected for the 12 permeate solutions (5% wt/wt). Spectral data were analyzed by hierarchical clustering analysis (HCA) with complete linkage. HCA classified LTP permeates in one cluster with 100% accuracy while HTP permeates were split into 2 sub-groups. The results showed that FFFS has potential to differentiate LTP permeates from HTP permeates by tryptophan fluorescence spectra.

Key Words: membrane processing, emission-excitation matrix

19 Transcriptomics characterization of genes involved in exopolysaccharide production in *Streptococcus thermophilus* ASCC1275 under the influence of various sugars. A. Padmanabhan*, Q. Wu, and N. P. Shah, *The University of Hong Kong, Hong Kong.*

One of the major determinants of exopolysaccharide (EPS) production is the availability of carbohydrate in the growth media. In our previous study, it was observed that the model dairy starter bacterium *Streptococcus thermophilus* ASCC 1275 (ST1275) produces high amount of EPS. In the current work, we investigated the influence of 3 major sugars, glucose, sucrose and lactose on the global transcriptomics of ST1275, to examine the expression of genes involved in EPS production. The bacterium was grown in M17 media supplemented with 1% of each sugar at 37°C and samples were collected at log phase (5 h) and stationary phase (10 h) for transcriptomics study. Overall, 233, 520, and 408 genes were upregulated in glucose, sucrose and lactose supplemented media, respectively. It was observed that there was a significant upregulation of various genes involved in EPS assembly (*eps*A, *eps* B, *eps* 1C, *eps* 1D, *eps* O, *eps* P), arginine/cysteine and methionine synthesis and phosphoenolpyruvate (PEP) transport system (PTS) in sucrose supplemented medium, which showed high EPS production, when compared with lactose and glucose supplemented media at 10 h. Even though, there was a major upregulation of most of the genes in EPS gene cluster and nucleotide sugar synthesis in lactose medium at 5 h, the same level of expression was not observed at 10 h due to rapid utilization of lactose in the medium and its unavailability thereafter. This suggests the systematic involvement of different sugars and pathways for their metabolism for increasing the EPS yield.

Key Words: *Streptococcus thermophilus*, exopolysaccharide, transcriptomics

20 Maintaining a high level of intact casein in Cheddar cheese during aging. B. M. Riebel*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], M. E. Johnson[2], and J. A. Lucey[1,2], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Center for Dairy Research, Madison, WI.*

As Cheddar cheese ripens, the original structure is broken down by proteolysis and calcium (Ca) solubilization. High levels of intact casein are needed for structure building in process cheese. Our goal was to minimize structural changes during ripening using 4 strategies: (1) use of milk with higher casein, (2) minimizing the rennet amount used, (3) using camel chymosin as the rennet, as it is less proteolytic than calf chymosin, and (4) applying high-pressure processing (HPP) to the cheese shortly after manufacture. Cheddar cheese was made from ultrafiltered (UF) milk. The cheesemilk protein and casein contents of were ~5.15% and 4.30%, respectively. Three types of cheeses were made (n = 4) using different rennet levels: (a) control, which had 2.0 μL rennet/50 g milk, (b) 25% reduced, and (c) 50% reduced. During cheesemaking, a licensed cheesemaker maintained high draining pH. Cheeses had similar moisture contents (~37%). Four days after the cheese was made, half of the samples from each vat were kept as controls; the other half underwent HPP at 600 MPa for 3 min. Loss tangent (LT) values of cheese during heating were measured by small strain oscillatory rheology. Intact casein was measured using Kjeldahl method to measure total and pH 4.6 soluble N. Acid/base titrations were used to determine the buffering capacity and insoluble Ca as a percentage of total Ca. Cheeses were aged for 8 m and made into process cheese at 2-wk, 2, 4, 6, and 8-m time points to determine performance changes of the cheese over time. Split-plot design was used for statistical analysis of data. Max LT values in process cheese increased with aging of the natural cheese; HPP decreased the max LT. Casein breakdown was slowed by use of the camel chymosin; during 6 m of aging, proteolysis was not affected by rennet levels or HPP. Due to the high natural cheese pH caused by the high draining pH, insoluble Ca contents remained high during ripening. HPP only influenced the rheological properties. The use of camel chymosin, concentrated UF milk, and maintaining high draining pH seem to have greater effects on retaining structure-building character than the rennet amount or HPP treatment.

Key Words: intact casein, process cheese, cheese ripening

21 Preparation of a non-surface-active solution from fluid milk for interfacial experiments of milk fat globule membrane polar lipids. L. M. Real Hernandez* and R. J. Flores, *The Ohio State University, Columbus, OH.*

The lack of a standard solution that resembles the solute composition and chemical properties of milk without having surface activity has led researchers studying the physicochemical properties of milk phospholipids and gangliosides at interfaces to use an array of buffers that mimic only a few parameters of fluid milk, such as pH and/or osmolarity. Polar lipid behavior is heavily influenced by the solutes present in their surroundings, so obtaining solutions that resemble fluid milk as much as possible, without having surface activity that can interfere with accurate data acquisition, is required for obtaining reliable results that can be applied to real food systems. In this work, a method for extracting surface- active compounds from milk, such as lipids and proteins, without significantly changing the integrity of the fluid milk is presented. Surface-active compounds are characterized as those that accumulate at the water/air interface and increase the surface pressure of that interface. By passing fluid milk through a 10-kDa molecular weight cut-off membrane ultrafiltration unit and then passing the resulting permeate through rounds of carbon filtration and aeration, a solution that is virtually non-surface-active can be created. No acid-base chemistry or excessive heat is used, and the presence of trace, fully soluble compounds can be retained unlike in simulated milk ultrafiltrate preparation. Equilibrium surface pressures of the permeate at various stages of refinement were obtained using a Langmuir trough, with crude permeate having an equilibrium surface pressure of 13 ± 1 mN/m. Surface pressures were obtained at least thrice from at least 3 different permeate samples. The stability of ganglioside monolayers on permeate at various stages of refinement show that ultrafiltration of fluid milk alone is not sufficient to obtain reliable data. With an increasing interest in the behavior of milk fat globule membrane lipids in fluid milk, this work improves model systems that heavily simplify the complexity of the matrix that milk fat globules present themselves in.

Key Words: ultrafiltration, water interface, membrane lipid

22 Tracking *Listeria* survival at different stages of ice cream manufacture. N. Neha* and S. Anand, *South Dakota State University, Brookings, SD.*

Recent recalls emphasize the need to find novel approaches for *Listeria* control in ice cream industry. Present study investigates survival of *Listeria* at different stages of ice cream manufacture. *Listeria innocua* ATCC 33090 (a surrogate) was spiked at mean log levels of 2, 3, and 4 log cfu/g in ice cream mix samples with total solid levels (32, 36, 42, 45%). The samples were pasteurized (69°C /30min) followed by aging (7°C), freezing (−4°C), and hardening (−40°C). Direct plating on selective agars was used to enumerate survivors, while any injured cells were resuscitated in *Listeria* enrichment broth, before plating. All trials were conducted in triplicates, and the data were statistically

analyzed. Although direct plating did not show any survivors in pasteurized mix, the enrichment protocol randomly recovered some injured cells. It is important to note that random presence of heat-injured cells was observed only at higher spiking levels (4.36 ± 0.13 log cfu/g). The scanning electron micrographs showed entrapment of some *Listeria* cells in the air pockets of ice cream mix, which might have led to injured cells. Binary models further established the dose levels to be a predictor of the presence of heat-injured cells of *Listeria.* Significantly, the heat-injured cells did not recover within ice cream mix itself during the different processing steps. In a parallel study, spiked pasteurized mix samples (2.65 ± 0.07 log cfu/g) were subjected to aging, freezing and hardening steps, followed by direct plating. No detrimental effect of these stages was observed on the intact cells of *Listeria.* It can be concluded that although a higher contamination with *Listeria* may lead to a random presence of injured cells, these cells do not recover under normal mix handling and processing conditions. On the contrary, any post-pasteurization contamination of mix could pose a potential risk from intact cells of *Listeria,* thus making a case for more robust risk assessment protocols.

Key Words: risk, listeria, ice cream

23 Subcritical hydrolysis of ice cream wastewater: Modeling and hydrolyzates properties. M. Enteshari* and S. Martínez-Monteagudo, *South Dakota State University, Brookings, SD.*

Dairy industry generates large quantities of wastewater (WW) from their routine cleanup operations. During the manufacturing of ice cream, the amount of WW generated is estimated to be between 8 and 12 L per kg of product. Ice cream WW is a complex mixture of suspended solids, soluble material, dispersed particles of different size (proteins and emulsified fat), and residues of cleaning agents. Such WW have proved to be problematic for the receiving effluent treatment plant. A promising method for WW conversion involves the hydrolysis of the compounds forming the colloidal mixture under subcritical conditions, which results in compounds of a lower molecular size that can be separated and further convert into valuable chemicals. In this study, the hydrolysis of ice cream WW was studied under subcritical conditions. The subcritical hydrolysis of ice cream WW was studied at 130–230°C, 20–60 bar, and 0–240 min in a continuous stirred-tank reactor. The degree of hydrolysis (DH) at different time intervals was measured by spectrophotometric method. Samples of ice cream WW were collected from the university dairy plant after the first step of a typical clean-in-place. The total protein of the ice-cream WW were 7.79 ± 1.33 on dry basis. Overall, the reaction time and temperature significantly increased the DH, reaching a maximum value of 40.99 ± 0.81, 34.44 ± 0.47, 20.61 ± 0.42, and 5.74 ± 0.36% after 200–240 min at 130, 170, 200, and 230°C, respectively. The experimental data were modeled using the Weibull distribution model showing a satisfactory correlation between experimental data and predicted values (R^2 = 0.981). After 240 min of reaction, the hydrolysates were recovered and their antiradical ability was measured through free radical scavenging (2,2-diphenyl-1-picrylhydrazyl) method. The inhibition of a free radical was found to increase linearly with the DH (R^2 = 0.991). The study outcomes presents an opportunity for utilizing subcritical hydrolysis to convert wastewater into valuable materials.

Key Words: ice cream wastewater, subcritical water, hydrolysis

24 Predicting quality attributes of yogurt-ice cream through fluorescence spectroscopy. N. Muhammad*[1], A. Sahar[1,2], N. Huma[1], A. Sameen[1], and U. Rahman[1], [1]*National Institute of Food Science and Technology (NIFSAT), Faculty of Food, Nutrition and Home Sciences (FFNHS), University of Agriculture Faisalabad (UAF), Faisalabad, Pakistan,* [2]*Department of Food Engineering, Faculty of Agricultural Engineering, UAF, Faisalabad, Pakistan.*

Yogurt is a healthy fermented dairy product which possesses various health promoting effects. Transforming yogurt into ice cream tends to increase its shelf life and overall acceptability by the people of all age groups. However, flavor and textural quality of ice cream is of prime importance from customer point of view. Several traditional methods have been used to evaluate the quality of ice cream during the storage. Nevertheless, a rapid prediction method to determine the quality of ice cream at any stage of storage is needed for on-line monitoring. Accordingly, the present study is planned to evaluate the impact of different yogurt concentrations (10–50%) on various compositional and quality attributes of yogurt ice cream [pH, acidity, meltdown properties, over run, SNF (solids-not-fat), total solids (TS), crude protein (CP), crude fat, ash] during storage of 2 mo and to explicate the potential of fluorescence spectroscopy for predicting the changes in these quality attributes of yogurt-ice cream during the storage period. The yogurt-ice cream was analyzed for various parameters; namely, titratable acidity, pH, TS, overrun, melt down, crude fat, CP, and ash %. In parallel, fluorescence spectra were also taken during the intervals at various excitation (250–510 nm) and emission wavelengths (300–620 nm). The findings concluded that the ranges of the selected parameters remained in the acceptable range during the storage of yogurt-ice cream. For instance, pH, meltdown ability, acidity, overrun, CP, SNF, TS, ash, and crude fat values ranged as 5.52–6.56, 7.97–16.10 g/100 g, 0.11–0.73, 30.03–44.50%, 3.48–3.58%, 21.47–23.38%, 31.85–33.22%, 1.10–1.70% and 9.33–11.50% respectively. The regression results revealed that fluorescence spectroscopy has a good prediction potential at various emission and excitation wavelengths (R^2 for pH = 0.97, R^2 for meltdown = 0.51, R^2 for acidity = 0.95, R^2 for overrun = 0.97, R^2 for CP = 0.99, R^2 for TS = 0.98, R^2 for SNF = 0.97, R^2 for crude fat = 0.98, R^2 for pH = 0.11) for determining the quality attributes of yogurt-ice cream during the storage.

Key Words: yogurt, fluorescence spectroscopy, chemometrics

25 Predicting butter adulteration with Fourier transform infrared spectroscopy and multi-variant analysis. A. Sahar*[1,2], M. U. Akram[2], U. Rahman[1], M. A. Khan[2], M. I. Khan[1], and I. Pasha[1], [1]*National Institute of Food Science and Technology (NIFSAT), Faculty of Food, Nutrition and Home Sciences (FFNHS), University of Agriculture Faisalabad (UAF), Faisalabad, Pakistan,* [2]*Department of Food Engineering, Faculty of Agricultural Engineering, UAF, Faisalabad, Pakistan.*

Mixing of food commodities with low-quality substances are being widely practiced around the globe that impose negative effects on the consumers' health. Addition of different vegetable oils in butter is a common practice that deteriorates the quality of butter. Suppliers are partially or completely replacing the butter fat with different low-quality vegetable oils and animal fats. Currently available methods used for detection of fat adulteration in different dairy products are color test and apparent solidification test. These methods are no doubt reliable but not comprehensive, require expensive and hazardous chemical reagents and are time consuming. Hence there is need to use some rapid and non-destructive methods which are suitable and can serve the purpose. Present study was aimed to detect the adulteration of vegetable oil in butter with FTIR spectroscopy. Butter samples with different concentrations of vegetable oil (10, 20, 30, 40, 50, and 60%) were prepared and FTIR spectra were collected (4000–650 cm^{-1}). PCA were applied on data set of FTIR spectra recorded on butter, vegetable oil and adulter

ated samples after removing the outliers using H-statistics. The PCA similarities maps defined by the principal components 1 and 2 for FTIR spectra showed that the first 2 principal components accounted for 98% of the total variance with a predominance of component 1 (85%). Better discrimination of butter, vegetable oil and adulterated samples were observed after removing outlier from the original data set. These results confirmed that the FTIR spectra retained information related to the molecular structure of butter and vegetable oil allowing discrimination of samples as a function of adulteration. The results obtained by PLS (partial least squares) regression model showed the values of R^2 equal to 0.88 for calibration and 0.68 for validation using 3 PLS factors. The good predictions strongly suggest that the FTIR spectra retain information on butter adulteration and that PLS regression can extract specific information regarding spectral variations due to the addition of vegetable oil in butter samples, which are helpful in predicting the adulteration in the butter samples.

Key Words: butter, adulteration, Fourier transform infrared (FTIR)

26 Intramammary infection in growing, nonlactating mammary glands. B. D. Enger*[1], C. E. Crutchfield[1], T. T. Yohe[1], K. M. Enger[1], S. C. Nickerson[2], C. L. M. Parsons[1], and R. M. Akers[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*University of Georgia, Athens, GA.*

Intramammary infections (IMI) are prevalent in lactating and nonlactating dairy cattle and their occurrence during pregnancy is concerning given the considerable amounts of mammary growth and development that occur during late gestation, in preparation for lactation. To date, the effects of IMI on mammary growth and development are largely unknown. This study's objectives were to determine how IMI affected total and differential mammary secretion somatic cell counts and characterize mammary morphology changes resulting from IMI in glands stimulated to grow with estradiol and progesterone. Mammary growth was induced in 19 nonpregnant, nonlactating dairy cows and 2 quarters free of IMI of each cow were subsequently infused with either saline (n = 19) or *Staphylococcus aureus* (n = 19). Mammary secretions were collected throughout the trial and mammary tissues were collected at either 5 or 10 d post-challenge. Secretion and tissue data were analyzed using a mixed model with treatment and euthanasia day as fixed effects and cow nested within euthanasia day as random. Infected quarter secretions contained a greater concentration of somatic cells than saline infused quarters (7.45 vs 6.77 ± 0.06 $\log_{10}$ cells/mL; $P < 0.001$), and contained a greater proportion of neutrophils than saline quarter secretions (47.2% vs 7.1% ± 2.3%; $P < 0.001$). Infected mammary tissues also exhibited greater degrees of immune cell infiltration than saline quarters. Lobules in *Staph. aureus* mammary tissues displayed a greater percentage of luminal space (7.7% vs 5.4% ± 0.6%; $P = 0.004$), a reduced percentage of epithelial area (33.3% vs 38.1% ± 1.1%; $P < 0.0001$), and tended to have a greater percentage of intralobular stromal area (59.0% vs 56.5% ± 1.3%; $P = 0.1$) than saline quarters. These results indicate that IMI in nonlactating glands that were stimulated to grow, elicited an immune response leading to an infiltration of immune cells into mammary tissues and secretions, which was associated with changes in mammary tissue structure. The observed reduction of epithelial areas suggest that IMI negatively affects mammary growth, which may result in reduced future milk yields and productive herd life.

Key Words: mastitis, dry cow, mammary growth

27 Genetic analysis of subclinical mastitis resistance in early lactation in first-parity cows. S. G. Narayana*[1,2], F. Miglior[2,3], S. A. Naqvi[1], F. Malchiodi[2], P. Martin[2], and H. W. Barkema[1], [1]*Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada,* [2]*CGIL, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Canadian Dairy Network, Guelph, ON, Canada.*

Subclinical mastitis (SCM) causes economic losses for producers by affecting milk production and leading to higher incidence of clinical mastitis and premature culling. The incidence of SCM in first-lactating cows is usually higher during early lactation. The SCC can be used for the diagnosis of subclinical mastitis. The objective of this study was to estimate genetic parameters for SCM in early lactation in first parity Holsteins. Test-day records for SCC were collected monthly between 2005 and 2009 in 90 Canadian herds participating in the national cohort of dairy farms of the Canadian Bovine Mastitis Research Network. Only the first test-day record available between 5 to 30 DIM was considered for the analysis. The final data set contained 8,518 records from first lactating Holstein heifers. Six alternative traits were defined as indicators of subclinical mastitis using different cut-off values of SCC (between 150,000 to 400,000 cells/mL). Linear and threshold animal models were used for the analysis. The prevalence of subclinical mastitis ranged from 15 to 24%. Estimated heritabilities from linear and threshold model ranged from 0.037 to 0.057 and 0.040 to 0.050, respectively. Strong genetic correlations were found among alternative SCC traits (from 0.90 to 0.99), indicating that these 6 traits were genetically similar. Despite a low heritability, estimated breeding values (EBV) predicted from both models showed a large genetic variation among sires. Higher EBV of SCM resistance corresponded to sires with higher percentage of healthy daughters. The percentage of diseased daughters varied between 5 to 13% and 19 to 33% among sires with best and worst EBV. The Spearman's rank correlations between EBVs of sires predicted from linear (0.76 to 0.95) and threshold (0.74 to 0.95) models were moderate to high. These results indicates that although the heritabilities were low, an exploitable genetic variation for SCM in early lactation exists.

Key Words: mastitis, heritability, somatic cell count

28 Predicting composition of empty body weight of Holstein heifers and cows. R. A. de Souza* and M. VandeHaar, *Michigan State University, East Lansing, MI.*

Our objective was to predict composition (% fat, % protein, % ash, and % water) as % of empty body weight (EBW) in heifers and cows using only data from Holsteins. We conducted mixed regressions analysis on 129 treatment means from 24 peer-reviewed publications. Methods to determine composition were direct analysis of EBW, analysis of carcass or rib, and dilution of deuterium oxide or urea. Means and standard deviations were 10 ± 5% fat, 19 ± 2% protein, 4 ± 1% ash, 66 ± 4% water, 118 ± 81 kg EBW, and 0.74 ± 0.25 kg daily gain (ADG) for growing animals and 20 ± 6% fat, 17 ± 2% protein, 5 ± 0.7% ash, 60 ± 3% water, 493 ± 64 kg EBW, and 2.9 ± 0.4 body condition score (BCS) for mature animals. We first used a mixed model with random effects of study and method to adjust dependent variables based on EBW, ADG, BCS, and possible 2-way interactions, with studies weighted by inverse of their variance. Second, adjusted compositions were modeled as functions of EBW, the natural logarithm of EBW (lnEBW), or EBW to the power of 0.75 (MEBW) to generate a base BW response. Finally, we examined effects of including ADG for heifers and BCS for cows. Cross-validation within the data set was used to select best fitting models based on concordance correlation coefficient (CCC), root means square error of prediction (RMSEP), and its decomposition. Including ADG for heifers and BCS for cows improved the overall prediction of body composition, and using lnEBW improved predictions of EBFat and EBWater. The proposed heifer models were: % Fat = −7.8 + 2.41 × lnEBW − 7.96 × ADG + 3.13 × lnEBW × ADG (CCC = 0.88, RMSEP = 1.77); % Protein = 27.5 − 1.62 × lnEBW − 1.20 × ADG (CCC = 0.43, RMSEP = 1.43); % Ash = 5.1 + 0.091 × lnEBW − 1.85 × ADG (CCC = 0.36, RMSEP = 0.79); and % Water = 90 − 3.41 × lnEBW + 0.990 × ADG − 1.74 × lnEBW × ADG (CCC = 0.69, RMSEP = 1.60). The proposed cow models were: % Fat = −24 + 6.43 × lnEBW + 1.82 × BCS (CCC = 0.54, RMSEP = 2.55); % Protein = 27 − 1.62 × lnEBW − 0.451 × BCS (CCC = 0.37, RMSEP = 0.98); % Ash = 5 + 0.091 × lnEBW − 0.340 × BCS (CCC = 0.30, RMSEP = 0.27); and % Water = 92.9 − 5.15

× lnEBW − 0.973 × BCS (CCC = 0.36, RMSEP = 2.34). These models could be further used to determine composition of BW gain.

Key Words: growth, body condition score, empty body weight

29 Manipulating serotonin pathway in dairy calves: Impact on blood hematology, growth, and health. M. G. Marrero-Pérez*, S. L. Field, B. Dado-Senn, A. L. Skibiel, D. R. Silva, and J. Laporta, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Serotonin, a monoamine derived from L-tryptophan, is naturally produced by peripheral tissues playing a role in several biological functions, including immunity. Here, we tested the safety and effectiveness of administering a serotonin precursor (5-hydroxy-L-tryptophan, 5-HTP) or a serotonin reuptake inhibitor (fluoxetine, FLX) to modulate serotonin bioavailability in dairy calves. Bull Holstein calves (21 ± 2 d old) were fed milk replacer (8 L/d) with either saline (CON, 8 mL/d, n = 8), FLX (40 mg/d, n = 8) or 5-HTP (90 mg/d, n = 8) for 10 consecutive d in a complete randomized block design. Heart rate (HR), respiration rate (RR), rectal temperature (RT), health and fecal scores were recorded daily. Hip height (HH) and body weight (BW) were measured at d-1, 5, and 10. Blood samples were collected at d-1 and daily (d 1–10) to measure serotonin and FLX concentrations. Blood hematology was assessed at d-1 and 10. Data were analyzed using repeated measure or one-way ANOVAs in SAS. Blood serotonin concentrations increased in calves fed 5-HTP starting on d 2, and decreased in calves fed FLX starting on d 6, compared with CON (6325, 2262 vs. 3166 ± 710 ng/mL, respectively, treatment by day interaction; $P < 0.001$). Health and fecal scores were similar among treatments. BW and HH of all calves increased throughout the experimental period, but calves fed 5-HTP gained more weight compared with CON (0.87 vs. 0.66 ± 0.12 kg/d, $P = 0.05$). RR was similar between treatments; but feeding 5-HTP lowered RT, while feeding FLX lowered HR, both relative to CON (37.6 vs. 38.0 ± 0.12°C, $P = 0.05$; and 107 vs. 114 ± 1.9 BPM, $P = 0.02$). Calves fed 5-HTP and FLX had greater red blood cells counts on d10 compared with CON (7.0, 7.5 vs. 6.3 ± 0.2 × 10^6/μL, respectively; $P < 0.05$). FLX fed calves had higher hemoglobin on d10 compared with CON (7.9 vs. 7.3 ± 0.2 g/dL, $P < 0.01$). There were no differences in total white blood cells counts between treatments, but 5-HTP fed calves tended to have more circulating neutrophils compared with CON (3.4 vs. 2.8 ± 0.3 × 10^3/μL, $P = 0.06$). Manipulation of serotonin pathway by supplementing FLX and 5-HTP is safe and may confer health and growth benefits to Holstein dairy calves.

Key Words: 5-HT, growth, immunity

30 Metabolic profile of Holstein heifers fed carinata meal compared with canola meal and a control diet. K. Rodriguez-Hernandez*[1,2], J. L. Anderson[1], J. A. Clapper[3], and G. A. Perry[3], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Matamoros, Coahuila, México,* [3]*Animal Science Department, South Dakota State University, Brookings, SD.*

Carinata meal is a developing oilseed meal that contains glucosinolates which may impair thyroid gland function and consequently metabolism and reproduction. Our objective was to compare the metabolic profile and onset of puberty of dairy heifers fed diets containing carinata meal, canola meal, or a control diet. A 16-wk randomized block design experiment with 36 Holstein heifers (6.3 ± 0.1 mo of age, and 207 ± 3 kg of BW) was conducted. Heifers were blocked by age. Treatments were (1) carinata meal (CRM), (2) canola meal (CAN), and (3) a control diet (CON) with most of the protein provided from soybean meal. Test meals were solvent extracted and included at 10% of diet DM. Diets were isocaloric and isonitrogenous and contained similar ingredients, other than the test feeds. Heifers were limit-fed rations at 2.4% of BW on DM basis using a Calan gate system. Jugular blood samples were collected 4 h post-feeding on 2 d during wk 0, 4, 8, 12, and 16 for metabolite and thyroid hormones analyses. To determine onset of puberty, blood samples were taken every 3 or 4 d for progesterone analysis. Data were analyzed using MIXED procedures with repeated measures in SAS 9.4. Puberty data were analyzed as binomial data (cycling or not cycling) and using repeated measures by 10-d and 10-kg intervals of age and BW. Significance was declared at $P < 0.05$. Glucose (75.9, 75.8, and 77.1 mg/dL; SEM = 1.65 for CRM, CAN and CON, respectively), cholesterol (81.7, 81.2, and 79.0 mg/dL; SEM = 2.95), triglycerides (23.4, 23.3, and 21.3 mg/dL; SEM = 1.13), plasma urea nitrogen (18.4, 18.0, and 17.2 mg/dL; SEM = 0.41), triiodothyronine (135.4, 140.7, and 141.6 ng/dL; SEM = 5.73), and thyroxine (4.7, 4.3, and 4.6 μg/dL; SEM = 0.17) concentrations were similar ($P > 0.05$). Age (297, 290, 294 d; SEM = 3.1) at puberty was similar among treatments. Heifers fed CRM and CON weighed less at puberty than heifers fed CAN (318 and 317 vs. 329 kg; SEM = 3.2; $P = 0.02$). Results demonstrate that heifers can be limit-fed diets with 10% CRM without negative effects on metabolic profile and onset of puberty, showing it is viable as a feed for heifers.

Key Words: carinata meal, metabolic profile, dairy heifer

31 Low-density lipoprotein ceramide accrual develops with steatosis, hyperlipidemia, and insulin antagonism during the transition from gestation to lactation. A. N. Davis*[1,2], J. E. Rico[1,2], W. A. Myers[1,2], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV.*

In nonruminants, low-density lipoprotein (LDL) C16:0 and C24:0 ceramide mediates insulin resistance. In dairy cattle, we have established that ceramide accumulates in plasma, liver, and skeletal muscle during the peripartum. Moreover, hyperlipidemia and steatosis promotes ceramide synthesis in cows. Liver-derived ceramide may contribute to impaired insulin action during the peripartum. Therefore, our objective was to characterize changes in lipoprotein ceramide levels in periparturient dairy cows. In a non-randomized controlled trial, 25 pregnant, multiparous lean (BCS 3.0 ± 0.2) or overconditioned (OVER; BCS 3.9 ± 0.3) Holstein cows were enrolled 28 d before expected parturition. Blood samples were routinely collected. Liver biopsies and the hyperinsulinemic-euglycemic clamp were performed 2 wk before and after parturition. Serum lipoproteins were isolated using fast protein liquid chromatography. Sphingolipids were quantified using mass spectrometry. Data were analyzed using a mixed model with repeated measures. Very low density lipoprotein (VLDL) total ceramide levels declined postpartum (730 vs. 1207 ng/mL; $P < 0.01$), whereas LDL total ceramide increased postpartum (1353 vs. 913 ng/mL; $P < 0.01$). C24:0 ceramide represented the most abundant ceramide in VLDL and LDL. OVER had lower VLDL C16:0 ceramide, relative to lean (71 vs. 86 ng/mL; $P < 0.01$). LDL C16:0 and C24:0 ceramide increased by d 10 postpartum, relative to d −12 (~50% increase; $P < 0.01$). Prepartum LDL C24:0 ceramide levels were greater in OVER (659 vs. 435 ng/mL; $P < 0.05$), relative to lean. The ratios of LDL C24:0 ceramide to sphingomyelin were greater in OVER ($P < 0.05$). LDL C24:0 ceramide were positively correlated with serum fatty acids ($P < 0.05$). Prepartum LDL total and C24:0 ceramide were positively correlated with liver lipid content ($P < 0.05$). Lastly, postpartum C16:0 LDL ceramide levels were inversely related to systemic insulin action (clamp index; $P = 0.05$). We

conclude that LDL ceramide accrual develops with steatosis, hyperlipidemia, and insulin antagonism in peripartal cows.

Key Words: ceramide, insulin sensitivity, peripartum

32 Aluminosilicate clay reduces the deleterious effects of an aflatoxin challenge on inflammation markers in lactating Holstein cows. R. T. Pate*[1], D. M. Paulus Compart[2], and F. C. Cardoso[1], [1]*University of Illinois, Department of Animal Sciences, Urbana, IL,* [2]*PMI Nutritional Additives, Shoreview, MN.*

Mitigation strategies are vital in minimizing the health and economic risks associated with dairy cattle exposure to aflatoxin (AF). The objective of this study was to determine the effects of a commercially available aluminosilicate clay in a traditional lactation diet during an AF challenge on inflammatory markers of multiparous lactating Holstein cows. Sixteen multiparous, lactating Holstein cows [BW = 758 ± 76 kg; DIM = 157 ± 43 d] were assigned to 1 of 4 treatments in a replicated 4 × 4 Latin Square design: no adsorbent and no AF challenge (CON), no adsorbent and AF challenge (POS), 113 g of aluminosilicate clay top-dressed on the TMR (adsorbent; PMI Nutritional Additives, Arden Hills, MN) with AF challenge (F4), and 227 g of adsorbent with AF challenge (F8). For each period, blood was sampled on d 14, 18, and 21, and liver was sampled on d 18. Gene expression and histological hepatocyte inflammation were assessed for liver tissue. One orthogonal contrast (POS vs. CON) was used along with the linear and quadratic effects of treatments POS, F4, and F8. Multivariable logistic mixed models considering the binary outcome variable histological hepatocyte inflammation score were constructed. Cows in CON had greater concentrations of serum cholesterol (202 mg/dL; $P < 0.001$) and plasma superoxide dismutase (2.8 U/mL; $P = 0.04$) than cows in POS (196 mg/dL and 2.6 U/mL, respectively). Plasma glutamate dehydrogenase increased as concentration of adsorbent in the diet increased (POS = 37.8, F4 = 39.3, F8 = 39.1 U/L; $P = 0.05$). *NFKB1* expression was higher ($P = 0.04$) in liver of cows in POS (0.78 ± 0.04) compared with cows in CON (0.70 ± 0.04). *MTOR* expression was higher ($P = 0.04$) in liver of cows in CON (1.19 ± 0.06) compared with cows in POS (0.96 ± 0.06). When compared with cows in CON, cows in POS had higher ($P = 0.05$) odds ratio for hepatocyte inflammation (OR = 5.14; 95%CI = 0.97–27.33). When compared with cows in F8, cows in POS tended ($P = 0.07$) to have higher odds ratio for hepatocyte inflammation (OR = 4.60; 95%CI = 0.90–23.66). In conclusion, AF exposure lead to reduced function and increased inflammation of the liver. Additionally, the adsorbent used helped to decrease hepatocyte inflammation and neither hindered nor improved blood metabolites and liver gene expression.

Key Words: aflatoxin, adsorbent, inflammation

33 Effects of feeding a *Saccharomyces cerevisiae* fermentation product during the periparturient period on performance of dairy cows fed postpartum diets differing in starch content. W. Shi*[1], C. E. Knoblock[1], I. Yoon[2], and M. Oba[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Diamond V, Cedar Rapids, IA.*

The objective of this study was to evaluate the effects of supplementing a *Saccharomyces cerevisiae* fermentation product (SCFP; NutriTek, Diamond V, Cedar Rapids, IA) during the periparturient period and dietary starch content during the first 3 wk after calving on the performance of dairy cows. From wk 4 before the expected calving date to 6 wk after calving, 117 Holstein cows were fed diets without (CON; n = 58) or with SCFP (SCFP; n = 59). All cows were fed a common basal diet (1.43 Mcal NE_L/kg DM; 13% starch) before calving. Cows within each treatment (CON or SCFP) were fed either a low- (LS; 21% starch; n = 59) or high-starch (HS; 27% starch; n = 58) diet from wk 1 to 3 after calving, and all cows were fed HS diets from wk 4 to 6 after calving. Cows were assigned to treatment balanced for parity, BCS, BW, and expected calving date. Data were analyzed using the FIT model procedure of JMP (SAS Institute Inc., Cary, NC). Although DMI was not different between LS and HS cows, milk yield was higher for cows fed the LS diets compared with those fed the HS diets at wk 1 to 3 after calving (34.1 vs. 32.1 kg/d; $P = 0.05$). Supplementation of SCFP had no effect on prepartum DMI, but SCFP cows had higher DMI than CON cows at d 1 (13.0 vs. 11.9 kg; $P = 0.02$) and d 5 after calving (15.5 vs. 14.1 kg; $P = 0.04$). However, overall DMI and milk yield from wk 1 to 3 after calving was not affected by SCFP supplementation. From wk 4 to 6 after calving, DMI tended to be lower for SCFP than CON cows (19.8 vs. 20.6 kg/d; $P = 0.10$), but 3.5% FCM yield (44.9 vs. 43.6 kg/d) was similar among treatments, resulting in greater feed efficiency for SCFP cows (3.5% FCM/DMI; 2.27 vs. 2.13; $P = 0.01$). These findings suggest that feeding low-starch diets during the immediate postpartum period can increase milk production of dairy cows fed a low-energy close-up diet, and that supplementation of SCFP in transition diets may increase feed intake around calving and feed efficiency in post-transition early lactation period.

Key Words: calving transition, dietary starch, *Saccharomyces cerevisiae* fermentation product

34 Dietary supplementation of *Scutellaria baicalensis* extract (SBE) during early lactation decreases milk somatic cells and increases whole lactation milk yield in dairy cattle. K. E. Olagaray*[1], M. J. Brouk[1], L. K. Mamedova[1], F. Robert[2], E. Dupuis[2], M. Zachut[3], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*CCPA Group, Janze, France,* [3]*Agriculture Research Organization, Volcani Center, Rishon Lezion, Israel.*

Multiparous Holstein cows (n = 122) were used in a randomized block design (blocked by parity, calving date, and risk factors) to determine the effect of SBE on 305-d milk yield and milk markers of inflammation and metabolism. Treatments were (1) control (CON), (2) 5-d administration of SBE (SBE5), and (3) 60-d administration of SBE (SBE60). Treatments were included in a treatment pellet that was identical to the control pellet except for the extract, both provided via an automated milking system beginning on d 1 of lactation. Milk samples were collected on d 1, 3, and once during d 5–12 of lactation, followed by weekly sampling until 120 d in milk. Milk samples collected in the first 2 wk were analyzed for biomarkers (haptoglobin, BHB, and glucose-6-phosphate [G6P]) and all samples were analyzed for components. Milk production, programmed pellet allocation, and actual provision of both pelleted feeds were recorded daily. Data were analyzed using the MIXED procedure in SAS. Treatment effects were evaluated by contrasts between CON and SBE5 and CON and SBE60 for both the treatment (TP; wk 1–9) and carryover (CP; wk 10–37) periods. Total pellet allocated was greater for SBE60 in both the TP ($P < 0.01$) and CP ($P = 0.02$), but was not different for SBE5 in either period. Treatments did not affect milk haptoglobin, BHB, or G6P. Whole-lactation milk yields were 11,245, 11,608, and 12,664 ± 465 kg for CON, SBE5, and SBE60, with differences between SBE60 and CON ($P = 0.03$). SBE5 did not alter milk components. SBE60 increased lactose and fat yields ($P < 0.05$) and tended to increase protein yield ($P = 0.09$) during TP; each increased during CP ($P < 0.05$). Somatic cell count decreased in SBE60 during TP ($P = 0.02$) but not CP. Mastitis incidence was lesser for SBE5 ($P = 0.04$) and SBE60 ($P = 0.05$) vs. CON. SBE60 tended to

decrease the hazard of leaving the herd compared with CON and SBE5 ($P = 0.07$). Overall, feeding SBE for 60 d postpartum increased 305-d milk yield, possibly related to decreased milk somatic cells.

Key Words: bioactive, polyphenol, transition cow

35 Bioavailability of rumen-protected histidine, lysine and methionine assessed by fecal amino acid excretion. S. E. Räisänen*[1], C. M. M. R. Martins[2], K. Nedelkov[3], J. Oh[1], M. T. Harper[1], X. Chen[4], C. Parys[5], R. A. Patton[6], M. Miura[7], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of São Paulo, Pirassununga, Brazil,* [3]*Trakia University, Stara Zagora, Bulgaria,* [4]*Lanzhou University, Lanzhou, Gansu, China,* [5]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany,* [6]*Nittany Dairy Nutrition Inc., Mifflinburg, PA,* [7]*Animal Nutrition Group, Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co. Inc., Kawasaki, Japan.*

The objective of this experiment was to assess bioavailability of rumen-protected His, Lys and Met products (RPAA). Eight Holstein cows (96 ± 21 DIM, 39.4 ± 7.0 kg/d milk yield), 4 of which rumen-cannulated, were used in a replicated 4 × 4 Latin square design experiment with four 25-d experimental periods. RPAA were fed daily to supply 20, 25, and 35 g/d of digestible (d)His, dLys and dMet, based on manufacturer's specification and dietary AA deficiencies. Treatments were combinations of RPAA products: MetA+LysA+HisA, MetB+LysB+HisB, MetC+LysC and MetD+LysC. Total fecal collection and blood sampling were performed during the last 3 d of each experimental period. Data were analyzed using the MIXED procedure of SAS with square, square(period), treatment and treatment × square in the model; cow(square) was random effect. Rumen by-pass AA (RBP, %) in each RPAA was determined in situ with the cannulated cows. RBP ranged ($P < 0.001$) from 29.3 to 98.0% for RPMet, from 33.3 to 84.7% for RPLys and was higher for HisA (89.7%) than for HisB (59.9%). Digestibility of AA from RPAA (%) was calculated as: (RBP AA, g – fecal AA excretion, g) ÷ RBP AA, g × 100, and bioavailability (BA, %) as: RBP, % × AA digestibility, % ÷ 100. Digestibility of Met from RPMet products varied from 86.1 to 97.8% ($P < 0.001$) and from 50.1 to 85.6% for RPLys ($P < 0.001$); dHis was not different for the 2 RPHis (85.6 and 88.5%; $P = 0.12$). BA of Met in RPMet varied ($P < 0.001$) from 25.8 to 95.3%, of Lys from 16.7 to 72.4%, and was 76.8 and 52.9% for HisA and HisB, respectively. Plasma Met concentration was higher for MetD (44.9 μ*M*; $P < 0.001$) compared with the other 3 RPMet (averaging 25.5 μ*M*). Plasma Lys concentration did not differ among RPLys (averaging 82.4 μ*M*, $P = 0.47$). Plasma His concentration was greater ($P = 0.002$) for HisA (73.9 μ*M*) than for HisB (55.5 μ*M*), reflecting the considerably greater dHis supply by HisA. This study showed that rumen degradability and bioavailability of AA from RPAA vary considerably and fecal AA output in relation to the amount of rumen by-pass AA can be used to estimate bioavailability of RPAA.

Key Words: rumen-protected amino acid, bioavailability, dairy cattle

Animal Behavior and Well-Being Platform Session: Assessment of Affective States of Dairy Cattle

36 The scientific assessment of affective states in dairy cattle. D. M. Weary*, *University of British Columbia, Vancouver, BC, Canada.*

How animals feel is both at the center of concerns about animal welfare and one of the most challenging questions for which to make strong scientific inferences. In this presentation, I will review various approaches used to investigate affective experiences in cattle, with a special focus on pain and fear (as much of the research is in this area). I will describe various methods available and argue that some of the most commonly used approaches suffer from important weaknesses. For example, provoked acute responses can be both highly variable and non-specific, meaning that both responses and non-responses are difficult to interpret. Stronger inferences can be drawn in cases where the functions of the responses are well understood, and with addition of appropriate control groups using drug treatments to target specific affective mechanism (e.g., analgesics in the case of pain). I will also review more elaborate approaches, including the use of approach-avoidance testing (that allows inferences about how much the affect matters to the animal), conditioned-place avoidance (that rely upon the memory of an experience), and drug-naming trials (that allow more specific inferences about affective states). Finally, I will discuss the potential of approaches that explore the longer-term impact of affective experiences, such a cognitive bias testing and anhedonia responses that may indicate mood changes and suggest more important welfare consequences.

Key Words: emotion, feeling, animal learning

37 Dairy calves anticipate the opportunity to access an alternative environment. H. W. Neave*[1], J. Webster[2], and G. Zobel[2], [1]*University of British Columbia, Vancouver, BC, Canada,* [2]*AgResearch Ltd., Hamilton, New Zealand.*

Anticipatory behavior for a reward has been used to assess emotional state in some farm animals, but not yet in dairy calves. We measured anticipatory behavior of calves for access to an alternative environment. Calves were randomly assigned in pairs to 1 of 2 housing treatments (n = 8 each): basic (2 m^2 bedded with river stones, used by some New Zealand farms) or enriched (5 m^2 bedded with wood shavings, an automated calf brush, manila rope, straw). Within housing treatment, each pair accessed either a basic (n = 7) or enriched (n = 9) alternative pen, resulting in calves experiencing a similar or different environment to their home pen. Calves were conditioned to anticipate entry to the alternative pen via a light signal, then a 1 min interval before pen access; access was permitted once per day for 3 d. Anticipatory behaviors (frequency of behavioral transitions; that is, changing between behaviors; duration of looking at or touching the light/door) were compared before and after the signal. The effect of treatment on these behaviors was analyzed in a repeated measures mixed model; values are reported as back-transformed means and confidence intervals. When access to the alternative pen was signaled, all calves increased anticipation ($P < 0.001$) [(4.9 vs 10.7 no. of behavioral transitions (4.2–5.8, 9.1–12.6); duration looking at or touching the light/door: 1.7 vs 31.7 s (0.73–3.1, 26.8–37.1)]. Basic-housed calves that gained access to an enriched alternative pen showed more behavioral transitions than enriched-housed calves that gained access to a basic or enriched alternative pen ($P = 0.05$) [(12.6 vs 9.5 vs 9.8 no. of behavioral transitions (10.5–15.5, 7.6–11.7, 8.1–11.7, respectively)]. Basic-housed calves left their home pen quicker compared with enriched-housed calves, regardless of the quality of alternative pen [6.0 vs 17.4 s (4.8–7.4, 13.8–21.9; $P < 0.01$)]. Calves anticipated access to an alternative environment, even if it was of lower quality than their home environment. However, basic-housed calves showed increased anticipation for the opportunity to leave their home pen, especially if they gained access to the enriched pen. The level of anticipation for a rewarding experience relates to the affective state of calves housed in enriched and basic environments.

Key Words: affect, animal welfare, enrichment

38 Effects of local anesthetic and/or systemic analgesia on pain associated with cautery disbudding in calves: A systematic review and meta-analyses. C. B. Winder*[1], C. L. Miltenburg[1], J. M. Sargeant[1,2], S. J. LeBlanc[1], D. B. Haley[1], K. D. Lissemore[1], M. A. Godkin[3], and T. F. Duffield[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Centre for Public Health and Zoonoses, University of Guelph, Guelph, ON, Canada,* [3]*Ontario Ministry of Agriculture, Food, and Rural Affairs, Elora, ON, Canada.*

Disbudding is a common management procedure performed on dairy farms, and when done without pain mitigation is viewed as a key welfare issue. Although use of pain control is increasing, full adoption of anesthesia and analgesia has not been achieved. The objective of this systematic review was to examine the effects of pain control practices for cautery disbudding on outcomes associated with pain, specifically: plasma cortisol concentration, pressure sensitivity of the horn bud, and validated pain behaviors. Intervention studies describing cautery disbudding in calves 12 wk of age or less were eligible, provided they compared local anesthesia, non-steroidal anti-inflammatory drug (NSAID), or both, to one or more of local anesthesia, NSAID, or no pain control. Of the 4,394 records identified, 21 articles comprising 23 studies were eligible for qualitative synthesis. Meta-analysis was performed for all outcomes measured at similar time points with more than 2 comparator groups. Local anesthetic was associated with reduced plasma cortisol until 2 h post-disbudding (30 min, n = 7 studies; 1 h, n = 8; 2 h, n = 5); however, a rise in cortisol was observed in the meta-analysis of studies reporting at 4 h post-disbudding (n = 5). The addition of NSAID to local anesthetic showed reduction in plasma cortisol at 4 h (n = 6), and a reduction in pressure sensitivity and pain behaviors in some analyses between 3 and 6 h post-disbudding (n = 3 to 6). Heterogeneity was present in several meta-analyses, including many examining pain behavior. This may reflect variation in measurement periods, and/or differences among NSAID treatments. Overall, a protective effect of local anesthetic was seen for acute pain, and the delayed rise in cortisol was mitigated by use of an NSAID, which also reduced pressure sensitivity and pain behaviors. These findings support the use of local anesthetic and an NSAID, but the magnitude and duration of the effect of NSAID treatment was not possible to deduce from the literature due to the variation between studies. We recommend adherence to reporting guidelines and consideration of more standardized outcome measurements, particularly for pain behaviors.

Key Words: pain, disbudding, systematic review

39 Development of a newborn calf vigor scoring system.
C. Murray-Kerr*[1], K. Leslie[2], S. Godden[3], S. McGuirk[4], and W. Knauer[3], [1]*Trouw Nutrition, Guelph, ON, Canada,* [2]*University of Guelph, Guelph, ON, Canada,* [3]*University of Minnesota, St. Paul, MN,* [4]*University of Wisconsin, Madison, WI.*

Calves that survive dystocia are often affected by injury, stress, pain, inflammation, hypoxia, acidosis and low overall vitality. Yet, vigor scoring systems, like the Apgar score used with human infants, have not been developed and validated. It was the objective of this research to quantify the effects of dystocia, to relate these effects to measurable signs of reduced vigor, and to develop a scoring system from these data. It was hypothesized that the proposed scoring system would be well-correlated with the physiological status of the calf, as well as future health and growth. Data from a comprehensive literature review and 3 separate investigations were used to develop the scoring system. Data were managed in Microsoft Excel and exported into Stata/IC 10 for variable screening and statistical modeling. Univariable or multivariable linear regression models were constructed to determine associations between specific physiological or clinical outcomes of interest. From these basic observations, a newborn calf VIGOR assessment tool was developed that could be quickly and easily performed by dairy producers. The final tool included 10 separate measures, under 5 categories. Visual appearance included measures of meconium staining and tongue/head swelling. Initiation of movement focused on time to sternal recumbency and standing. General responsiveness was assessed through 4 measures, including suckling reflex, the straw tickle test, tongue pinch, and eye reflex. Oxygenation was classified by mucous membrane color. Finally, the rates of heart beats and respirations per minute were scored in 3 broad categories. Using these measures, calving assistance was highly associated with decreasing newborn calf vitality. However, increased time between birth and VIGOR assessment significantly improved the score, due to a longer recovery interval following birth. Associations of meconium staining and eye reflex with assistance at calving were not consistent and should be re-evaluated. In conclusion, the proposed VIGOR Scoring System is ready for dissemination and large-scale assessment, as well as the development of an iPad app for convenient data collection, storage and analysis.

Key Words: newborn, calf, vigor

40 Effect of implementing a novel calf vitality scoring system and early intervention program on pain management in newborn dairy calves.
S. Godden[1], W. Knauer[1], K. Leslie*[2], C. Murray-Kerr[2], S. McGuirk[3], H. Coetzee[4], D. Haines[5], C. Gapinski[1], K. Yorek[1], and R. Hullinsky[1], [1]*University of Minnesota, St. Paul, MN,* [2]*University of Guelph, Guelph, ON, Canada,* [3]*University of Wisconsin, Madison, WI,* [4]*Kansas State University, Manhattan, KS,* [5]*University of Saskatchewan, Saskatoon, SK, Canada.*

Calves experiencing dystocia frequently suffer from tissue injury and associated inflammation and pain. The study objective was to conduct a randomized controlled field trial to evaluate the effect of implementing a novel program that includes initial assessment of newborns using the VIGOR Scoring System (U of Guelph) followed by provision of a non-steroidal anti-inflammatory (NSAID) to calves with low vigor scores, on outcomes reflecting calf well-being and health. The study was conducted in the summer of 2017 on a 5,000-cow dairy in MN. Live born calves underwent enrollment within 3 h of birth (T0), including recording of weight and rectal temperature, collection of a venous blood sample, vitality assessment using the VIGOR System and colostrum feeding. The calf was then randomly assigned to either the treatment (TX) or control program (CON). Within the TX program, calves with a low VIGOR score (<21) were administered a gel capsule per os containing meloxicam (approximately 1 mg/kg). Between 24 and 36 h post-enrollment (T24) calves were reassessed using the VIGOR system, temperature recorded and a second blood sample collected. Paired (T0 and T24) plasma samples from 300 randomly selected calves were submitted for determination of plasma prostaglandin E_2 (PGE_2, pg/mL) and cortisol (ng/mL) concentrations. Of 1,483 calves enrolled (CON = 760; TX = 723), 68% and 32%, respectively, achieved a high (≥21) or low (<21) VIGOR score at T0. Multivariable linear regression models showed that at T0, calves with low VIGOR scores were heavier, were from calvings with higher dystocia scores (DS; 0 to 5) and had increased plasma PGE_2 concentrations (BWt = 40.2 kg; DS = 0.50; $\log_{10}PGE_2$ = 1.63) as compared with calves with high VIGOR scores (BWt = 39.4 kg; DS = 0.37; $\log_{10}PGE_2$ = 1.53) ($P < 0.05$). Meloxicam treatment of low VIGOR calves at T0 resulted in a larger reduction and lower plasma PGE_2 concentrations by T24 ($\log_{10}PGE_2$ = 0.76), as compared with untreated low VIGOR calves ($\log_{10}PGE_2$ = 0.99). We conclude that newborn calves with a low VIGOR score experienced increased inflammation as compared with calves with a high VIGOR score, and that meloxicam treatment of low VIGOR calves significantly alleviated inflammation by 24 h of age.

Key Words: newborn, calf, vigor

41 Can calving assistance influence dairy cows' lying times?
M. V. Robichaud*[1,2], D. L. Pearl[2], J. Rushen[3], S. M. Godden[4], S. J. LeBlanc[2], A. Marie de Passillé[3], and D. B. Haley[2], [1]*Université Laval, Québec, QC, Canada,* [2]*University of Guelph, Guelph, ON, Canada,* [3]*University of British Columbia, Vancouver, BC, Canada,* [4]*University of Minnesota, St. Paul, MN.*

Assistance is often provided to cows and heifers during calving by dairy producers and veterinarians to minimize morbidity and mortality. Even though calving is believed to be painful for both cows and calves, especially when strong assistance is provided, the animals' discomfort is difficult to measure. Many research studies have used lying behavior as a non-invasive indicator of discomfort and pain in dairy cattle. The aim of our study was to evaluate the impacts of various characteristics of calving events on the lying behavior of dairy cows. The lying time of 153 Holstein cows was measured continuously for 10 d after calving using accelerometers, as part of randomized controlled trial on the timing of calving assistance. Of the 153 cows, 74 calved unassisted and 79 received assistance during calving. Among the 79 assisted cows, 56 received early assistance, defined as pulling the calf approximately 15 min after first appearance of both hooves, and 23 cows received late assistance, defined as pulling the calf approximately 60 min after first appearance of both hooves. For 57 of the assisted cows, information on the duration of the pull and the average force applied during the assistance was recorded using an electronic load cell. Univariable and multivariable mixed models that included a random intercept for animal and random slope for days after calving were used to assess the associations between lying time and calving event characteristics. Cows that were assisted during calving had significantly shorter daily lying times compared with cows that calved unassisted ($P = 0.002$). Among the 79 assisted cows, the timing of the assistance (early versus late) did not significantly influence the daily lying time ($P > 0.05$). Moreover, the length and force of assistance was not associated with the cows' daily lying time post calving ($P > 0.05$). It appears that regardless of the timing, duration, and strength of pulling (within the ranges observed in this study), calving assistance may have reduced the discomfort of the dairy cows.

Key Words: calving assistance, lying time, force of pulling

42 Pain mitigation in cattle following soft tissue surgery. A. D. Futrell*[1,2], J. M. Caldwell[1], P. D. Krawczel[2], B. K. Whitlock[1], and D. E. Anderson[1], [1]*University of Tennessee College of Veterinary Medicine, Knoxville, TN,* [2]*Animal Science Department, University of Tennessee Institute of Agriculture, Knoxville, TN.*

Mitigation of pain for surgical procedures has become a topic of concern for the public, producers, and veterinarians. The objective of this study was to determine the efficacy of meloxicam for mitigation of pain in adult lactating dairy cattle following a right side laparotomy with omentopexy. Twenty-four dairy cattle >20 mo old and between 50 and 188 d in milk (mean 121 d) were enrolled. Cattle were acclimated for 7 d, assigned to blocks based on days in milk, milk yield, and pregnancy status, and randomly assigned to groups (MEL or CON). The study had 2 phases; sham (d 0–14) and surgery (d 15–28). On d 0, a sham procedure was performed on all animals. Cattle were prepared for surgery including local blocks with lidocaine. Injectable meloxicam (MEL) or saline placebo (CON) was administered (dose: 0.5 mg/kg) 5 min before simulated surgery (restraint for 30 min). On d 15, a right flank laparotomy, brief abdominal exploration, and omentopexy was performed on all animals. Meloxicam and saline were administered before surgery to each respective group. Blood was collected via a jugular catheter at hours 0, 2, 4, 8, 12, 24, 36, 48, 60, and 72 respective to the sham and surgery for cortisol, and at hours 0, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 for haptoglobin, PGE2, and fibrinogen. Mean nociceptive threshold (MNT) was measured using an algometer and collected at hours 0, 1, 4, and 8 after sham and hours 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 after surgery. Infrared thermography (IRT) was taken of the incision site at hours 0, 1, 4, and 8 h after sham and 0, 2, 4, 8, 12, 24, 36, 48, 60, and 72 after surgery. Haptoglobin was significantly increased in the CON 72 h post-surgery (CON mean 476.6 +/− 110.7 µg/mL; MEL mean 202.5 +/− 92.1 µg/mL; $P = 0.01$). Cortisol was significantly increased in the CON 4 h post-surgery (CON mean 22.7 +/− 4.9 ng/mL; MEL mean 9.5 +/−2.0 ng/ml; $P = 0.009$). There was no difference for fibrinogen ($P = 0.08$), PGE2 ($P = 0.1$), MNT ($P = 0.14$) or IRT ($P = 0.68$). The results demonstrate that meloxicam significantly reduced some indirect measures of pain and suggests meloxicam is effective in mitigating post-operative pain in adult lactating dairy cattle.

Key Words: pain, meloxicam, cortisol

43 Comparison between conventional culture, MALDI-TOF, and 16S rRNA for test agreement in diagnosis of bacteria in individual cow milk samples. D. J. Wilson*[1], J. Middleton[2], P. Adkins[2], and G. M. Goodell[3], [1]*Utah State University, Logan, UT,* [2]*University of Missouri, Columbia, MO,* [3]*The Dairy Authority, Greeley, CO.*

Comparison of culture, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), and 16S rRNA genomic sequencing to identify mastitis pathogens was the objective. All quarter milks submitted to The Dairy Authority (TDA) laboratory on one day were streaked (10 μL) onto Columbia blood agar and MacConkey agar. Plates with colonies were number coded, paraffin sealed and shipped overnight with cold packs to the University of Missouri (MU). Culture at TDA identified bacteria to genus level except for speciation of *S. aureus* and *E. coli*. PCR was negative for *Mycoplasma* spp. At MU, a MALDI-TOF mass spectrometer tested colonies in duplicate. Comparison with the Biotyper database of known bacteria produced scoring between 1.7 and 1.99 for genus level identification and ≥2.0 for species level. 16S rRNA colony lysate PCR products were Sanger sequenced, and sequences were compared with GenBank data using nucleotide-BLAST at MU. All microbiologists were blind to other results. Culture and MALDI-TOF tested 181 isolates; 16S rRNA tested 179 (2 lost during storage). In accordance with culture, *S. aureus* and *E. coli* agreement was to species level, all others to genus level. This was a test of agreement, not sensitivity or specificity (no "gold standard"). Overall agreement between the 3 diagnostic methods was 87% (155/179). Agreement between MALDI-TOF and 16S rRNA was 98% (176/179). That assumes agreement for 29 isolates called *E. coli* by culture and MALDI-TOF that 16S rRNA defined as *E. coli* or *Shigella* spp. Most bacteria were identified with good agreement among all 3 methods by McNemar's test, including 94% (80/85) of isolates defined by culture as *Staph* spp., with 22 isolates defined by culture as *S. aureus, Enterobacter* spp., *Klebsiella* spp., *Pasteurella* spp., or *T. pyogenes* showing 100% agreement among all 3 methods. Many members of the dairy industry are comfortable using either bacterial culture or MALDI-TOF for routine milk bacteria diagnosis; 16S rRNA is mainly a research tool. The results suggest that all 3 methods are valuable tools for the dairy industry.

Key Words: mastitis, MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight), 16S rRNA

44 Investigation of risk factors of subclinical mastitis in large-scale dairy farms. Y. F. Zhong*, Y. M. Wu, and J. X. Liu, *Institute of Dairy Institute, Zhejiang University, Hangzhou, China.*

Mastitis has been an important disease because of its common occurrence and resulting significant loss of profit. This study was conducted to investigate the risk factors related to subclinical mastitis (SCM) in large-scale farms. Eleven dairy farms with over 1,000 dairy cows were selected in Zhejiang province, China. A questionnaire on management factors potentially associated with SCM was designed and completed with the help of the managers from all the investigated farms. Aspects of management included in the questionnaire consisted of the general of farm and stalls, management of diet and water supply, cleaning of stalls, operation and hygiene of calving and milking, strategy of drying off, and detection and treatment of mastitis. The incidence rate of SCM was calculated according to the Dairy Herd Improvement (DHI) data. In this study, the SCM was defined as the SCC over 4×10^4/mL milk. Correlation analysis was conducted to identify the herd management factors that were associated with an increased rate of subclinical mastitis. One-way ANOVA was used to analyze the cow factors from DHI data in SPSS 19.0. The average incidence of SCM in these farms was 17.8% in 2015. The incidence rate of SCM increased with the increasing parity and days in milk. Within a year, the incidence of SCM was highest in summer and lowest in winter. Six management factors significantly were identified to be associated with the incidence of SCM, including type of stalls ($P < 0.01$), milking system ($P < 0.01$), cleaning frequency of stall ($P < 0.01$), usage of milking gloves ($P < 0.01$), individual pen for calving ($P < 0.01$), and duration staying with lactating cows before calving ($P < 0.01$). The result of this study indicated that udder health in large dairy farms can be affected by several factors especially the hygiene of milking and calving. It warrants further investigated how mastitis caused by different management methods affects physiological changes of dairy cows.

Key Words: large-scale dairy farm, risk factors, subclinical mastitis

45 Use of electrical conductivity for the differentiation of mastitis-causing pathogens. S. Paudyal*[1], P. Melendez[2], D. Manriquez[1], A. Velasquez[1], P. Pinedo[1], and G. Pena[3], [1]*Colorado State University, Fort Collins, CO,* [2]*University of Missouri, Columbia, MO,* [3]*Zoetis, Parsippany, NJ.*

Mastitis is one of the most prevalent and costly diseases in dairy operations. Key components for adequate mastitis control are the detection of early stages of infection, as well as the selection of therapy based on the causal pathogen associated with infection. Our objective was to characterize the pattern of electrical conductivity (EC), provided by an in-line mastitis detection system, considering specific mastitis-causing pathogen group involvement. Cows (n = 200) identified by the system with a deviation >15% in the manufacturer's (Afimilk Ltd., Kibbutz Afikim, Israel) proprietary algorithm for EC (HEC) were considered cases and enrolled in the study. One control (CON), defined as within normal ranges for EC, was matched to each case and monitored for milk yield (MY) and EC for ± 10 d. A sterile pooled milk sample was collected from each cow for bacteriological culture. Pathogens were categorized into gram-positive (GP), gram-negative (GN), other (OTH), and no growth (NO). Data were submitted for repeated measures analysis (PROC MIXED, SAS), with EC as dependent variable. EC status (HEC or CON), bacterial categories, and milking relative to d of enrolment were considered independent fixed variables and farm was included as a random effect in the model. For HEC animals, EC was greater in NO than in GN ($P = 0.036$) but EC was not different among other pathogen groups. For CON animals, EC was greatest in OTH compared with NO ($P = 0.03$), GP ($P = 0.03$), and GN ($P = 0.07$). However, EC was not different when comparing between GP and GN. For HEC animals, MY was not significantly different among pathogen groups. For CON, GN had greater MY than NO ($P = 0.006$) and GP ($P = 0.02$). For HEC and CON animals, EC was greater in multiparous cows than in primiparous cows ($P < 0.001$). State of lactation had no effect in CON animals whereas, for HEC cows, EC was greater in animals in early ($P < 0.0001$) and late lactation ($P = 0.0015$), compared with mid lactation cows. Thus, it is concluded that EC variation cannot solely be attributed to pathogen groups and multiple factors should be considered in developing mastitis pathogen detection models based on EC.

Key Words: electrical conductivity, mastitis

46 Flax oil supplementation affects systemic blood biomarkers and polymorphonuclear leukocytes mRNA expression in neonatal dairy calves. F. Rosa*[1], C. R. Schossow[1], N. A. Carpinelli[1], E. Trevisi[2], J. L. Anderson[1], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Polyunsaturated fatty acids have been observed to reduce inflammatory response. The adaptation of neonatal calves to an extrauterine environment is commonly associated with inflammatory-like conditions. Thus, our objective was to evaluate the effects of supplementing flax oil on immune function reflected in the profiles of systemic blood biomarkers and mRNA in polymorphonuclear leukocytes (PMNL). Thirty-six Holstein dairy calves housed in individual hutches were used in a randomized complete block design from birth until 12 wk of age. A subset of 16 calves (n = 8/trt) was used for immune function analysis. Treatments were control (CON) with no supplement or 80 g/d of flax oil (FLAX) with the milk. Calves were fed 2.8 L/d of pasteurized milk 2 × /d during wk 1 to 5. Starter pellets and water were fed ad libitum. Blood samples were taken at 1, 7, and 14 d of age for biomarker profiling of metabolism, oxidative stress, and inflammation as well as PMNL isolation. RNA was extracted from isolated PMNL, and concentration and viability were assessed through flow cytometry (Attune NxT; Invitrogen). Target genes evaluated in PMNL function involved inflammation, cellular receptors, and oxidative stress. Data were analyzed using the MIXED procedure of SAS. There was a significant ($P = 0.04$) diet by time (D × T) interaction in the mRNA expression of *SELL* (L-Selectin), a cell surface receptor, where an upregulation ($P = 0.03$) of *SELL* in FLAX calves at 7 d of age was observed. A trend ($P = 0.14$) for a D × T effect was observed in *IL1B* (interleukin 1β), an inflammatory cytokine, which resulted in a trend ($P = 0.06$) for lower expression of *IL1B* in FLAX calves at 7 d of age. The overall *MPO* (myeloperoxidase) mRNA expression tended ($P = 0.07$) to be upregulated in FLAX calves, which is commonly associated with PMNL activity. Results suggest that early life supplementation of flax oil to neonatal calves may benefit their transition into an extrauterine environment not only by mediating the inflammatory response, but enhancing the PMNL ability to detect potential infection sites through cell receptors such L-selectin.

Key Words: calves, inflammation, flax oil

47 Validation of methods to practically evaluate failure of passive transfer in calves arriving to a veal facility. D. L. Renaud*, T. F. Duffield, S. J. LeBlanc, and D. F. Kelton, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Providing a sufficient quantity of good quality colostrum is critical for male and female calves to reduce the risk of disease and mortality. Practical tests have not been validated at arrival to veal facilities to determine failure of passive transfer (FPT). There are many challenges to validation including the lack of information regarding the age of the calf and the high prevalence of dehydration. The objective of this study was to validate a semiquantitative IgG test using whole blood and a digital refractometer using serum to determine passive transfer status. A total of 149 Holstein calves were evaluated at arrival to a milk-fed veal facility for dehydration status and had blood drawn to evaluate passive transfer. Serum IgG was determined by radial immunodiffusion (RID), whereas, serum total protein (STP) was evaluated using a digital refractometer (Misco Palm Abbe) and a semiquantitative test (ZAPvet Bovine IgG test) was used on whole blood to determine failure of passive transfer. A nonparametric receiver operating characteristic (ROC) curve was generated to compare STP and IgG levels. Sensitivity, specificity, positive predictive values and negative predictive values were calculated for STP and the semiquantitative IgG test using RID as a gold standard test. A total of 31 calves (21%) had a level of serum IgG <1,000 mg/dL. The level of total protein was well correlated with the level of IgG yielding an R^2 value of 0.75. The cut point to determine passive transfer on serum was ≥5.1 g/dL, yielding a sensitivity of 84% and specificity of 90%. The semiquantitative test performed poorly on whole blood with a sensitivity of 77% and a specificity of 44%. This study demonstrates that serum total protein is a reliable measure to evaluate passive transfer status and can be used despite high levels of dehydration and the inability to determine the calf's age.

Key Words: male calf, colostrum, total protein

48 Mycotoxin occurrence in 2017 US corn. P. N. Gott*[1], E. G. Hendel[1], U. Hofstetter-Schähs[2], and G. R. Murugesan[1], [1]*Biomin America Inc., Overland Park, KS,* [2]*Biomin Holding GmbH, Getzersdorf, Lower Austria, Austria.*

Mycotoxins, secondary metabolites produced by molds, can occur in many types of grains and forages as well as other feedstuffs such as fruits and nuts which are consumed by livestock and humans. These toxic substances frequently negatively affect animal health and performance. A survey was conducted to determine the occurrence of mycotoxins in the 2017 US corn crop to assess the potential risk posed to livestock. A total of 442 corn-based samples (grain, fermented feeds, and by-products) were collected from August 2017 to January 2018 from 29 states. The majority of samples originated from the Midwest. Samples were analyzed at Romer Labs (Union, MO) for the presence of 17 mycotoxins by the liquid chromatography tandem mass spectrometry (LC-MS/MS) method. These mycotoxins were categorized into 6 groups: Type B trichothecenes including deoxynivalenol (DON), fumonisins (FUM), zearalenone (ZEN), aflatoxins (Afla), Type A trichothecenes including T-2 toxin (T-2), and ochratoxin A (OTA). Eighty-eight percent of samples had at least one mycotoxin detected while 45% of samples were contaminated with more than one mycotoxin. The percent of positive samples, mean of positives [ppb], standard error of the mean (SEM) of positives [ppb], and maximum of positives [ppb] for the 6 major mycotoxin groups are presented in Table 1. Deoxynivalenol was detected in 75% of samples (−14 percentage points vs. 2016 Biomin survey data) while FUM were present in 43% of samples (−29 percentage points vs.

Table 1 (Abstr. 48). Summary of mycotoxin analyses

Parameter	DON	FUM	ZEN	Afla	T-2	OTA
Positive samples (%)	75	43	29	4	0	<1
Mean of positives (ppb)	1,026	2,298	248	12	—	600
SEM[1] of positives (ppb)	179	397	55	4	—	NA
Maximum contamination (ppb)	54,149	64,500	5,556	67	—	600

[1]Standard error of mean.

2016 Biomin survey data). Zearalenone was the third most frequently occurring mycotoxin, detected in 29% of samples (−28 percentage points vs. 2016 Biomin survey data). Deoxynivalenol, with additional potential concerns from FUM and ZEN, pose the greatest mycotoxin threats to animal health and productivity due to their frequent occurrence in 2017 US corn sourced predominantly from the Midwest.

Key Words: mycotoxin, deoxynivalenol, fumonisin

49 Feeding NutriTek reduces linear scores and clinical mastitis cases. J. D. Ferguson[1], M. A. Sattler[2], D. L. Hanson*[2], C. P. Davis[2], T. S. Edrington[2], and I. Yoon[2], [1]*University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA,* [2]*Diamond V, Cedar Rapids, IA.*

The objective of the current research was to examine the association of feeding NutriTek, a *Saccharomyces cerevisiae* fermentation product (Diamond V, Cedar Rapids, IA) in close-up and lactating cows with udder health. Production records and clinical mastitis event data were collected from 25 herds from 5 regions of the US using back-up files from herd DairyComp305 collected from June to September 2017. This provided production and event records that ranged from 400 to 890 d following NutriTek feeding. Herds ranged in 305-d milk production from 7,800 to 12,700 kg, and the number of cows in the adult herd ranged from 880 to 10,727 cows. Test day records were extracted for herds that recorded linear scores (LS) by cow, event data for mastitis, date of calving, and date of herd removal for an equal period of time before and after feeding NutriTek. Fifteen herds had test day records with LS data and all 25 herds had event data. A total of 1,444,450 production records were available for analysis: 702,449 records were for the period before, and 742,000 records post NutriTek feeding. Lactation records were categorized by herd, cow, lactation number (where available), date fresh, and date of test. Data for LS were analyzed using SAS statistical software and analyzed by herd using Proc Mixed with period of NutriTek feeding, test period, and month of test as independent variables. Cow was treated as a repeated observation. Least squares means for TRT (NutriTek or no NutriTek) were estimated for each herd. Clinical mastitis prevalence was tested by herd using Proc Logistic in SAS. Mastitis was the independent variable and TRT period and month of calving were the independent variables. Sixteen herds had a reduction ($P < 0.05$) in the prevalence of clinical mastitis cases and 8 herds had a tendency ($P < 0.10$) for a reduction in mean LS during the NutriTek feeding period. A purely random sampling should have resulted in only one-third of herds improving their linear scores and mastitis cases indicating that the herds fed NutriTek improved more than expected (odds ratio $P < 0.05$). Causative mechanisms for the above observations need to be explored to more fully describe this association.

Key Words: *Saccharomyces* fermentation product, mastitis, linear score

50 Frequency of antimicrobial usage on treatment for bacterial diseases occurring in cows on large dairy farms. J. Leite de Campos*[1], A. Steinberger[2], T. Goldberg[2], N. Safdar[2], J. Shutske[2], A. Sethi[2], G. Suen[2], and P. Ruegg[1], [1]*Michigan State University, East Lansing, MI,* [2]*University of Wisconsin-Madison, Madison, WI.*

Use of antimicrobials for treatment of animals is under scrutiny and knowledge about frequency of antimicrobial treatment in dairy cows is needed. The objective of this study was to determine associations of farm size and milk production with antibiotic usage (AMU) on larger WI dairy farms. Eligible farms contained >250 lactating cows and met specified criteria for recording diseases and treatments in DairyComp 305. Enrolled farms (n = 38) submitted data before a farm visit during which an interview was performed to validate disease diagnosis, definitions and recording accuracy. Farms contained 52,563 cows and an average of 1251 (±332 SE) lactating cows per herd. Herds were categorized as "medium" (<1,000 total cows (n = 22) or "large" (≥1,000 (n = 16) and "low" (≤12,700 kg/cow (n = 22) or "high" (>12,700 (n = 16) producers. One-way ANOVA was used to identify associations of categories (production or herd size) with selected disease frequencies and AMU rate. Descriptive statistic was used to calculate the frequency and rate of disease. Frequencies (events/100 cows/yr) of diseases were 34.2 (±3.9), 27.8 (±3.2), 79.7 (±1.2), 3.4 (±0.644), 2.3 (±0.373), 10.5 (±1.7), 3.7 (±0.8) for all mastitis, clinical mastitis, dry cow treatment, respiratory disease, displaced abomasum, metritis and retained placenta, respectively. Frequencies of antibiotic treatment (treatments/100cases/yr) of mastitis, clinical mastitis, dry cow treatment, respiratory disease, displaced abomasum, metritis and retained placenta were 72.7 (±5.2), 69.1 (±5.5), 98.5 (±0.8), 84.5 (±4.5), 74.0 (±5.4), 78.9 (±5.5), 48.2 (±7.2) respectively. No associations between milk production or farm size were identified for mastitis (including clinical cases), displaced abomasum or dry cow treatment. Respiratory disease treatment rate was associated with herd size (0.78, ± 0.05 (medium), (0.93, ± 0.6 (large); $P = 0.105$) and production (0.77 ± 0.05 (low), (0.94 ± 0.06 (high) $P = 0.105$) as well as metritis treatment rate (0.69 ± 0.06 (low), (0.92 ± 0.08 (high); $P = 0.03$) and retained placenta treatment rate (0.33 ± 0.08 (low), (0.68 ± 0.1 (high) $P = 0.01$) were associated with production. Diseases and AMU varied among farms and for some diseases, production and herd size category were positively associated with AMU.

Key Words: antibiotic, treatment frequency, dairy

51 Residual effects of maternal consumption of metal amino acid complexes in offspring inflammatory and oxidative status during the weaning period. R. C. B. Grazziotin*[1], C. B. Jacometo[2], M. Socha[3], E. Trevisi[4], J. J. Loor[5], and J. S. Osorio[1], [1]*South Dakota State University, Brookings, SD,* [2]*Universidad de la Salle, Bogota, DC, Colombia,* [3]*Zinpro Corporation, Eden Prairie, MN,* [4]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [5]*University of Illinois, Urbana Champaign, IL.*

Maternal diets fed during late pregnancy (close-up) to dairy cows have been observed to have a significant effect on the offspring's immune status and health during the neonatal stage. Our objective was to evaluate the effect of maternal consumption of organic trace minerals supplemented during late pregnancy on offspring's immune and oxidative status during the weaning period. Forty multiparous Holstein cows were supplemented for 30 d prepartum to supply 40, 20, 5, and 1 mg/kg of Zn, Mn, Cu, and Co from either metal amino acid complexes (AAC) or sulfate (INO) sources (total diet contained supplemental 75, 65, 11, and 1 mg/kg of Zn, Mn, Cu, and Co, and additional Zn, Mn, and Co provided by sulfates). A subset of calves (n = 8/trt) born to dams enrolled in the trial was used for growth and performance data and blood immunometabolic markers. Calves were housed in individual hutches from birth to 7 wk of age and intakes of milk replacer and starter were measured daily. Calves were fed the same nutritional program with 2 × /d feeding milk replacer from wk 1 to 5, 1 × /d feeding during wk 6 (36 to 42 d of age), and weaning at wk 7 (43 d of age). Starter grain was offered ab libitum. Fecal and respiratory scores were recorded daily. Blood samples were taken pre-weaning and post-weaning at 40 and 50 d of age, respectively. Body weight (BW) and withers height (WH) were recorded weekly. Data were analyzed using the MIXED procedure of SAS. Overall BW was not affected ($P = 0.77$) by maternal

AAC, however, there was a trend ($P = 0.07$) for greater withers height in AAC calves. The AAC calves had overall lower paraoxonase ($P = 0.02$) and myeloperoxidase($P = 0.03$), while greater ferric reducing antioxidant power (FRAP; $P = 0.04$) in comparison to INO calves. A trend for lower glutamic-oxaloacetic transaminase (GOT; $P = 0.06$), γ-glutamyltransferase (GGT; $P = 0.06$), and reactive oxygen metabolites (ROM; $P = 0.10$) was observed in AAC calves than INO. These data suggest that maternal consumption of AAC can have long-lasting effects in the offspring by reducing oxidative stress and potentially alter liver function during stress periods such as weaning.

Key Words: weaning, trace minerals, calves

52 Experimental *Staphylococcus aureus* mastitis teat-dip infection model for evaluation of efficacy of vaccine against *Staphylococcus aureus* intramammary infection. O. K. Dego*, R. Abdi, and R. Almeida, *The University of Tennessee, Knoxville, TN.*

Staphylococcus aureus is the most frequent and major pathogen of mammary glands of dairy cows. There is increasing need to develop protective vaccine against *S. aureus* IMI. A good experimental infection model is required to evaluate vaccine efficacy. The intramammary infusion of *S. aureus* is a reliable method in terms of causing infection. However, intramammary infusion is unrealistic method in terms of mimicking natural IMI because it overwhelm the host immune mediated defenses since large number of bacteria were directly delivered into the intramammary area bypassing innate and acquired immune defenses at teat opening. Therefore, a challenge model that is similar to natural infection is required to evaluate protective effect of new vaccine against *S. aureus* mastitis. The objective of this study was to develop experimental *S. aureus* mastitis teat-dip infection model. A total of 8 dairy cows at early dry period were divided into group 1 (n = 5 cows) and group 2 (n = 3 cows). Cows in Group 1 were challenged with *S. aureus* strain SAUT1 by dipping all 4 teats in a suspension of *S. aureus* at a concentration of 10^6 cfu/mL Tryptic soy broth (TSB) medium. Similarly, all 4 teats of cows in the group 2 (Control) were dipped in PBS. The challenge strain was grown to mid log phase in TSB at 37°C and cows were challenged after morning milking. Results showed that out of 5 *S. aureus* challenged cows 80% (n = 4 out of 5) and 20% (n = 1 out of 5) were infected subclinically and clinically respectively. At d 3 of challenge, at least one quarter of each cow was shedding *S. aureus* in milk resulting in 100% infection. At d 6 of challenge, 100% of challenged quarters were shedding *S. aureus* in milk. We also further evaluated the protective effects of experimental vaccine against *S. aureus* intramammary infection (IMI) using this model and found promising results. We concluded that teat dipping in the *S. aureus* bacterial suspension at cell density of 10^6 cfu/mL of growth medium is good experimental intramammary infection model to induce *S. aureus* IMI.

Key Words: intramammary infection, model, dairy cow

53 Understanding and addressing nutrient losses to the environment from livestock production. G. Zwicke*, *USDA-NRCS, Air Quality and Atmospheric Change Team, Fort Collins, CO.*

Air emissions from livestock production systems have become a considerable regulatory focal point in recent years. Transport of those air emissions can further result in additional ecosystem impacts far beyond the emission point in some cases, so a better understanding of the mechanisms related to generation, emission, and transport of these air emissions is critical to mitigating their potential impacts. In much the same way that releases of nitrogen (N), phosphorus (P), and potassium (K) are related to nutrient management and subsequent water quality impacts, air releases can be generally related to nitrogen (N), carbon (C), and sulfur (S). This paper investigates the reasons for the new-found interest in air emissions of N, C, and S; how those emissions are generated, emitted, and transported; and how professionals working to assist the livestock production industry can affect the generation, emission, and transport of those emissions.

Key Words: emissions, regulatory, ecosystem

54 Mitigation of greenhouse gases emissions from dairies (the cow, the manure, and the field). M. A. Wattiaux*[1], R. D. Jackson[2], and R. A. Larson[3], [1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI,* [2]*Department of Agronomy, University of Wisconsin-Madison, Madison, WI,* [3]*Department of Biological Systems Engineering, University of Wisconsin-Madison, Madison, WI.*

In the United States, approximately 70% of fluid milk carbon footprint occurs on-farm primarily from cows [enteric methane (CH_4)], from manure [CH_4 and nitrous oxide (N_2O)], and from cropland [carbon dioxide (CO_2) and N_2O]. In spite of some methodological inconsistencies attributional life cycle assessments (LCA) have consistently reported farm-gate emissions around 1.00 kg CO_2 equivalent per kg of milk. Variations among farms within a production system are large and often greater than across production systems (conventional, grazing or organic). Dry matter intake is the main driver of daily enteric CH_4 emission. Thus, reduction of enteric CH_4 per kg of milk depends heavily on feed-to-milk conversion efficiency. Consequently, better herd health, increased productive life (decrease culling rate), reduction in replacement herd, and in the long term, genetic selection for feed efficiency are effective practices that lowers milk carbon footprint. Dietary composition (type and level of carbohydrates, fiber, and fat, as well as feed additives) has direct effects on enteric CH_4 and indirect effects on N_2O because dietary crude protein determines manure N content and vulnerability to gaseous N emissions. In the manure management chain, aerobic conditions (e.g., aerated compost) depress CH_4 but stimulate N_2O emission, and the reverse is true for anaerobic manure systems (e.g., liquid storage systems). Mitigation options in the field have focused primarily on reducing the loss of CO_2 (soil organic matter) and reducing N_2O emission. Introductions of perennial crops or winter crops contribute carbon storage in plants and soils. Reduced or no till and the incorporation of organic matter (manure or crop residues) also favor carbon sequestration in soils, but may enhance N_2O emission. Although attributional LCA emphasizes increasing productivity and efficiency of farm (sub-) systems, and recovering energy (e.g., manure bio-digestion) and nutrients, recent consequential (expanded boundaries) LCA has suggested important trade-offs that cannot be ignored if the goal is to reduce actual emissions rather than the carbon foot print of the dairy sector.

Key Words: climate change

55 Modifications to the CNCPS related to environmental issues—Capability to evaluate greenhouse gasses, nitrogen and phosphorus excretion at the farm level. M. E. Van Amburgh* and L. E. Chase, *Cornell University, Ithaca, NY.*

The Cornell Net Carbohydrate and Protein System (CNCPS) was developed to evaluate nutritional adequacy and predict nutrient supply and requirements in cattle under most management and environmental conditions. Evaluations of this capability have been published and the model is in continuous development (Tylutki et al., 2008; Van Amburgh et al., 2015). As model precision increases, the ability to develop or integrate extant equations to predict excretion of feces and urine and gasses from digestion also increases as those predictions are outcomes of predicting metabolizable energy and protein supply. For example, the ability to predict urinary and fecal nitrogen (N) was through evaluation of extant equations (feces) and development of a new equation that predicted urinary excretion (Higgs, et al., 2012). Implementation of this capability in 8 herds in NY was shown to reduce manure N excretion by 14% (22 kg/cow/yr) resulting in $163 increased income over feed costs (Chase, 2018). Greenhouse gas (GHG) production from cattle has been extensively studied and modeled as part of the development of DE, ME and NE equations, thus if the model is adequate at predicting those variables, predictions of GHG should be less complicated. Extant equations from Casper and Mertens, (2010) for CO2 and Mills et al. (2003) for methane were tested and adapted and were very robust at predicting GHG emissions. Total emission of CO_2 per cow was positively related to total milk yield ($R^2 = 0.69$). However, CO_2 emissions per kilogram of milk as a function of milk yield (kg CO_2/kg milk) resulted in a negative relationship, ($R^2 = 0.81$). The average value of CO_2 emission per unit of milk yield was 0.353 kg CO_2/kg, with minimum and maximum values of 0.283 and 0.423 kg CO_2/kg milk, respectively. Methane emissions per kg DMI ranged from 0.021 to 0.027 with a mean of 0.024 kg. Methane emissions were positively correlated with milk yield (slope = 0.004; $R^2 = 0.68$) but negative correlated when expressed as a function of milk yield (slope = −0.26; $R^2 = 0.88$) (Van Amburgh et al., 2015).

Key Words: environment, efficiency, CNCPS

56 Genetic and environmental analysis of diseases with major economic impact in Israeli Holsteins. J. I. Weller*[1], E. Ezra[2], and M. van Straten[3], [1]*ARO, The Volcani Center, Rishon LeZion, Israel,* [2]*Israel Cattle Breeders Association, Caesaria Industrial Park, Israel,* [3]*Hachaklait, Mutual Society for Veterinary Services, Caesarea Industrial Park, Israel.*

Incidence of ketosis, metritis, mastitis, and retained placenta were studied on Israeli Holstein cows calving between 2008 and 2017. These diseases were selected based on their economic impact. Ketosis, metritis, and retained placenta were scored dichotomously. Mastitis was scored as absent, a single occurrence during the lactation or more than once. Ketosis and metritis were recorded during the first 21 d after calving, retained placenta during the first 5 d after calving, and mastitis up to 305 d in milk. The effects of herd-year-season, calving age, month of calving, gestation length and occurrence of dystocia were included in the first parity analysis models. All effects were significant ($P < 0.001$) for metritis and retained placenta. For ketosis all effects were significant, except for gestation length. For mastitis, only the effects of herd-year-season and calving age were significant. First-parity heritabilities and genetic and environmental correlations among these diseases and the traits included in the Israeli breeding index were computed by the MTC REML individual animal model program. Heritabilities and genetic and environmental correlations among the disease traits are in Table 1. All correlations were positive, but the highest correlation, between ketosis and metritis, was only 0.26. Genetic correlations between the disease traits and milk production traits were all "positive," but all correlations were <0.25. Since mastitis is farmer recorded, a truncated data set was analyzed including only herd-year with >50 cows and >4% mastitis. Genetic correlations between mastitis and lactation somatic cell score were higher in second and third parities, and heritabilities for mastitis were slightly higher.

Table 1 (Abstr. 56). Heritabilities and genetic (above the diagonal) and environmental (below the diagonal) correlations among the disease traits in 229,571 first-parity cows[1]

Trait	Ketosis	Metritis	Mastitis	Retained placenta
Ketosis	**0.07**	0.27	0.00	0.05
Metritis	0.16	**0.08**	0.03	0.10
Mastitis	0.00	0.01	**0.05**	0.01
Retained placenta	0.06	0.14	0.01	**0.06**

[1]All standard errors were < 0.01.

Key Words: ketosis, metritis, mastitis

57 Gene mapping and gene-set analysis for milk fever in Holstein dairy cattle. H. A. Pacheco[1,2], A. Sigdel[1], C. K. Mak[1], K. N. Galvão[1], L. T. Dias[2], and F. Peñagaricano*[1], [1]*University of Florida, Gainesville, FL,* [2]*Federal University of Paraná, Curitiba, PR, Brazil.*

Milk fever (MF) is an important metabolic disorder of dairy cows around the time of calving. MF leads to important economic losses due to deaths, reduction in milk production and productive lifespan, as well as costs associated with both prevention and treatment. The objective of this study was to unravel the genomic architecture underlying MF in Holstein dairy cattle. Data consisted of 28k producer-recorded MF event records from 14k cows. The analysis included a whole-genome scan to identify genetic variants and genes regulating MF, and a subsequent gene set analysis for detecting pathways and biological mechanisms associated with MF. The association analysis identified several regions located on BTA6, BTA7, BTA14, BTA16, BTA17, and BTA23 that explained a significant amount of genetic variance for MF. These regions harbor several genes; for example, *GC*, *CAMK2A*, *CAMK1G* and *CPNE5*, that are directly involved in calcium and vitamin D metabolism. Notably, these regions also harbor microRNAs that regulate the expression of genes implicated in calcium ion transmembrane transport, such as *CACNA1D* and *NCS1*. Moreover, the gene set analyses revealed several significant functional categories, including endorphins, potassium channels, phosphatidylinositol phosphates, and NFATC transcription factors. Most of these terms are associated either with hypocalcemia or the cascade of events that occur during MF. Overall, our study contributes to a better understanding of the genetic control of this complex disease. These findings can provide opportunities for improving MF in dairy cattle through marker-assisted selection.

Key Words: enrichment analysis, hypocalcemia, whole-genome scan

58 Identification of genomic regions associated with resistance to clinical mastitis in US Holstein cattle. J. B. Cole*[1], K. L. P. Gaddis[2], C. Willard[1], D. J. Null[1], C. Maltecca[3], and J. S. Clay[4], [1]*Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD,* [2]*Council on Dairy Cattle Breeding, Bowie, MD,* [3]*Department of Animal Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC,* [4]*Dairy Records Management Systems, Raleigh, NC.*

The objective of this research was to identify genomic regions associated with clinical mastitis (MAST) in US Holsteins using producer-reported data. Genome-wide association studies (GWAS) were performed on deregressed PTA using GEMMA v. 0.94. Genotypes included 60,671 SNP for all predictor bulls (n = 35,724) and 35,000 cows sampled from the predictor population of 112,895 females. Autosomal SNP with Wald P-values $\leq 5 \times 10^{-8}$ were assigned to the closest annotated gene within 25 kbp using BEDTools v. 2.21.0 and the UMD3.1.1 assembly of the *Bos taurus* genome, and gene functions were determined by a review of the literature. Genes associated with MAST included *CARD14* (80.16 Mbp on BTA17) and *RPTOR* (52.30 Mbp on BTA19), both of which were previously reported to have significant associations with clinical mastitis in Holsteins. Other genes of interest included: *MGAT5* (63.11 Mbp on BTA2), which regulates the biosynthesis of glycoprotein oligosaccharides; CGNL1 (52.83 Mbp on BTA10), which is involved in the formation and maintenance of tight cell-cell junctions and mediates junction assembly and maintenance; EPAS1 (28.57 Mbp on BTA11), a transcription factor associated with blood vessel development and the expression of endothelial growth factor; and *ANGPT1* (59.13 Mbp on BTA14), which is associated with vascular development and angiogenesis. These genes are of interest because they may be involved in the development and defense of the mammary gland, and possibly associated with changes in milk composition in response to infections of the udder. However, these results represent only statistical associations, and functional validation is needed to determine if these effects are causal, or simply represent correlations with other processes that may represent true causal mechanisms.

Key Words: clinical mastitis, disease resistance, genomic evaluation

59 Single-step genome-wide association study of digital dermatitis and sole ulcer in Holstein cattle. F. Malchiodi*[1], L. F. Brito[1], A.-M. Christen[2], A. Fleming[1], D. F. Kelton[3], F. S. Schenkel[1], and F. Miglior[1,4], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Valacta, Sainte-Anne-de-Bellevue, QC, Canada,* [3]*Department of Population Medicine, OVC, University of Guelph, Guelph, ON, Canada,* [4]*Canadian Dairy Network, Guelph, ON, Canada.*

Digital dermatitis and sole ulcer represent the most prevalent lesions in Canada, with almost 20% and 8% of cows affected by these lesions, respectively. This study aimed to perform a genome-wide association study (GWAS) and functional analysis to uncover genomic regions associated with digital dermatitis and sole ulcer. Hoof lesions were recorded by 51 hoof trimmers during the routine trimming activity in 1,080 Canadian herds between 2009 and 2016. Hoof lesions were coded as binary traits (0; 1), where 1 was assigned to the presence of a lesion in any claw. The final data set contained 249,709 observations from 105,450 animals, while the pedigree file contained 351,215 animals. Of those animals, 20,188 were genotyped either with 50K SNP panel or a low-density panel and imputed to 50K. The model implemented in the weighted single-step GWAS included the fixed effects of herd-date of hoof trimming, hoof trimmer, parity at trimming, stage of lactation at trimming, and the random additive genetic animal and permanent environmental effects. Important genomic regions associated with digital dermatitis and sole ulcer were identified and a list of functional candidate genes within or next to these regions was created.

Key Words: digital dermatitis, sole ulcer, ssGWAS

60 Additive genetic effect of cow on pathogen-specific single-quarter udder infection and differential somatic cell count. E. A. Lozada-Soto*[1], K. Anderson[2], C. Maltecca[1], and F. Tiezzi[1], [1]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [2]*Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.*

Genetic selection toward mastitis resistance has not advanced substantially in the past. The detection of new traits that will improve selection is needed. The aim of this study was to examine the feasibility of using threshold concentration values of somatic cells as traits in selection against mastitis susceptibility. Quarter milk samples (n = 965) were collected at d 4 and d 11 post calving from Holstein and Jersey cows reared at the Dairy Education Unit at North Carolina State University. Cell counts for total leucocytes (TLC), neutrophils (N), macrophages (M), and lymphocytes (L) were obtained via differential somatic cell count. Intramammary infection with major and minor pathogens, as well as fungi, was determined via microbiological culturing on duplicate quarter milk samples. Log-transformed scores for the cell counts were calculated. MCMCglmm was used to fit various models for the following dependent variables: TLC, N, M, L, major pathogen presence, minor pathogen presence, fungi presence, and cell count thresholds for major pathogens. The animal and the week of sampling were treated as random, while breed, lactation, sampling day, sampling time, and quarter position were treated as fixed. The variance due to the animal was 0.56, 0.65, 0.47, 0.57, 12.57, 2.38, 10.22, 4.87, 3.84, and 4.41 for the variables TLC, N, M, L, major pathogen presence, minor pathogen presence, fungi presence, TLC threshold, N threshold, M threshold, and L threshold respectively. The variance due to the week of sampling obtained was negligible for all variables considered. The effect of sampling day was significant ($P < 0.001$) for the variables TLC, N, M, L, TLC threshold, N threshold, and M threshold. Lactation was significant for the presence of major pathogens. The effects of breed, sampling day, and quarter position were not significant for any variable. A second model included the additive genetic effect of the cow using pedigree information. Heritability results will be presented.

Key Words: mastitis, differential somatic cell count, genetics.

62 Multitrait modeling of first versus later parities for US yield, somatic cell score, and fertility traits. P. M. VanRaden* and M. E. Tooker, *USDA Animal Genomics and Improvement Laboratory, Beltsville, MD.*

Genetic merits in first vs. later parity with correlations <1 were compared with official repeatability models using 88 million lactation records of 34 million cows for yield traits and fewer records for SCS and 2 cow fertility traits. Estimated genetic correlations of first with later parity ranged from 0.85 for SCS to 0.95 for fertility traits. These estimates were also applied to permanent environmental and herd-by-sire interaction effects that were constants within later parities and correlated with first-parity effects. Previous parity variance adjustments were removed. Computation took twice as many processors and required more iteration because of slightly slower convergence. All-parity merit combined first and later merit with weights of 0.33 and 0.67, respectively; genetic correlations of all-parity merit with either first or later merit ranged from 0.96 to 0.99. For all bulls progeny-tested since 1995, correlations with official evaluations were very high (0.999) for all traits. Correlations for the 2 most recent years of progeny-tested bulls were lower (0.991 for SCS to 0.997 for fat yield) because many of these bulls had only or mostly first-parity daughters. Computed reliabilities of these recent bulls averaged 3 percentage points less than official reliabilities. With the latest 5 yr of data removed, correlations of truncated and current evaluations were compared for the most recently proven US bulls. Correlations for SCS were higher for the new model than for the traditional model for Holsteins (0.875 vs. 0.867) and Brown Swiss (0.800 vs. 0.76) but not for Jerseys (0.822 vs. 0.826). Correlations for yield traits did not improve for any breed. In a separate test, modeling maturity effects using random regressions on parity gave predictions very similar to modeling first vs. later records. Modeling lactations as correlated traits can possibly reduce biases from early daughters and slightly improve stability for SCS when bulls transition from genomic predictions to observed daughter records, but did not improve correlations with future evaluations for other traits.

Key Words: maturity effect, parity, genetic correlation

63 Relationships between daughter phenotypes and sire PTA for production and fertility traits in US organic Holstein cows. L. C. Hardie*[1], I. W. Haagen[1], L. Han[1], B. J. Heins[2], D. D. Fitzsimmons[3], and C. D. Dechow[1], [1]*Pennsylvania State University, University Park, PA,* [2]*University of Minnesota, Minneapolis, MN,* [3]*Alfred State University, Alfred, NY.*

The objective of this study was to evaluate the relationship between daughter performance on US organic farms with sire predicted transmitting ability (PTA) estimated through national evaluations. Production and fertility data were collected from 3,002 Holstein cows housed on 9 commercial organic farms across the US. Only records from the year of organic certification through 2017 were used, and for a cow to be included in the data set, her first parturition must have occurred during or after the year of organic certification. Daughters belonged to 513 sires with the most commonly used sire having 185 daughters. We regressed 1,560 daughter records of 305-d mature equivalent (ME305) milk, fat, and protein yield on sire PTA for milk, fat, protein, respectively, and we regressed 1,624 records of average lactation SCS, on sire PTA for

SCS. For analyses of fertility, we regressed 5,432 daughter phenotypes for days open on sire PTA for daughter pregnancy rate (DPR) and cow conception rate. Fixed class effects of age group at parturition within lactation group (lactations 1, 2, 3, ≥ 4) and random effects of herd-year-season and cow were included in all models. Analyses were performed in SAS using mixed linear models. Regression coefficients on sire PTA were 0.87 ± 0.15, 0.62 ± 0.15, 0.86 ± 0.16 for ME305 milk, fat, and protein, respectively, and 1.43 ± 0.25 for SCS. Expectations for these coefficients were 1. The regression coefficients for days open on sire PTA for cow conception rate and DPR were −1.38 ± 0.38 and −1.72 ± 0.45 d per unit PTA, respectively. Nationally, the expectation is 4 fewer days open per unit PTA of DPR. Overall, regression coefficients were smaller in magnitude than expected indicating that the genetic variation of Holstein sires is not fully expressed on these farms, with the exception of the regression on SCS. However, regression coefficients carried the expected sign, suggesting that use of sire PTAs estimated through national evaluations should foster genetic improvement for production and fertility on US organic farms.

Key Words: regression, organic, genetics

64 Genetic analysis of heat tolerance for conception rate in US Holstein cows. A. Sigdel*[1], J. A. Vaca[1], I. Aguilar[2], R. Abdollahi-Arpanahi[1,3], and F. Peñagaricano[1], [1]*University of Florida, Gainesville, FL,* [2]*Instituto Nacional de Investigación Agropecuaria, Las Brujas, Canelones, Uruguay,* [3]*University of Tehran, Tehran, Pakdasht, Iran.*

Heat stress has adverse effects on the reproductive performance of dairy cows. Even with heat abatement systems, conception rates are negatively affected during heat conditions. Genetic selection is an attractive alternative for reducing the effects of heat stress on dairy cattle fertility. The first goal of this study was to estimate genetic components of conception rate across lactations considering heat stress. The second goal was to identify genomic regions, individual genes, and pathways responsible for thermotolerance. Data included 74,221 pregnancy records on 13,704 Holstein cows. Multi-trait repeatability test-day models with random regressions on THI values were used to estimate variance components. The models included herd-test-day and DIM classes as fixed effects, and generic and heat tolerance additive direct genetic and permanent environmental as random effects. Genetic variance for heat tolerance increased by 80% from first to second parity and 66% from second to third parity, suggesting that cows become more sensitive to heat stress as they age. Heritability estimates for conception rate at THI 78 were between 0.02 and 0.03, whereas genetic correlations between general merit and thermotolerance were always negative, ranging from −0.35 (0.21) to −0.82 (0.08). Whole-genome scans were performed using ssGBLUP. One region on BTA23 that harbors *PADI6* and *HCRTR2* was found to be strongly associated with general merit across all parities. Gene *PADI6* influences oocyte competence whereas *HCRTR2* is implicated in maternal recognition of pregnancy and implantation. Genomic regions on BTA10 and BTA11 were found to be strongly associated with thermotolerance; these genomic regions harbor *RGS6* and *PRKCE*, genes implicated in intracellular protein trafficking and heat shock response. Gene-set analysis revealed several functional categories, such as protein refolding, lipid modification, and gap junction, that are involved in biological processes and functions closely related to conception and pregnancy maintenance. Overall, this study contributes to a better understanding of the genetics underlying heat stress and points out novel opportunities for improving thermotolerance in dairy cattle.

Key Words: heat stress, fertility, gene mapping

65 Genetic dissection of sire conception rate in US Jersey cattle. F. M. Rezende*, G. O. Dietsch, and F. Peñagaricano, *University of Florida, Gainesville, FL.*

Fertility is arguably a very important economic trait in dairy cattle. Despite recent advances, reproductive performance remains suboptimal in most dairy herds, resulting in significant economic losses for the dairy industry. Recent studies suggest that a significant percentage of reproductive failure is attributable to bull subfertility. As such, our main objective was to dissect the genetic architecture of sire fertility in US Jersey cattle. The data set included 1.5k Jersey bulls with sire conception rate (SCR) records and 96k single nucleotide polymorphism (SNP) markers spanning the whole genome. The analysis included whole-genome scans for both additive and non-additive effects, and subsequent functional enrichment analyses using KEGG Pathway, Gene Ontology (GO) and Medical Subject Headings (MeSH) databases. The association analyses identified 10 different regions associated with Jersey bull fertility. These 10 1.5 Mb SNP windows jointly accounted for roughly 7% of the SCR additive genetic variance. Several candidate genes were annotated in these regions, such as *PKDREJ*, *STX2*, *PDHB*, and *RhoA*. These genes are directly involved in spermatogenesis, sperm differentiation, sperm capacitation and fertilization process. Moreover, the non-additive scan revealed 3 putative fertility genes, *FER1L5*, *CNNM4* and *DNAH3*, with marked recessive effects. The gene-set analysis identified several significant functional terms, including *gap junction*, *MAPK signaling pathway*, *regulation of cation channel activity*, *pyrophosphatase activity, synaptic vesicle exocytosis, GTPase activity, membrane fusion*, and *calcium channels*. Most of these terms are known to be involved in biological processes and functions closely related to male fertility. Overall, our results contribute to the identification of genomic regions and pathways underlying sire fertility in Jersey cattle, which may point out new strategies for improving service sire fertility via marker-assisted selection.

Key Words: bull fertility, gene set analysis, whole-genome scan

Dairy Foods: Joint ADSA-American Society of Nutrition Symposium: New Views on Milk and Human Health

68 Milk glycobiome and impact on human health. J. B. German*[1,2], [1]*University of California-Davis, Davis, CA,* [2]*Foods for Health Institute, University of California-Davis, Davis, CA.*

The evolutionary origin of lactation and the composition, structures and functions of milk's biopolymers highlight the Darwinian pressure on lactation. Lactation selected for biopolymers with considerable structural complexity that in turn provide functions from the mammary gland through the digestive system of the infant. Milk is an extensively glycosylated biological fluid whose glycan structures and functions are only recently emerging, although just one of the roles, feeding and fueling a unique microbiological community within the lower intestine of the infant is an astonishing example of evolutionary composition for biological function. Milk contains glycans, complex polymers of sugars whose stereospecific linkages are not matched by glycosidic enzymes within the mammalian infant gut. Hence, these glycan polymers travel to the lower intestine undigested. On reaching this microbe-rich environment, bacteria compete to access and ferment the sugars via different hydrolytic strategies. One specific strain of bacteria, *Bifidobacteriun longum* ssp. *infantis*, (*B. infantis*) is uniquely equipped genetically with a repertoire of genes encoding enzymes capable of taking up, hydrolyzing and metabolizing the complex glycans of human milk. This combination of a distinct food supply and unique genetic capability results in a profound effect on the composition and metabolic products of the entire microbial community within the lower intestine of breast-fed infants. The intestinal community is dominated by *B. infantis*, its metabolites and its interactions with the host. The role of *B. infantis* and its metabolites in shielding the infant from the growth of gram-negative enteropathogens and their endotoxins is a clear benefit and consistent with a selective advantage for this aspect of lactation evolution.

Key Words: lactation, oligosaccharides, microbiome

69 Bioactive peptides in dairy products. N. P. Shah*, *The University of Hong Kong, Hong Kong.*

The functionality of dairy proteins is further enhanced upon liberation of bioactive peptides by proteolysis caused by naturally occurring enzymes in milk, and those in bacteria from starter cultures and probiotics. Among various bioactive peptides, antihypertensive peptides have been studied extensively. These peptides inhibit angiotensin I-converting enzyme (ACE), the key enzyme responsible for the regulation of blood pressure via the renin-angiotensin system. ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor; ACE also hydrolyzes and inactivates bradykinin, a potent vasodilator. Therefore, excessive activity of ACE leads to an increased rate of vasoconstriction and development of high blood pressure. Inhibitory peptides block the ACE-mediated production of angiotensin II, and the reduction in ACE activity results in enhanced levels of bradykinin, resulting in an overall antihypertensive effect. Various food-derived peptides possess ACE inhibitory (ACE-I) properties. Caseins are important sources of those peptides. Biological significance of ACE-I peptides, their impact on human health and incorporation in functional foods have been the subject of intense research. Some antihypertensive peptides present in sour milk resist in vivo degradation and exert antihypertensive activity through the inhibition of ACE in the aorta. Most of the studies on the ACE-I production by fermentation are performed with selected strains of lactic acid bacteria mainly *L. helveticus* and direct hydrolysis of milk proteins with purified enzymes. A variety of naturally formed bioactive peptides have been found in fermented dairy products such as yogurt, sour milk, and cheese. These peptides are known to have multifunctional properties including immunostimulatory, opioid, hypotensive, antithrombotic, and antimicrobial activities. Several commercial products with highly proteolytic strains of *L. helveticus* have been developed and marketed to possess hypotensive activity including Calpis sour milk, prepared by fermenting milk using mixed culture containing *L. helveticus* CM4 (CP790) and *Saccharomyces cerevisia*e, which are responsible for the release of 2 tripeptides, Val-Pro-Pro and Ile-Pro-Pro, and Evolus (Valio Ltd., Finland), which is produced by highly proteolytic *L. helveticus* LBK-16H.

Key Words: bioactive peptide, angiotensin I-converting enzyme, *L. helveticus*

70 Milk fat implications on human health: The emerging scientific evidence. M. Torres-Gonzalez*, *National Dairy Council, Rosemont, IL.*

It has been thought for more than 50 years that saturated fat consumption is associated with increased risk of cardiovascular disease (CVD), because it raises LDL-cholesterol, a biomarker thought to better predict CVD risk. Because of this, current dietary guidelines recommend to limit saturated fat and/or saturated fat sources such as whole milk dairy foods. In fact, recommendations for dairy products consumption are limited to low-fat and fat-free versions. However, it has to be recognized that saturated fat is not a single compound but rather comprises a broad spectrum of fatty acids, and specifically, saturated fat in dairy foods is rich in different classes of saturated fatty acids that exert differential effects on metabolic and health outcomes. Additionally, emerging scientific evidence suggests that saturated might not be harmful as it has been thought, and related with whole milk dairy products (milk, cheese and yogurt) the evidence suggests that it might be associated with lower risk of chronic diseases. Thus, the objectives for this presentation attendees are (1) to review the current nutrition landscape and barriers for saturated fat, (2) to summarize the emerging scientific evidence around saturated fat and its association with cardiometabolic diseases, (3) to summarize the emerging body of evidence on dairy foods and their connection with cardiovascular disease, obesity, type 2 diabetes.

Key Words: milk fat, human health, saturated fat

71 Milk, calcium, and human health. B. R. Martin* and C. M. Weaver, *Purdue University, West Lafayette, IN.*

Calcium and vitamin D are among the key nutrients contributing to growth and bone development. It is estimated that approximately 40 to 50% of consumed calcium comes from fluid milk and approximately an additional 20% from other dairy sources. Building peak bone mass to optimal levels during childhood and adolescence will reduce the risk of fracture later in life. A National Osteoporosis Position Statement evaluated the strength of the lifestyle factors on developing peak bone mass (Weaver et al., Osteoporosis International 2016). Dietary calcium and physical activity have the strongest level of evidence for positive effect followed by dairy intake and vitamin D. Later in life, the goal of lifestyle modification is to reduce bone loss. Calcium and vitamin D have been

the most studied nutrients partly because they are most often deficient. The reported effectiveness of added calcium and vitamin D from food, beverages, or supplements is mixed. Increased calcium intake has not led to lower body weight unless as part of a weight loss diet. Evidence of the recent concern of high calcium intakes on soft tissue calcification is considered insufficient to alter public health recommendations. It is prudent to have a diet that includes adequate amounts of calcium. Cow's milk and dairy products are nutrient dense and bioavailable sources.

Key Words: calcium, vitamin D, osteoporosis

72 Effects of supplementing a xylanase enzyme on production performance of high-producing Holstein cows consuming diets containing corn or sorghum silage as the forage source. Y. Yang*[1], G. Ferreira[1], and B. T. Campbell[2], [1]*Department of Dairy Science, Virginia Tech, Blacksburg, VA,* [2]*DSM Nutritional Products, Parsippany, NJ.*

The objective of this study was to evaluate the effects of supplementing a xylanase enzyme (Ronozyme WX) on production performance of dairy cows consuming diets containing corn or sorghum silage as the forage source. Four primiparous (BW 581 ± 47 kg, DIM 47 ± 14 d) and 20 multiparous (BW 707 ± 67 kg, DIM 51 ± 14 d) Holstein cows were assigned to 1 of 4 diets in a replicated 4 × 4 Latin square design with 19-d periods. With a 2 × 2 factorial arrangement (i.e., silage type and xylanase), treatments consisted of (1) corn silage-based diet (CORN-XYL), (2) corn silage-based diet with xylanase (CORN+XYL), (3) sorghum silage-based diet (SORG-XYL), and (4) sorghum silage-based diet with xylanase (SORG+XYL). Diets contained (DM basis) 33% silage (either corn or sorghum), 8% grass hay, and 59% concentrate. Xylanase was included at a rate equal to 1.5 g product/kg DM. Cows were fed once daily by means of a Calan gate system. All variables were analyzed with the MIXED procedure in SAS, and the model included the fixed effects of square, treatment, and square by treatment interaction, and the random effects of period, cow within square, and the random residual error. XYL did not affect any of the variables of production performance ($P > 0.09$). Xylanase supplementation did not interact with silage type ($P > 0.19$). Dry matter intake was greater for CORN than for SORG ($P < 0.01$; 28.8 vs. 25.5 kg/d). Similarly, milk yield was greater for CORN than for SORG ($P < 0.01$; 51.6 vs. 48.9 kg/d). Milk fat concentration ($P < 0.01$; 3.84 vs. 3.30%) and milk fat yield ($P < 0.01$; 1.81 vs. 1.68 kg/d) were greater for SORG than for CORN. Silage type did not affect milk protein ($P > 0.59$) or milk lactose ($P > 0.50$) concentrations (2.90 and 4.79%, respectively). CORN yielded more milk protein ($P < 0.01$; 1.48 vs. 1.36 kg/d) and more milk lactose ($P < 0.01$; 2.45 vs. 2.29 kg/d) than SORG. CORN resulted in lower milk urea nitrogen than SORG ($P < 0.01$; 12.3 vs. 14.1 mg/L). Silage type did not affect 3.5% fat-corrected milk ($P > 0.24$; 50.0 kg/d). In conclusion, xylanase supplementation did not affect production performance when feeding either corn-based or sorghum-based diets to high-producing dairy cows.

Key Words: xylanase, corn silage, sorghum silage

73 Effects of a brown midrib corn silage hybrid with enhanced starch digestibility on production and nutrient digestibility in lactating dairy cows. A. Tebbe* and W. Weiss, *Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.*

Feeding corn silage hybrids selected for high fiber digestibility can increase energy intake and milk production, but these hybrids often have reduced starch digestibility. A hybrid with enhanced fiber and starch characteristics may improve feed efficiency. The objective was to determine the nutritional value of corn silage from a brown midrib (*bm3*) hybrid selected for floury endosperm (*bm3*-Plus; Unified, Dow AgroSciences, Indianapolis, IN) compared with an isogenic *bm3* and a conventional dual-purpose (DP) hybrid. Twenty-one Holstein cows averaging 105 DIM were used in 7 replicated 3 × 3 Latin squares with 28 d periods. Two of the 7 squares were used to measure nutrient digestibility via total collection of urine and feces. Cows were blocked by parity and randomly assigned to 1 of 3 diets formulated to contain similar concentrations of corn silage (46% of DM) and nutrients (34.3% NDF, 23.2% starch, and 17.0% CP) but contain different corn silage hybrids: (1) DP (38.6% NDF, 36.9% starch), (2) *bm3* (40.2% NDF, 29.9% starch), and (3) *bm3*-Plus (41.0% NDF, 29.8% starch). Based on in vitro digestibility, the *bm3* hybrids had less digestible starch (3 h incubation) and higher NDF digestibility compared with DP; however, between *bm3* hybrids, in vitro starch digestibility was similar. Hybrid did not affect DMI (average 25.7 kg/d; $P > 0.10$) or in vivo NDF digestibility (average 51.5%; $P > 0.10$). Feeding *bm3*-Plus increased ($P < 0.09$) yields of milk and milk energy about 2 kg/d and 1.6 Mcal/d, respectively, compared with DP and *bm3* hybrids. The *bm3*-Plus diet increased ($P < 0.01$) feed efficiency nearly 6% compared with *bm3* and DP (milk energy/DMI; 1.10 vs 1.04 Mcal/kg) likely from greater ($P < 0.04$) total-tract starch digestibility (88.3 vs 85.0%). Compared with standard *bm3*, cows consuming *bm3*-Plus had less biohydrogenation intermediates in milk fat and greater milk fat content (3.1 vs 2.9%; $P < 0.04$) indicating potentially better rumen conditions. Feeding silages with both enhanced fiber and starch digestibility had improved nutritional value compared with a conventional hybrid or a hybrid selected for greater fiber digestibility.

Key Words: corn silage hybrids, digestibility, brown midrib

74 Composition and digestibility of cell walls from corn stems and corn leaves according to plant cutting height. G. Ferreira* and C. L. Teets, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

The objective of this study was to compare cell wall (CW) composition and in vitro neutral detergent fiber digestibility (IVNDFD) of stems and leaf blades from phytomers positioned at different cutting heights within corn plants. Five corn plants from 14 diverse corn silage hybrids (95 to 120 relative maturity) were obtained from a corn hybrid testing trial performed by Virginia Cooperative Extension. Plots consisted of 4 4-m-long rows separated by a 76-cm inter-row space. Tissues were harvested at a single day, when the crops were at 1/4- to 3/4-milkline stage of maturity. Stem internodes (STEM) and leaf blades (BLADE) from the 2th (BASAL) and the 4th (UPPER) phytomers positioned above the nodal roots were obtained. After grinding samples with a cyclone mill (2-mm screen), IVNDFD were determined using a DaisyII Incubator. After re-grinding samples with a cyclone mill (0.5-mm screen), cell walls were extracted and protein-free lignin concentration was determined using the acetyl-bromide procedure. Data were analyzed using the MIXED Procedure of SAS, and the model included the random effect of hybrid and the fixed effects of tissue, phytomer, and their interaction. BLADE had greater CW concentrations than STEM ($P < 0.01$; 55.1 vs. 48.4% DM, respectively). BASAL blades had greater CW concentrations than UPPER blades (60.4 vs. 49.9% DM, respectively), while stems had similar CW concentrations among phytomers ($P > 0.75$; 48.4%). BLADE had greater IVNDFD than STEM ($P < 0.01$; 73.9 vs. 48.9% NDF, respectively). UPPER stems had greater IVNDFD than BASAL stems ($P < 0.01$; 50.9 vs. 46.6% NDF, respectively), while blades had similar IVNDFD among phytomers ($P > 0.34$; 73.9% NDF). STEM had greater lignin concentrations than BLADE (30.2 vs. 25.5% CW). Lignin concentrations, in either stems ($P > 0.50$) or blades ($P > 0.88$), did not differ among phytomers. Lignin concentration in the CW did not correlate with IVNDFD in either blades ($r = 0.02$; $P > 0.78$) or stems ($r = 0.19$; $P > 0.33$). In conclusion, cutting height can affect the IVNDFD

of the stem but not the blade, and the difference in IVNDFD of the stem at different cutting heights is not related to lignification of the CW.

Key Words: cutting height, lignin, fiber digestibility

75 Fermentation quality, and in vitro digestibility of alfalfa and red clover silages treated with pre-fermented juice. Y. Jiang*[1], Y. L. Xue[2], D. Vyas[1], L. Sun[2], G. M. Yin[2], Y. Y. Zhang[2], S. B. Liu[2], Z. Yu[3], Q. Z. Sun[4], and A. T. Adesogan[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Inner Mongolia Academy of Agriculture and Animal Husbandry Science, Hohhot, Inner Mongolia, China,* [3]*Department of Animal Sciences, China Agricultural University, Beijing, China,* [4]*Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Hohhot, Inner Mongolia, China.*

This study was conducted to evaluate the effects of pre-fermented juice (PFJ) prepared from red clover (RC) or alfalfa (AF) on fermentation quality and in vitro digestibility of RC and AF silages. The RC (Experiment 1) and AF (Experiment 2) were harvested at the early-bud stage, chopped to 1–2 cm lengths, mixed with distilled water (1:9 ratio) and ensiled in anaerobic jars for 48 h at 30°C. Subsequently, lactic acid bacteria (LAB) in the RC and AF PFJ filtrates were counted. In Experiment 1, RC was chopped, wilted to 40% DM and ensiled in vacuum-sealed plastic bags (18 × 26 cm) in triplicate for 60 d after applying following treatments: (1) Control (deionized water), (2) PFJ-RC (1×10^6 cfu of LAB/g fresh weight); and (3) PFJ-AF (1×10^6 cfu of LAB /g fresh weight). In Experiment 2, AF was treated and ensiled similarly. Data were analyzed using GLM procedure of SAS. Compared with the control, treating RC silage with PFJ-RC reduced ($P < 0.05$) pH (4.63 vs. 5.31), NH_3N/total-N (4.5 vs.10.6%) and acetic acid concentration (0.6 vs. 1.4%) but increased ($P < 0.05$) DM (39.3 vs. 38.4%), WSC (2.4 vs. 2.0) and lactic acid (LA) concentrations (4.2 vs. 0.8%) as well as LAB counts (8.2 vs. 6.1 cfu/g) and in vitro DM digestibility (IVDMD, 66.4 vs. 62.4%). Compared with PFJ-AF, treating RC with PFJ-RC reduced ($P < 0.05$) pH and IVDMD but increased NH_3N/total-N and LA concentrations and LAB counts. Compared with the control, AF silages treated with PFJ-AF had lower ($P < 0.05$) pH (4.3 vs. 5.4) and NH_3N/total-N (3.1 vs. 10.6%), and higher ($P < 0.05$) DM (38.1 vs. 37.1%) and LA concentrations (4.5 vs. 0.9%) as well as LAB counts (8.2 vs. 6.3 cfu/g) and IVDMD (71 vs. 63%). Compared with PFJ-RC, applying PFJ-AF to AF silages reduced ($P < 0.05$) pH and NH_3N/total-N and increased ($P < 0.05$) LA concentration, LAB counts and IVDMD. Overall, ensiling red clover or alfalfa with PFJs improved the fermentation quality and IVDMD and reduced proteolysis. For red clover or alfalfa, applying PFJ prepared from the same forage was more effective at improving the fermentation quality than using PFJ prepared from the other forage.

Key Words: alfalfa, red clover, pre-fermented juice

76 Temporal trends in financial performance of spring-calving pasture-based dairy farms segregated by profit or feed use category. G. Ramsbottom*[1], B. Horan[2], K. M. Pierce[3], D. P. Berry[2], and J. R. Roche[4,5], [1]*Teagasc, Oak Park, Carlow, Ireland,* [2]*Teagasc, Animal and Pastureland Research and Innovation Centre, Moorepark, Fermoy, Cork, Ireland,* [3]*School of Agriculture and Food Science, UCD, Belfield, Dublin, Ireland,* [4]*Dairy NZ, Private Bag* 3221*, Hamilton, New Zealand,* [5]*School of Biological Sciences, University of Auckland, Auckland, New Zealand.*

Greater milk price volatility has raised questions about how future pasture-based farm systems should be adapted to maximize farm profitability. We evaluated the factors associated with economic robustness in pasture-based dairy systems using the financial results of all of the dairy farms with longitudinally complete records over 8 yr (n = 315), which allowed us to assess the effects of volatile milk prices, input costs, and adverse weather challenges on net profit/ha. A series of analyses were undertaken to characterize the longitudinal trends in physical and financial performance for farms ranked by average operating profit (i.e., net profit/ha) and, separately, according to the quantity of feed purchased. All analyses were undertaken using a mixed model framework in PROC MIXED where herd nested within region was included as a repeated effect with an autoregressive covariance structure assumed among records within herd. Study farms expanded and intensified during the study period, with increases in milk fat and protein yield per cow (+15%; $P < 0.001$) over the 8 yr; mean annual pasture DM consumed/ha also increased linearly (+19%; $P < 0.05$). When nested within region and ranked by quartiles (highest to lowest) for 8-yr average farm net profit/ha, the highest profit quartile were, on average, smaller farms with greater technical efficiency; farm net profit per hectare were €1,611, €1,189, €937 and €630 for the highest, second highest, second lowest and lowest profit quartiles respectively. Pasture-based production systems with greater use of non-pasture feeds achieved greater ($P < 0.05$) milk production/cow, but not per hectare, and consistently lower farm profitability across years. Results indicate that high profit farms are also most 'resilient', exhibiting a greater capacity to recover profit after low milk price events or adverse weather yr. The results reinforce the association between utilization of grazed pasture and net farm profit. Furthermore, the results highlight that importing more than 10% of the cow's diet as a supplement to pasture can reduce farm profitability, even in high milk price years.

Key Words: dairy, systems, resilience

77 Mycotoxin occurrence in southern US pasture grasses. P. N. Gott*[1], A. Stam[2], A. Johns[3], B. A. Bell[1], S. M. Mendoza[1], E. G. Hendel[1], U. Hofstetter-Schähs[4], T. Jenkins[4], and G. R. Murugesan[1], [1]*Biomin America Inc., Overland Park, KS,* [2]*Federally Recognized Tribal Extension Program, University of Florida, Okeechobee, FL,* [3]*Seminole Tribe of Florida Inc., Okeechobee, FL,* [4]*Biomin Holding GmbH, Getzersdorf, Lower Austria, Austria.*

A survey was conducted in the southern United States to investigate the occurrence of mycotoxins in pasture grasses. An initial survey in south central Florida indicated zearalenone (ZEN) in common Bermudagrass (*Cynodon dactylon*) as the major challenge. The survey was expanded to cover a greater geographic area and additional grass species to further explore mycotoxin exposure in grazing livestock. Grass was hand-plucked to simulate cattle foraging behavior. Samples comprised of 25–30 subsamples collected throughout each pasture were screened for the presence of mycotoxins at Activation Laboratories (Ancaster, ON, Canada; 15 mycotoxins) or Romer Labs (Union, MO; 17 mycotoxins) by the liquid chromatography tandem mass spectrometry method. Mycotoxin levels for positive samples are presented on a dry basis in parts per billion (ppb). A total of 291 samples were collected from March 2016 through January 2018, with 271 being bermudagrass, 12 were limpograss (*Hemarthria altissima*), 5 were bahiagrass (*Paspalum notatum*), one was stargrass (*Cynodon* spp.), and 2 were unidentified. One or more mycotoxins were detected in 200 (68.7%) samples overall. The main mycotoxin detected was ZEN in 179 (61.5%; mean of positives 1459 ppb; found in all species) of total samples and 175 (64.6%; mean 1484 ppb) of bermudagrass samples. ZEN did not occur as frequently in limpograss samples (16.7%; mean 135 ppb), but 11 (91.7%; mean 3508 ppb) tested positive for type B trichothecenes (B-Trich; includ

ing deoxynivalenol, nivalenol, and fusarenon X). Only 9 (3.3%; mean 200 ppb) of bermudagrass samples had detectable levels of B-Trich. However, type A trichothecenes (T-2 or HT-2 toxin) were detected in 43 (15.9%; mean 1492 ppb) bermudagrass samples. The preliminary results of the mycotoxin survey in Southern US grasses indicate that a variety of mycotoxins can occur at levels that may pose challenges to cattle reproduction, health, and performance. Further research is needed to better understand if specific mycotoxins are associated with certain grass species, the potential for seasonal patterns of contamination, and the greater scope of this potential challenge to grazing livestock.

Key Words: mycotoxin, pasture, zearalenone

78 Post-ruminal choline ion supplementation during a feed restriction-induced negative energy balance alters milk production and liver triacylglycerol concentration in Holstein cows. D. N. Coleman*[1], M. Vailati-Riboni[1], A. A. Elolimy[1], F. C. Cardoso[1], M. Miura[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Ajinomoto Co. Inc., Tokyo, Japan.*

The objective was to investigate the effects of post-ruminal choline ion (CHO) supplementation to cows during a feed restriction-induced negative energy balance on metabolism, liver triacylglycerol (TG), and milk production. Ten primiparous rumen-cannulated Holstein cows (158 ± 24 DIM) were used in a replicated 5 × 5 Latin square design with 4 d treatment periods (d 1–4), and 10 d recovery periods (d 5–14). Treatments were (1) ad libitum intake with abomasal infusion of water (A0), (2) restricted intake (60% of net energy for lactation requirements) with abomasal infusion of water (R0), (3) restricted intake with abomasal infusion of 6.25 g/d CHO (R6.25), (4) restricted intake with abomasal infusion of 12.5 g/d CHO (R12.5), and (5) restricted intake with abomasal infusion of 25 g/d CHO (R25). Liver biopsies were performed in the morning on d 5 after the abomasal infusion ended, and tissue analyzed for TG content. Body weight and BCS were recorded on d 1 and 5. Blood was collected on d 1–5 for analysis of nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB). Milk production was recorded daily and composite samples were analyzed for components. Two contrasts, CONT1 (A0 vs. R0) and CONT2 (R0 vs. the average of 6.25, 12.5 and 25 g/d CHO), and tests of linear and quadratic treatment effects of CHO dose were performed. BW, milk yield (MY) ($P < 0.0001$) and energy balance was lower in R0 and milk fat % greater compared with A0. Feed restriction also increased liver TG and plasma concentrations of NEFA and BHB. Supplementation of CHO vs. R0 decreased NEFA and milk fat % and increased MY. Supplemental CHO led to a linear increase in MY and a linear decrease in milk fat %. There was a linear decrease in liver TG with CHO supplementation (R0: 3.48 mg/g wet tissue, R6.25: 2.70 mg/g, R12.5: 1.96 mg/g, R25: 2.50 mg/g). These results suggest that supplementation of CHO at 25 g/d during a feed restriction-induced negative energy balance leads to the greatest improvement in MY, whereas 12.5 g/d CHO leads to greater improvement in liver TG storage. The mechanisms associated with these responses merit further research.

Key Words: choline, feed restriction, metabolism

79 Methionine supply during the periparturient period alters plasma amino acid profiles and liver metabolism in dairy cows. F. Batistel*[1], R. R. C. Yambao[1], Y.-X. Pan[1], C. Parys[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

The objective of this study was to investigate the effect of methionine supply during the periparturient period on plasma amino acid concentrations and liver metabolism (TCA cycle, 1-carbon metabolism, transsulfuration pathway, and gluconeogenesis) in dairy cows. Multiparous cows were used in a block design and assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH). Mepron was fed from −28 to 30 d relative to parturition at a rate of 0.09% and 0.10% of DMI during the prepartum and postpartum period, respectively. That rate ensured that the ratio of Lys to Met in the metabolizable protein was close to 2.8:1. Blood was sampled from from 15 cows/treatment at −14, +7, +21, and +30 d relative to calving date. Liver was sampled from 8 cows/treatment at −10, +10, and +30 d relative to parturition. Targeted metabolomics (LC-MS) was performed to quantify 32 metabolites. Activity of betaine-homocysteine S-methyltransferase (BHMT), methionine synthase (MTR), and cystathionine-β-synthase (CBS) was measured. mRNA expression of the other enzymes was measured by RT-PCR. Compared with control, feeding MET increased DMI in the prepartum and postpartum period as well as milk yield. A treatment × time was observed for the plasma concentration of Asn, Cys, and Gly due to higher concentrations in MET-supplemented cows during the prepartum period. Compared with control, MET-fed cows had greater concentration of Met, Lys, Thr, Leu, Val, and Phe. Liver from MET cows had greater concentrations of metabolites related to the TCA cycle (isocitric acid, α-ketoglutaric acid, and malic acid), 1-carbon metabolism (Met) and transsulfuration pathway (glutathione and Tau) than control cows. Activity of CBS was greater in MET-supplemented cows compared with control. Compared with control, MET cows had greater mRNA expression of enzymes related to the TCA cycle (*ACO2* and *FH*), 1-carbon metabolism (*MAT1*), transsulfuration pathway (*CBS*), and gluconeogenesis (*PCK1*). Results indicate that feeding MET to achieve a Lys to Met ratio of 2.8:1 during the periparturient period increases blood amino acid concentrations, oxidation of substrates and synthesis of antioxidants in the liver.

Key Words: amino acids, metabolomics

80 Insulin sensitivity and glucose utilization in response to methionine supply during the periparturient period in dairy cows. F. Batistel*[1], C. C. I. Garces[1], C. Parys[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

The objective was to examine the effect of methionine supply during the periparturient period on peripheral insulin sensitivity via glucose tolerance test and glucose utilization by skeletal muscle in dairy cows. Multiparous cows were randomly assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH). Mepron was fed from −28 to 30 d relative to parturition at a rate of 0.09% and 0.10% of DMI during the prepartum and postpartum period, respectively, to ensure a ratio 2.8:1 Lys to Met in the metabolizable protein. The glucose tolerance test was conducted at day −12 and +12 relative to calving in 12 cows per treatment. A sterile solution of 50% dextrose was administered intravenously at a dose of 0.25 g of glucose per kg of BW over the course of 5 min. Blood samples were collected from the tail vein or artery at −15, −5, 5, 10, 15, 30, 60, 120 min relative to administration of glucose. Hind-leg muscle was sampled via puncture biopsy at day −10 and +10 relative to parturition and was used to measure protein expression of the insulin-sensitive transporter SLC2A4. In the prepartum period, increased MET supply was associated with greater plasma glucose baseline values, maximum concentration of glucose after infusion, and reduced time required for plasma glucose concentration to return to baseline values and plasma glucose area under the curve (AUC). A similar response was observed for the insulin after glucose infusion; MET led to greater plasma insulin baseline values, maximum concentration of insulin after infusion, and plasma insulin AUC while the time required for plasma insulin concentration to return to baseline values was not affected by MET. Protein expression of SLC2A4 was greater in the MET-supplemented cows during the prepartum period. During the postpartum period, MET-supply increased plasma glucose AUC and none of the other parameters measured were affected by treat

ments. Overall, the data indicate differences in glucose utilization during the prepartum and postpartum period in response to methionine supply.

Key Words: metabolism, muscle

81 Postpartum supplementation with rumen-protected branched-chain amino acids: Effects on production and plasma metabolites. F. A. Leal Yepes*[1,2], S. Mann[2], T. R. Overton[1], J. J. Wakshlag[2], and D. V. Nydam[2], [1]*College of Agriculture and Life Sciences, Ithaca, NY,* [2]*College of Veterinary Medicine, Ithaca, NY.*

The objective was to evaluate the effect of rumen protected branched-chain amino acids (RPBCAA; 375 g of 27% L-leucine, 85 g of 48% L-isoleucine and 91 g of 67% L-valine) with or without propylene glycol (PG) oral supplementation on milk production, dry matter intake, free fatty acids (NEFA), BHB), and plasma urea nitrogen (PUN) during the early postpartum period in dairy cows. Multiparous Holsteins were enrolled in blocks of 3 and randomly assigned to either the control group or one of the 2 treatments from calving until 35 d. The Control group (n = 26) received 200g of dry molasses; the RPBCAA group (n = 23) received RPBCAA mixed with 200g of dry molasses; the RPBCAA plus PG (RPBCAAPG) group (n = 25) received RPBCAA mixed with 200g of dry molasses plus 300 mL of PG once daily from calving until 7 DIM. Blood was sampled 3 times per week from calving until 21 DIM. Milk yield, energy corrected milk (ECM), dry matter intake (DMI) and PUN were analyzed using repeated measurements ANOVA (Table 1). Chi-squared tests were generated for differences among groups in the number of hyperketonemia events (HYK), defined as concentration of BHB in plasma ≥1.2mmol/L during the first 15 DIM. The number of plasma samples classified as HYK was 57, 22 and 44 in groups control, RPBCAA and RPBCAAPG, respectively ($P = 0.001$). BCAA are mainly oxidized by the tricarboxylic acid cycle to produce ATP during catabolic states. Therefore, the use of RPBCAA might be a feasible and manageable option to reduce the incidence of HYK in dairy cows during early lactation. The higher PUN in the treatments groups reflects the inclusion of BCAA in the ration.

Table 1 (Abstr. 81).

Measurement	BCAA	BCAA+PG	Control	*P*-value
CP, % DM	16.0	15.8	15.6	0.36
Milk yield, kg/d	43.9 ± 2.4	44.9 ±2.4	41.9 ± 2.4	0.20
ECM, kg/d	52.4 ± 2.0	53.2 ± 2.0	50.4 ± 1.6	0.10
DMI, kg/d	23.7 ± 0.4	24.0 ± 0.4	23.2 ± 0.4	0.30
NEFA, µEq/L	548 ± 2.9	504 ± 2.9	580 ± 2.9	0.52
BHB, mmol/L	0.80 ± 1.07	0.86 ± 1.0	0.96 ± 1.1	0.06
PUN, mg/dL	10.2 ± 1	9.56± 1	8.37 ± 1	0.0006

Key Words: branched-chain amino acids, hyperketonemia, milk yield

82 Reducing milking frequency from three to twice a day during the first month of lactation improves energy balance and metabolic status of cows with minor effects on yields. U. Moallem*[1], H. Kamer[1,2], A. Hod[1,2], L. Livshits[1], G. Kra[1], S. Jacoby[1], Y. Portnick[1], and M. Zachut[1], [1]*Department of Ruminants Science, Volcani Center, Rishon LeZion, Israel,* [2]*Department of Animal Science, the Hebrew University of Jerusalem, Rehovot, Israel.*

Reducing the milk production during early lactation might be of interest to improve the energy balance (EB) of high yielding dairy cows; therefore, the objectives were to test the effects of reducing the milking frequency from 3 to twice a day during the first 30 DIM, on yields, intake, efficiency, and the metabolic status. Forty-two multiparous cows were divided into 2 groups according to previous lactation performance, parity, and body weight. The control cows were milked 3 times a day (3ML), and the treatment cows were milked twice a day until 30 DIM (2ML), and then 3 times a day. Both groups were followed until 100 DIM. Milk samples were taken twice a week from 3 or 2 consecutive milkings until 45 DIM for milk solids analysis. Individual DMI, milk yields, and BW were recorded daily. Blood samples were taken 3 times weekly from 14 d prepartum until 45 DIM. Data were analyzed using the PROC MIXED model of SAS for the first 30 DIM, and then from 31-to 100 DIM for carry-over effects. Milk yields during the first 30 DIM were 9.4% higher (44.3 and 40.5 kg/d, respectively; $P < 0.01$), milk-fat percentage was lower (4.21 and 4.57%, respectively; $P < 0.001$), and yields of all milk solids were higher in the 3ML cows than in the 2ML cows. DMI and FCM (4%) were similar between groups and the EB during the first 30 DIM was better in the 2ML than in the 3ML cows (1.28 and −1.74, respectively; $P < 0.007$); milk/DMI, but not FCM/DMI, was higher in the 3ML cows. From 31 to 100 DIM, no differences were observed in milk yield (56.9 kg/d for both groups), milk solids yields, DMI or milk/DMI; however, fat percentage was higher in the 2ML and the EB was better in the 3ML cows. Blood glucose concentrations between 0 and 30 DIM were higher ($P < 0.0003$), β-hydroxybutyrate were lower ($P < 0.02$), NEFA were higher ($P < 0.002$), and insulin were lower ($P < 0.08$) in the 2ML than in the 3ML cows. In conclusion, reducing the milking frequency to twice a day during the first 30 DIM improved the EB and the metabolic status, with minor effects on production.

Key Words: milking frequency, energy balance, metabolic status

83 Endocrine effects of milking frequency and anti-inflammatory treatment in early lactation. C. M. Ylioja*, M. Garcia, L. K. Mamedova, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

Inflammatory signals in early lactation may aid in the allocation of nutrients toward milk production through altered sensitivity of endocrine signals. We sought to measure effects of anti-inflammatory treatment, as well as the impact of reducing nutrient demand by the mammary gland, on endocrine signaling in early lactation. Multiparous Holstein cows were enrolled at calving and randomly assigned to either sodium salicylate (SS; 2 g/L) or control (CTL; molasses carrier) treatment, administered via drinking water for the first 5 d of lactation, and to a milking frequency (MF) of either once (1×) or 3 times daily (3×), in a 2 × 2 factorial design (n = 8–9). Mixed models were used to assess repeated measures over time. Analysis of plasma samples collected daily before feeding on d 1 to 5 showed that insulin levels were decreased with more frequent milking (0.32 vs. 0.24 ± 0.02 ng/mL; $P = 0.02$), and also decreased over time compared with 1× (interaction $P = 0.02$). Insulin was also decreased with SS treatment (0.31 vs. 0.25 ± 0.02 ng/mL for CTL and SS, respectively; $P = 0.04$). Treatment × MF interactions for plasma glucagon ($P = 0.05$) and resistin ($P = 0.05$) revealed increases in both hormones only in 3 × cows treated with SS. Glucagon concentration was greater on d 5 for 3 × vs. 1 × cows (35.5 vs. 24.9 ± 2.9 pg/mL; $P = 0.01$). Circulating TNFα was also increased by frequent milking (56.1 vs. 34.1 ± 5.3 pg/mL; $P < 0.01$). On d 5 of the study, cows underwent a glucose clamp protocol with a 2-h euglycemic phase followed by a 2-h hyperinsulinemic-euglycemic phase; blood samples were collected in the last 30 min of each phase. During hyperinsulinemia, SS increased resistin compared with CTL (124 vs. 64 ± 19 pg/mL; $P =$

0.03). Hyperinsulinemia decreased glucagon only in the 3 × cows (MF × phase interaction $P = 0.05$). These results suggest that greater MF (and negative energy balance) may promote inflammatory signaling and alter adipose and pancreatic sensitivity to endocrine signals in early lactation. Manipulation by SS treatment triggers additional endocrine responses to maintain metabolic homeostasis in this window of time.

Key Words: inflammation, insulin sensitivity, endocrine

84 Expression and activity of the branched-chain α-keto acid dehydrogenase (BCKDH) in different tissues of early-lactating dairy cows. L. A. Webb*[1], H. Sauerwein[1], D. von Soosten[2], S. Dänicke[2], and H. Sadri[3,1], [1]*Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, North Rhine-Westphalia, Germany,* [2]*Institute of Animal Nutrition, Friedrich-Loeffler Institut, Federal Research Institute for Animal Health, Brunswick, Lower Saxony, Germany,* [3]*Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.*

Break-down of branched-chain amino acids (BCAA) plays a major role in the metabolic adaptation to the increased energy needs due to lactation. We aimed to specify the potential role of various metabolically active tissues in BCAA catabolism of early-lactating dairy cows. Liver, muscle (*M. semitendinosus*), mammary gland (MG), subcutaneous (scAT; tail head) and visceral adipose tissue (vAT; omental fat) of 25 primiparous Holstein cows were collected on d 1, 42 and 105 postpartum (p.p.) during slaughter and examined for mRNA and protein abundance and the activity of the rate-limiting enzyme BCKDH. Target genes were quantified by qPCR, protein abundance was measured via Simple Western, and enzyme activity was determined spectrophotometrically. Data were analyzed using a Linear Mixed Model (fixed: tissue, time, tissue × time; random: cow; post-hoc: Bonferroni; SPSS). The mRNA abundance of the α subunit of the BCKDH E1 component (*BCKDHA*) in muscle was lower ($P < 0.05$) compared with those in liver, MG, and scAT. The mRNA abundance of the β subunit of the BCKDH E1 component (*BCKDHB*) was about 5-fold higher in liver than in any other tissue ($P < 0.001$). Both *BCKDHA* and *BCKDHB* mRNA in MG and vAT were affected by time ($P < 0.05$): Abundance on d 105 was on average twice as high as on d 1 p.p. (all $P < 0.05$ except for *BCKDHB* in vAT, $P = 0.06$). The BCKDHA protein abundance was greatest in liver and MG, followed by the 2 AT and then muscle ($P < 0.001$). At all 3 time points, the BCKDH activity was around 7-fold greater in liver compared with MG ($P < 0.001$). In liver, an increase of BCKDH activity from d 1 to d 42 p.p. was observed ($P = 0.02$), whereas no time-dependent changes were detected in MG. Muscle and AT did not show any measurable BCKDH activity. During early lactation, liver seems to be a physiologically significant site of BCAA catabolism, while the further involvement of muscle, beyond being the main supply of these AA especially in times of insufficient intake, remains unclear. The observed lower activity in MG indicates that BCAA might be spared from oxidation possibly for de novo protein synthesis.

Key Words: branched-chain α-keto acid dehydrogenase (BCKDH), tissue regulation, dairy cow

85 Characterizing changes in the proteome of high-density lipoprotein over the transition period in dairy cows. E. Behling-Kelly*[1], S. Davidson[2], D. Nydam[1], F. Leal-Yepes[1], and S. Mann[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Cincinnati, Cincinnati, OH.*

High-density lipoproteins (HDL) across all species, are largely known for their role in cholesterol transport. Human HDL contain over 95 different proteins, and only one-third are dedicated to lipid transport. The HDL proteome consists largely of proteins with anti-oxidant, complement regulatory, proteolytic, or antithrombotic activity. Several physiologic and pathologic states are associated with changes in the HDL proteome. The transition period in a dairy cow, a high-risk time for development of production-related diseases, is marked by profound changes in lipid metabolism. In this study, quantitative proteomic analysis was performed on HDL samples from 28 clinically healthy, multiparous Holstein cows at 2 weeks prepartum (wk −2), week of calving (wk 0) and 2 wk postpartum (wk +2). HDL were isolated by density gradient ultracentrifugation. Samples were equivalently loaded (75 µg protein) with N15 labeled human ApoA1 as an additional loading control and analyzed using ESI-MS/MS QStar XL mass spectrometer (Applied Biosystem). Generated peaks were scanned against the UniProtKB/Swiss-Prot Protein Knowledgebase (release 57.0, 03/2009) using the X!Tandem search engine. Relative protein counts were analyzed by repeated measures ANOVA. 81 unique sequences were identified (95% probability match). The majority of proteins were similar to those identified on human HDL with the salient exception of glycosylation dependent cell adhesion molecule 1, which has not been found on human HDL. Relative protein count of apolipoprotein CII (ApoCII) was significantly different across all time points studied (0.9 wk −2, 2.8 wk 0, 4.7 wk +2, $P < 0.05$, n = 4 per time point). ApoCII is postulated to have lipopolysaccharide-neutralizing effects. Relative amount of serum amyloid A was also divergent (0.4 wk −2, 1.8 wk 0, 1.0 wk +2, $P < 0.05$, n = 4 per time point), with smaller changes noted in α 1 antiprotease, paraoxonase, and 3 different glycoproteins. Proportion of HDL was not different across time points (75.7% ± 5.4 wk −2, 83.7% ± 6.5 wk 0, 85.3% ± 3.8% wk+2, $P > 0.06$). In addition to the well-recognized decline in HDL that occurs during transition, our data indicate the composition of HDL varies across this time as well.

Key Words: lipoprotein, proteome, transition

86 Association between bile acid with energy balance, and glucose to insulin ratio during the transition period. S. H. Cheong*, E. Behling-Kelly, W. R. Butler, and M. S. Roberson, *Cornell University, Ithaca, NY.*

Bile acids are traditionally known for their role in lipid digestion and recently bile acids have been shown to have potential endocrine activity. Pregnancy state elevates bile acid concentration in humans, but the effects of pregnancy on bile acid concentrations have not been studied in cattle. Our objective was to report the bile acid profile during the transition period and to determine if bile acid concentrations are associated with energy balance, glucose and insulin. Multiparous Holstein cows (n = 48) were characterized for plasma total bile acid concentration, energy balance, nonesterified fatty acids (NEFA), BHB, glucose and insulin at 5 time points: 3 wk prepartum (wk −3), 2 wk prepartum (wk −2), week of calving (Wk0), first week postpartum (wk +1) and second week postpartum (wk+2). Blood was collected at approximately 0900 h and fresh TMR was given at 0800 h daily. Cows were individually fed in tie-stalls with daily feed offered and refused weighted to determine feed intake. Average daily energy balance was calculated based on energy intake and energy requirements for maintenance, pregnancy or lactation for each of the time periods. Data were analyzed as repeated measures using PROC MIXED of SAS. Total bile acid concentration was significantly lower during pregnancy and increased postpartum (in µmol/L, wk −3 = 15.7 ± 1.3, wk −2 = 14.9 ± 1.3, wk 0 = 38.7 ± 2.6, wk +1 = 65.1 ± 5.0, wk +2 = 75.2 ± 9.1; $P < 0.001$). Plasma total bile

acid concentration was positively associated with NEFA ($P < 0.001$) and BHB ($P = 0.009$); and negatively associated with energy balance ($P < 0.001$) and insulin ($P < 0.001$); but not associated with glucose ($P = 0.19$). Glucose to insulin ratio was also higher in cows with increased plasma total bile acid ($P < 0.001$). Taken together, increased plasma total bile acid was associated with poorer energy balance and higher glucose to insulin ratio during the transition period but further studies are warranted. *This work was supported by the USDA National Institute of Food and Agriculture, Research Animal Health project NYCV-480867.*

Key Words: bile acids, energy balance, insulin resistance

87 PBMC mitochondrial enzyme activity in high- and low-producing Holstein cows during early lactation. A. Niesen*[1], H. Rossow[1], and O. Genther-Schroeder[2], [1]*UC Davis, Davis, CA,* [2]*Purina Animal Nutrition Center, Gray Summit, MO.*

Mitochondria are central to metabolism and the primary energy producers for all biosynthesis, including lactation. The objective of this study was to determine if high and low producing dairy cows exhibit differences in mitochondrial enzyme activities during early lactation. Fifty-six Holstein cows were assigned to one of 4 groups: primiparous high or low ECM (41.8 vs 33.2 kg/d) and multiparous high or low ECM (56.8 vs 44.3 kg/d). Group assignments were made after data were collected by averaging ECM for primiparous cows and then for multiparous cows and assigning below average cows to the low group and above average cows to the high group. Whole blood samples were collected at one time point within (70 ± 11 DIM) and processed for crude mitochondrial extracts from peripheral blood mononuclear cells (PBMCs). Mitochondrial function of the extracts was assessed by measuring the activity rates of citrate synthase, complex I, complex IV, and complex V using kits from Abcam (Cambridge, MA). Milk samples were collected 9 times within a week of blood collection and analyzed for major components using a MilkoScan FT2 by FOSS (Mulgrave, Australia). Data were analyzed using the Mixed procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) for high and low ECM with cow as the experimental unit of interest and dependent variables parity, and DIM as a covariate. Complex V enzyme activities tended to be higher in multiparous than primiparous cows (0.30 vs. 0.20 mOD/min/ug) respectively; ($P = 0.1$). Citrate synthase activity was lower in multiparous animals than primiparous (0.92 vs.1.3 mOD/min/ug) respectively; ($P = 0.006$). Complex I activity was lower in low producing cows than high producing cows for both primiparous and multiparous groups (0.022 vs. 0.020 mOD/min/ug), (0.028 vs. 0.013 mOD/min/ug), respectively; ($P = 0.04$) and not affected by parity ($P > 0.1$). These findings suggest that complex I enzyme activity may be a marker of ability to produce milk and support previous findings that mitochondrial density (citrate synthase activity) decreases with age in dairy cattle.

Key Words: mitochondria, lactation, PBMC

88 Effects of level of DCAD and duration of feeding on responses to glucose tolerance test and insulin challenge in prepartum dairy cows. A. Vieira-Neto*[1], C. Lopera[1], R. Zimpel[1], F. R. Lopes Jr.[1], P. Molinari[1], B. Faria[1], M. L. Gambarini[1], E. Block[2], W. W. Thatcher[1], C. Nelson[1], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*Church and Dwight Animal Nutrition, Ewing, NJ.*

Objectives were to determine the effects of acidogenic diets (AD) fed for 21 or 42 d on glucose metabolism and tissue insulin responsiveness. Ninety Holstein cows at 230 d of gestation were randomly assigned to receive diets with −70 or −180 mEq/kg DM for 21 (Short) or 42 d (Long). Cows in Short received a diet with +110 mEq/kg from 233 to 254 d of gestation. Therefore, in the first 21 d cows were fed +110, −70, or −180 mEq/kg DM, whereas in the last 21 d of gestation they were fed either −70 or −180 mEq/kg DM. The glucose tolerance tests (GTT) were performed at 250 and 270 d of gestation by infusing 0.25 g of dextrose/kg BW within 2 min. The following day, cows received 0.1 IU of insulin/kg BW intravenously (insulin challenge, IC). Jugular blood was sampled at min −15, −5, and 0 to establish a baseline, and from 5 to 180 min relative to infusions; concentrations of glucose, NEFA, and insulin were determined and area under the curve (AUC) and change in AUC relative to baseline (rAUC) were calculated. Liver was sampled after the GTT and subcutaneous adipose tissue was sampled after the GTT and IC for RT-qPCR. Data were analyzed by ANOVA with mixed models using the Mixed procedure of SAS. At GTT250, the rAUC for glucose increased ($P = 0.04$) in cows fed AD (+110 = 264.5, −70 = 285.5, and −180 = 270.4 ± 6.4 m*M*/min), which is explained by the smaller ($P < 0.04$) rAUC for insulin in cows fed AD (+110 = 1,420, −70 = 1,079, −180 = 852 ± 208 ng/mL/min). At IC250, cows fed AD tended ($P = 0.06$) to have increased NEFA (+110 = 0.24, −70 = 0.30, and −180 = 0.33 ± 0.04 m*M*). At GTT270, cows in Long had greater ($P < 0.05$) rAUC for glucose than cows in Short (Long = 1,201 vs. Short = 1,110 ± 61 m*M*/min), which is explained by the smaller ($P < 0.04$) rAUC for insulin in Long than Short (Long = 969 vs. Short = 1,409 ± 157 ng/mL/min). Hepatic expression of *G6PC*, *PCK1*, *PCK2*, and *PC* did not differ among treatments after GTT250 and GTT270. Treatments did not affect adipose tissue expression of *ATGL*, *ACC*, *B2AR*, *HSL*, and *PLIN1*. Metabolic acidosis induced by AD reduced insulin release in response to a GTT and adipose tissue responsiveness to an IC. Feeding AD for 42 d reduced insulin release in response to GTT.

Key Words: adipose tissue, DCAD, glucose

89 Association of residual feed intake with abundance of ruminal bacteria and biopolymer hydrolyzing enzyme activities during the peripartal period and early lactation in Holstein dairy cows. A. Elolimy*[1], J. Arroyo[1,2], F. Batistel[1], M. Iakiviak[1], and J. Loor[1,3], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Departamento de Nutrición Animal, Instituto de Producción Animal, Facultad de Veterinaria, Universidad de la Republica, San José, Uruguay,* [3]*Division of Nutritional Sciences, Illinois Informatics Institute, University of Illinois, Urbana, IL.*

Residual feed intake (RFI) in dairy cattle typically calculated at peak lactation is a measure of feed efficiency independent of milk production level. The objective of this study was to evaluate differences in ruminal bacteria, biopolymer hydrolyzing enzyme activities, and overall performance between the most- and the least-efficient dairy cows during the peripartal period. Twenty multiparous Holstein dairy cows with ad libitum access to a total mixed ration from d −10 to d +60 relative to the calving date were used. Cows were classified into most-efficient (i.e., with low RFI, n = 10) and least-efficient (i.e., with high RFI, n = 10) based on a linear regression model involving DMI, FCM, changes in BW, and metabolic BW. The most-efficient cows had ~2.6 kg/d lower DMI ($P < 0.05$) at wk 4, 6, 7, and 8 compared with the least-efficient cows. In addition, the most-efficient cows had greater relative abundance of total ruminal bacterial community ($P < 0.05$) during the peripartal period. Compared with the least-efficient cows, the most-efficient cows had 4-fold greater relative abundance of *Succinivibrio dextrinosolvens* ($P < 0.05$) at d −10 and d +10 around parturition and tended ($P < 0.10$) to have greater abundance of *Fibrobacter succinogenes* and *Megasphaera elsdenii*. In contrast, the relative abundance of *Butyrivibrio proteoclasticus* and *Streptococcus bovis* was lower ($P < 0.05$) whereas

Succinimonas amylolytica and *Prevotella bryantii* tended to be lower ($P < 0.10$) in the most-efficient cows around calving. During the peripartal period, the most-efficient cows had lower enzymatic activities of cellulase, amylase, and protease compared with the least-efficient cows. The results suggest that shifts in ruminal bacteria and digestive enzyme activities during the peripartal period could, at least in part, be a mechanism for better feed efficiency in dairy cows.

Key Words: residual feed intake (RFI), bacteria, enzyme

90 Validating a "heat stress" model: The effects of an electric heat blanket and nutritional plane on lactating dairy cows. M. Al-Qaisi*, E. J. Mayorga, E. A. Horst, S. K. Kvidera, A. J. Kramer, C. S. McCarthy, M. A. Abeyta, S. L. Potner, B. M. Goetz, H. A. Ramirez Ramirez, J. A. D. R. N. Appuhamy, L. L. Timms, and L. H. Baumgard, *Department of Animal Science, Iowa State University, Ames, IA.*

Study objectives were to determine whether electric heat blanket (EHB)-induced hyperthermia affects body temperature indices and production parameters similar to those generated in climate-controlled chambers. Multiparous Holstein cows (27) were subjected to 2 experimental periods (P): During P1 (4 d), cows were in thermoneutral conditions with ad libitum feed intake. During P2 (4 d), cows were assigned to 1 of 2 treatments: (1) thermoneutral conditions and pair-fed (PF; n = 8), (2) heat stress induced artificially by an EHB (Thermotex Therapy Systems Ltd. Calgary, Canada), with ad libitum feed intake (n = 19). Overall, the EHB markedly increased rectal temperature, vaginal temperature, skin temperature, respiration rate, and heart rate (1.3°C, 1.4°C, 1.1°C, 2-fold, and 15 bpm, respectively; $P < 0.01$) relative to PF cows. The EHB reduced DMI by 45% and by design PF cows had similar pattern of decreased DMI. Milk yield decreased in EHB and PF cows by 22.4% (9.8 kg) and 10.4% (4.2 kg), respectively ($P = 0.03$), indicating that reduced DMI accounted for only ~50% of decreased milk yield. Relative to PF cows, MUN increased (20.4%; $P < 0.01$) in EHB cows; however, no other differences were observed in milk composition between treatments. The EHB decreased blood total CO_2, partial CO_2, HCO_3, and base excess levels (15, 13, 15, and 78%, respectively; $P < 0.01$) when compared with PF group. The EHB increased hematocrit and hemoglobin (both indicators of dehydration) by (9%; $P < 0.01$) compared with PF cows. A treatment by day interaction was detected for plasma NEFA levels; as they progressively increased in PF controls but remained unchanged in EHB cows ($P < 0.01$). BUN and BHBA increased in EHB cows (31 and 83%, respectively; $P < 0.01$) relative to PF controls. No differences in circulating glucose were observed; however, insulin levels tended to increase (74%; $P = 0.07$) in the EHB cows compared with PF controls. In summary, employing the EHB increased body temperature indices, reduced productivity, and altered metabolism similar to climate controlled chamber studies.

Key Words: heat stress, insulin

91 Dry period heat stress reduces dam, daughter, and granddaughter productivity. J. Laporta*, F. C. Ferreira, B. Dado-Senn, A. De Vries, and G. E. Dahl, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Dry period heat stress impairs dairy cows' subsequent lactation performance. We hypothesize that exposure of late-gestation dams to dry period heat stress also exerts transgenerational effects on their daughters' and granddaughters' productivity. We tested this hypothesis using lactation records from previous experiments conducted during 9 summers at the University of Florida (2007–2012, 2014, 2016, and 2017, THI >68 for all years). A total of 154 multiparous dams were provided active cooling ($CL_{F0,}$ shade, fans and soakers, n = 75) or shade only ($HT_{F0,}$ n = 79) during a 46 d dry period. Milk yield, fat and protein records (Afimilk Ltd.) of the dams, daughters (CL_{F1} n = 64, HT_{F1} n = 44) and granddaughters (CL_{F2} n = 24, HT_{F2} n = 13) of those dams were used to estimate ECM from calving to 35 wk in milk (WIM) for 2 consecutive lactations. Data were analyzed using mix models in SAS with WIM as repeated measure and dam within treatment as random effect. Dry HT_{F0} dams produced 2.3 kg less milk/d in their subsequent lactation than dry CL_{F0} dams [ECM: 34.2 vs. 36.5 ± 0.07 kg, respectively; $P < 0.01$]. Daughters born to HT_{F0} dams, who experienced heat conditions through the intrauterine environment, produced 3.7 kg less milk/d during their first lactation [ECM: 28.6 vs.. 32.3 ± 0.08 kg for HT_{F1} and CL_{F1}, respectively; $P < 0.01$], and 3.4 kg/d less during their second lactation [ECM: 33.2 vs.. 36.6 ± 0.13 kg, WIM by treatment interaction; $P < 0.01$], compared with those born to CL_{F0} dams. Similarly, the granddaughters of HT_{F0} dams, born to HT_{F1}, produced 3.7 kg less milk/d during their first lactation [ECM: 28.5 vs.. 32.2 ± 0.13 kg for HT_{F2} and CL_{F2}, respectively; $P < 0.01$] and 6.5 kg/d less in their second lactation [ECM: 34.0 vs.. 40.5 ± 0.21 kg, WIM by treatment interaction, $P < 0.01$] compared with granddaughters of CL_{F0} dams born to CL_{F1}. Dry period heat stress not only impairs dam's subsequent lactation but also has carryover effects on the productivity of daughters and granddaughters 2 generations later. Dry-period cooling is crucial to rescue milk loss of the dam, but also to prevent milk losses of their daughters and the granddaughters.

Key Words: milk, environment, cooling

92 Relationship between environments, vaginal temperature, and behaviors of lactating cows on pasture. T. N. Marins*, R. M. Orellana, J. K. Bernard, and S. Tao, *University of Georgia, Tifton, GA.*

Understanding environmental impacts on physiology and behavior is critical to improve animal welfare and performance. Our objective was to identify correlations between environments, vaginal temperature (VT) and behaviors of grazing lactating dairy cows on pasture during summer. Lactating cows (n = 119, 29–30/farm) from 4 farms were randomly enrolled. Each farm had different milking schedules and cooling facilities in holding pens and feedlots. Pivots equipped for evaporative cooling were provided during day but not night on all farms. The VT was recorded every 5 min. Times of lying and standing were recorded every 15 min using Smarttag Leg (Nedap) for 6 consecutive days. Meteorological variables were measured on pasture, and temperature humidity index (THI) calculated. Data were averaged or summed every 30 min and only the data collected between milkings (on pasture during day and night, Table 1) were used for correlation analyses by PROC GLM procedure of SAS 9.4. At day, VT had strongest correlation with dry bulb temperature (= 36.79+0.08 × dry bulb temperature, $r^2 = 0.254$, $P < 0.001$), but lying time (= 28.21–0.58 × black globe temperature, $r^2 = 0.172$, $P < 0.001$) and standing time (= −0.88+0.58 × black globe temperature, $r^2 = 0.185$, $P < 0.001$) had strongest correlations with black globe temperature. At night, VT had strongest correlation (= 35.50+0.05 × THI, $r^2 = 0.041$, $P < 0.001$) with THI, but lying time (= 80.23–2.62 × dry bulb temperature, $r^2 = 0.168$, $P < 0.001$) and standing time (= −38.44+1.99 × dry bulb temperature, $r^2 = 0.12$, $P < 0.001$) had strongest correlations with dry bulb temperature. In conclusion, at day under pivots, VT had the strongest correlation with dry bulb temperature, but black globe temperature explained more of the variation of lying and standing times. At night, THI is a better variable to predict VT, and lying and standing times had strongest correlations with dry bulb temperature.

Table 1 (Abstr. 92). Mean or sum of environmental and behavioral variables every 30 min on pasture

Variable	Day	Night
Dry bulb temperature, °C	29.4	24.1
Relative humidity, %	74.0	93.9
Black globe temperature, °C	37.4	23.7
THI	80.7	74.7
VT, °C	39.3	39.5
Lying time, min	6.4	17.0
Standing time, min	20.7	9.5

Key Words: vaginal temperature, behavior, grazing

93 Differential gene expression in the rumen epithelium of heat stressed Holstein heifers. A. Bedford*[1], L. Beckett[1], T. T. Yohe[1], L. Harthan[1], C. Wang[1], N. Jiang[1], H. Schramm[2], M. Hanigan[1], K. M. Daniels[1], and R. R. White[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA.*

Heat stressed cattle have depressed feed intake. However, performance reductions observed during heat stress are greater than could be expected from depressed intake alone. This disparity suggests that digestive, absorptive, or post-absorptive shifts in physiology may also influence the degree to which animals respond to heat stress. Our objective was to investigate the effect of heat stress on the expression of genes in the rumen epithelium associated with the transport and metabolism of volatile fatty acids (VFA), testing the hypothesis that absorptive function of the epithelium is impaired by heat stress. Eight ruminally cannulated Holstein heifers (200 kg) were used in a factorial, repeated measures experiment with 2 treatments and 2 periods. During the first period, animals were provided feed ad libitum and housed at 20°C. During the second period, one group (HS) was housed at 30°C and fed ad libitum. The other group (PF) was housed at 20°C and pair-fed to match the intake of heifers in the HS group. During each period, animals were exposed to the heat or intake treatment for 9 d and the 10th d of each period was used for papillae biopsy sample collection. During the second period, indicators of heat stress including feed intake, respiration rate, heart rate, and body temperature were significantly different between PF and HS animals ($P < 0.05$). There was a significant feed intake effect on BHD1 expression ($P = 0.04$), and a tendency for a heat stress effect ($P = 0.08$), with expression increasing in both cases. This data indicates increased β-hydroxybutyrate production. Differential expression of epithelial transporters responsible for VFA uptake into the epithelium (MCT4 and NH3), and transport into the blood (NHE1 and NHE2) was observed. Expression of MCT4 was significantly affected by feed intake ($P = 0.003$) as were all NHE genes (NHE1, NHE2, and NHE3; $P < 0.05$). These results indicate that both heat stress and feed intake can affect gene expression in the rumen epithelium. With low feed intake, the epithelium appears to increase capacity for VFA absorption and transport. However, during heat stress, it appears that butyrate metabolism and transport capacity is decreased.

Key Words: heat stress, gene expression, rumen epithelium

94 Relationship between the accumulative effects of heat stress and Holstein dairy cows' milk performances in eastern Canada. V. Ouellet*[1], V. E. Cabrera[2], L. Fadul-Pacheco[3,4], P. Grenier[5], and É. Charbonneau[1], [1]*Département des sciences animales, Université Laval, Québec, QC, Canada,* [2]*Department of dairy science, University of Wisconsin, Madison, WI,* [3]*Valacta, Saint-Anne-de-Bellevue, QC, Canada,* [4]*Department of Animal Science, McGill University, Saint-Anne-de-Bellevue, QC, Canada,* [5]*Consortium Ouranos, Montréal, QC, Canada.*

Heat stress (HS) is known to cause a strain on performances in dairy cows that experience high temperatures and humidity for long periods throughout the year. However, research on its consequences on dairy cows' production performances in colder areas of the globe is limited. Our objective was to examine the relation between the accumulative effects of HS and daily milk, fat, and protein yields (kg/d), and milk composition (%) in Holstein dairy cows located in eastern Canada. The initial data set used in this study comprised 606,031 test-day (TD) records from 16,446 Holstein dairy cows of different lactations (2.7 ± 1.7). Weather data (2010–2015) were retrieved from the closest meteorological stations. Daily maximum temperature-humidity index (THI_{max}) was calculated using maximum ambient daily temperature and average relative humidity and merged with corresponding TD. This was also done for the 7 d before TD. The accumulative effects of HS were evaluated by calculating the number of consecutive d with a $THI_{max} > 65$. Number of consecutive days were then grouped in categories: 0 = 0 d with HS; 1 = 1 or 2 d; 2 = 3 or 4 d; 3 = 5 or 6 d; 4 = 7 or 8 d with HS. Effects of the categories on TD milk production and composition were assessed using the HPMIXED procedure of SAS. Fixed effects included in the model were DIM, exp DIM (calculated as $e^{-0.05DIM}$), genetic value, and categories of consecutive d with HS. Random effects were year(herd), calving month × year(herd), the calving month × year(herd(cow)). Cows subjected to more consecutive days with HS had lower ($P < 0.05$) ECM, fat, and protein yields, and fat, and protein concentration in milk compared with cows subjected to conditions not susceptible to induce HS. The cows most impacted by HS were of third lactation and higher, with declines of 4.1 and 2.9% of fat and protein yields and 6.5 and 4.0% of fat, and protein concentration in milk between category 0 and 4. Establishing actual effects of HS on dairy cows' milk performances in cooler climates may help producers to better adapt to climate change and to develop appropriate management countermeasures.

Key Words: dairy cow, temperature-humidity index, heat stress

95 Effects of fully acidified close-up diets and dietary calcium content on production and milk composition of transition dairy cows. K. M. Glosson*[1], X. Zhang[2], S. S. Bascom[3], A. D. Rowson[3], and J. K. Drackley[1], [1]*University of Illinois, Department of Animal Sciences, Urbana, IL,* [2]*Institute of Animal Nutrition, Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Sichuan Agricultural University, , Chengdu, Sichuan, China,* [3]*Phibro Animal Health Corp., Teaneck, NJ.*

Dry cow dietary strategies of decreasing dietary Ca and using acidogenic diets with a negative dietary cation-anion difference (DCAD) vary in effectiveness to reduce subclinical hypocalcemia (SCH). The objective of this study was to determine the effects of 3 dietary strategies for close-up dry cows on dry matter intake (DMI) and production in multiparous Holstein cows (n = 81). Cows were assigned to 1 of 3 treatments during the close-up dry period (−28 d to calving): (1) a positive DCAD diet with low dietary Ca (0.4% DM; CON); (2) a fully acidified DCAD diet (urine pH 5.7) with low dietary Ca (0.4% DM; LOW); or (3) a fully acidified DCAD diet (urine pH 5.7) with high dietary Ca (2.0% DM; HIGH). The DCAD was decreased by addition of a commercial anionic product (Animate; Phibro Animal Health) and Ca was increased by adding $CaCO_3$. All cows were fed CON during the far-off dry cow period (−50 to −28 d) and then all groups were fed the same lactation diet postpartum. Cows were milked 3x/d and production was summarized by week. Intake was recorded daily and DMI was calculated

weekly. Milk composition (3 consecutive milkings) was determined once weekly during the fresh period. The MIXED procedure in SAS was used to contrast: 1) CON vs the average of LOW and HIGH; and 2) LOW vs HIGH. Cows receiving CON had greater close-up DMI ($P < 0.01$). After calving, cows fed LOW or HIGH had greater DMI (18.9 and 19.5 kg/d) and greater milk yield (44.6 and 45.2 kg/d) when compared with cows fed CON (17.0, $P < 0.01$ and 42.0 kg/d, $P < 0.05$, respectively). Cows given CON had a greater milk fat percentage ($P < 0.01$), but protein (%) and component production (kg/d) did not differ among treatments ($P > 0.10$). Milk fat and protein were used to calculate the fat to protein ratio (FPR), which indicates subclinical ketosis (SCK) when above 1.4. Cows given CON had a higher FPR (1.57) than those given LOW or HIGH (1.36 and 1.28), while cows given LOW or HIGH had greater postpartum DMI and milk yield. Prepartum dietary Ca did not affect these variables in cows fed a negative DCAD close-up diet.

Key Words: fully acidified close up diet, production, calcium

96 Determining the change in body weight per unit of body condition score in Holstein cows. R. A. de Souza* and M. Vande-Haar, *Michigan State University, East Lansing, MI.*

Our goal was to quantify the change in BW (ΔBW) per change in body condition score (ΔBCS). Our database had 69 studies of 2,181 Holstein cows (75 to 263 DIM) with weekly records of BW, BCS, and DMI. The average interval between consecutive BCS for each animal was 38 d. Means and standard deviations were 644 ± 83 kg BW, 3.1 ± 0.4 BCS, and 25 ± 4 kg DMI with 12 ± 22 ΔBW, 0.07 ± 0.18 ΔBCS, and 0.0 ± 2.6 ΔDMI for the period. Using HPMIXED from SAS 9.4, a mixed regression with random effects of animal, study, and location and fixed effects (as described next) was fitted to determine the ΔBW per ΔBCS. To determine the best model to predict ΔBW, we developed 4 candidate models with 2 levels of complexity for fixed effects with and without effects of parity. A simple model contained only linear effects of initial BCS, initial BW, ΔDMI, and ΔBCS, whereas a complex model also included 2-way interactions of all main effects and quadratic and cubic polynomials. Cross-validation across study was used to select the best fitting model based on concordance correlation coefficient (CCC), root mean square error of prediction (RMSEP). Parity was highly significant but had little impact on CCC and RMSEP. Model complexity also had little impact on CCC but the simple models had lowest RMSEP. The interaction of ΔBCS with initial BCS was not significant ($P = 0.8$), so we conclude that ΔBW per ΔBCS does not depend on starting BCS. Our proposed model is $\Delta BW = -17 + 0.042 \times BW + 2.7 \times \Delta DMI + 54 \times \Delta BCS + PARITY_i$ (CCC = 0.77, RMSEP = 20), where $PARITY_i$ of 1, 2, and 3 or more, adds 0, −3.6, and −5.9, respectively. With this equation, a 1-unit ΔBCS is associated with ΔBW of 54 kg, which equals 8.4% of live BW or 9% of empty BW (assuming gut fill is 5.2 x DMI). Using the same statistical approach for early lactation (<75 d-in-milk), ΔBW is 64 kg per 1-unit ΔBCS, or 10% of live BW. This is substantially less than the value used in the NRC 2001 of 14%. These data should be limited to the typical ΔBCS of most cows, which range from BCS 2.0 to 4.0.

Key Words: body condition score, body weight, model

97 Estrus detection with an activity and rumination monitoring system in an organic grazing and in a low-input conventional herd. G. M. Pereira*, B. J. Heins, M. Endres, and K. Minegishi, *University of Minnesota, St. Paul, MN.*

Estrus detection using an activity and rumination system (ARS) was evaluated in an organic grazing and in a low-input conventional seasonally calving herd. Estrus prediction models were created using raw data provided by the ARS. The study was conducted from June 2014 to August 2017 at the University of Minnesota West Central Research and Outreach Center, Morris, MN. All cows were fitted with an ARS tag (HR-LD tags; SCR Engineers Ltd., Netanya, Israel) at calving, and the tag was removed at dry off. Cows calved in the spring and autumn and were bred in the summer and winter. During each breeding season (4 summer and 3 winter breeding seasons), activity and rumination (daily and 2-h periods) were monitored electronically using the ARS tags. Activity was reported in activity units, and rumination was reported in min per 2-h block and min per d from SCR DataFlow II software. Estrus alerts of individual cows provided by the SCR DataFlow II software were used to determine if the alert coincided with the breeding date of a cow. The gold standard for this study were breeding dates of cows that were determined by breeder evaluation of an Estrotect patch placed on the rump of a cow. The study included 1,463 breeding dates across the 4-yr period. The ARS had a sensitivity of 56.7%, a specificity of 99.3% and a positive predictive value of 59.8% for the organic herd, and a sensitivity of 70.1%, a specificity of 99.2% and a positive predictive value of 66.3% for the low-input herd across breeding seasons. Higher sensitivity indicates that estrus alerts provided by the ARS coincide with true estrus events, defined by the current gold standard. Custom models illustrated the potential tradeoffs that can be achieved with ARS raw data, and the models had a sensitivity from 73.0% to 84.2% and a specificity from 94.3% to 97.7%. Adjusting the threshold of estrus detection may provide producers more control of ARS generated estrus alerts depending on the breeding season. The ARS evaluated in this study showed potential for estrus detection in grazing and low-input dairy herds.

Key Words: automated estrus detection, grazing, low-input dairy

98 Evaluation of G7G-Ovsynch protocol with or without heat detection and milk pregnancy-associated glycoproteins as non-invasive pregnancy diagnosis method. A. H. Shahzad*[1], A. Abbas[2], R. S. Baloch[2], I. Khan[1], and S. Abbas[1], [1]*University of Veterinary and Animal Sciences, Lahore, Lahore, Punjab, Pakistan,* [2]*Livestock and Dairy Development, Punjab, Lahore, Punjab, Pakistan.*

Important components of reproductive efficiency are, in dairy industry, fertility and early pregnancy diagnosis on global level. The objectives of the present study were to appraise the G7G-Ovsynch protocol in postpartum Holstein cattle with or without Estrotect heat detector and to evaluate ELISA-based milk pregnancy-associated glycoproteins (PAG), optical density (OD), as a non-invasive method of pregnancy diagnosis (PD). A total of 178 multiparous postpartum cows were divided either in (1) G7G-Ovsynch-FTAI (n = 88) or (2) G7G-Ovsynch-AIDE (n = 90). Pregnancy was observed on d 30, PAI via ultrasonography (US), P4 and PAGs while on d 60, 90 with US. Pregnancy rate (PR) was compared by chi-squared. Milk PAGs and plasma P4 conc. were analyzed using REG procedures of SAS ($P < 0.05$). The PR on all 3 timelines was 49% (43/88) versus 55.60% (50/90); ($P = 0.30$) on d30, 44% (39/88) versus 49% (44/90); ($P = 0.54$) on d60 and 43% (38/88) versus 47% (42/90); ($P = 0.70$) on d90 PAI in G7G-Ovsynch-FTAI and G7G-Ovsynch-AIDE groups respectively. Pregnancy loss (PL) was 9% (4/43), 3% (1/38) in G7G-Ovsynch-FTAI versus 12% (6/50) and 5% (2/44) on d 60 ($P = 0.70$) and 90 ($P = 0.66$) in G7G-Ovsynch-AIDE group respectively. Overall PL on d90 PAI was 12 versus 16% in G7G-Ovsynch-FTAI and G7G-Ovsynch-AIDE groups respectively. The OD of milk PAGs (n = 16) was, mean ± SEM, 0.75 ± 0.08 on d30 PAI. Duplicate aliquot of milk samples that were stored at 4°C, resulted in slight reduction of OD (0.55 ± 0.057) after 60 d of storage period. P4 profile (ng/mL; mean ± SEM) was measured (n = 16) by RIA and was 6.0 ± 0.31 on d30, 6.68

± 0.42 on d60 and 7.11 ± 0.39 on d90 PAI. Sensitivity (Se), Specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of milk PAG-based test in present study was 100% in comparison with US (gold standard) on d30 PAI. Similarly, Se, Sp, PPV and NPV of plasma based P4 test in present study was 100% (92.6–100), 94% (84–99), 94% (84–98) and 100% respectively. In conclusion, use of Estrotect has resulted in increased PR, although non-significant, and milk PAG test is another viable PD method on d30 PAI, even with a lapse of 60 d storage.

Key Words: postpartum Holstein cow, G7G-Ovsynch, milk pregnancy-associated glycoproteins (PAG)

99 Factors associated with low colostrum yield in Jersey cattle. K. Gavin[1], H. Neibergs[1], A. Hoffman[2], J. Kiser[1], M. Cornmesser[1], S. A. Haredasht[3], B. Martinex-Lopez[3], J. Wenz[1], and D. Moore*[1], [1]*Washington State University, Pullman, WA,* [2]*Sunrise Veterinary Service, Dalhart, TX,* [3]*University of California, Davis, CA.*

Some US dairy herds have reported seasonal low to no colostrum production. However, little information regarding this problem exists. The purpose of this study was to describe the phenomenon and identify potential risk factors. A 2,500-cow commercial Jersey dairy farm was enrolled. Cow information on parity, previous lactation length, previous 305ME milk production, and dry period length were collected from the farm's dairy management software. First-milking colostrum weight and calf information were recorded just after calving. Over a year of enrollment, 1,845 cows and 1,143 heifers (38%) calved with colostrum weights recorded. Average colostrum yield differed across the year; 6.6 ± 3.2 kg per cow in June 2016, 2.5 ± 2.4 kg in December 2016, and 4.8 ± 3.2 kg in May 2017 ($P < 0.01$). Multiparous cows had a larger decline in colostrum production June to December (6.6 ± 3.2 kg to 1.3 ± 1.6 kg) compared with primiparous animals (6.5 ± 3.2 to 4.2 ± 2.3 kg per cow) ($P < 0.001$). Average colostrum production decreased by 0.17 kg per cow per week during this time, 0.22 kg for multiparous and 0.08 kg for primiparous cows ($P < 0.01$). A multivariate logistic regression model was constructed for multiparous cows only for the probability of low colostrum yield (<2.7 kg at first milking). Dry period length, calf sex, singleton or twin, age at freshening, month of calving and previous lactation length were associated with low colostrum yield ($P < 0.05$). Pedigree analysis for cows calving October 23, 2016 through January 21, 2017, showed that extremes of colostrum production followed some sire lines: low (<0.45 kg; n = 102) and high (>5.44 kg; n = 112) yield. Using a single-factor ANOVA, colostrum weight for offspring were different between sire lines ($P = 0.0002$). A cross-correlation function analysis between the time series for colostrum yield and photoperiod revealed a high correlation (0.91 ± 0.13) at the time of calving and one month prior (0.84 ± 0.14) for multiparous cows, higher correlations than for primiparous cows (0.53 ± 0.13 and 0.48 ± 0.14, respectively). These data indicate that photoperiod, in some cow families, may be involved with seasonal low colostrum production in Jersey cows.

Key Words: colostrum production, photoperiod, pedigree analysis

100 Improving nutritional accuracy and economics through multiple ration-grouping strategy. J. A. Barrientos Blanco*, V. Cabrera, and R. D. Shaver, *University of Wisconsin Madison, Madison, WI.*

Study objective was to evaluate implementation of nutritional grouping strategy (NGS) to improve nutritional accuracy and reduce feed costs for lactating cows 148 ± 65 DIM. A 2,500-cow Wisconsin commercial dairy farm was used in the study. Three months (June–August 2017) of data, including individual cow reproductive and productive performances, were used to simulate NGS. Three pens (450 cows total) of multiparous cows currently grouped in 3 pens indiscriminantly, were regrouped using k-mean algorithm based on their net energy (NE) and metabolizable protein (MP) requirements. An equation was developed to calculate a diet accuracy index (DAI = |[Diet nutrient/PENs DMI] – [nutrient requirement/required DMI]|), utilized to assess and compare nutritional accuracy of feeding cows clustered by NGS to the farm grouping strategy (FGS). The lower the DAI value, the more accurate the diet. Minimum least squares algorithm (L2 - norm) was used to calculate the most representative NE and MP requirements per pen from NGS. These NE and MP requirement values where utilized to formulate diets using the same recipe and equal or lower cost than the current farm diet. Supplied NE, MP by the diets, DAI and cost per pen at the farm, were compared with the simulated pens using NGS. Results from the simulation suggest that NGS increases diet accuracy for supply of NE and MP per pen (see Table 1). DAI values were lower for NGS in both NE and MP. Total cost of 3 mo of feeding lactating cows using FGS was $51,894, whereas it was $43,041 for NGS; $8,943 diet cost savings. Implementing NGS in groups of lactating cows has potential to increase nutritional accuracy of diets and savings through decreased diet costs.

Key Words: feeding costs, diet accuracy, nutritional grouping

Table 1 (Abstr. 100). Diet accuracy index (DAI) of diet NE and MP supply for FGS vs. NGS

	PGS			NGS			FGS-NGS
Nutrient	Pen 1	Pen 2	Pen 3	Pen 1	Pen 2	Pen 3	All pens
DAI NE (Mcal/kg)	0.246±0.424	0.086±0.667	0.081±0.602	0.031±0.043	0.028±0.020	0.032±0.026	0.107
DAI MP (g/kg)	9.693±7.238	8.846±6.270	9.058±5.827	3.299±4.272	2.934±2.541	3.812±3.040	5.861

Reproduction: Joint ADSA-SSR Symposium: The Immune–Reproduction Nexus—The Good, the Bad, and the Ugly

101 The ugliness at the evolutionary root of mammalian pregnancy. G. Wagner*, *Yale University, New Haven, CT.*

While viviparity has evolved many times in vertebrates and even several insects and velvet worms, the fetal maternal relationship in eutherian mammals (placental mammals) stands out as unique. To my knowledge, there is no other animal group where the fetus is affecting the maternal tissue to such an extent as in eutherian mammals. The situation, however, is complicated by the fact that eutherians present a variety of fetal-maternal relationships, from highly invasive hemochorial placentation to largely "non-invasive" epitherliochorial placenation in what colloquially is called "farm animals" and their relatives. A key insight from modern molecular phylogenies is that the most recent common ancestor of placental mammals had an invasive placentation and that the less invasive placentation present in farm animals is a secondary development. The key problem then is to understand how the evolution of invasive placentation and extended gestation in the stem lineage of eutherian mammals was achieved at a mechanistic level. Based on our research on the gray short tailed opossum, *Monodelphis domestica*, I will present evidence supporting the following scenario: the therian ancestor; that is, the most recent common ancestor of marsupials and eutherians, had a situation similar to that as it exists in the opossum, where the embryo remains separated from the uterine tissue by a shell coat for most of gestation, and once hatched, it remains attached to the uterus only for a very short time. A consequence of fetal attachment is an acute inflammatory reaction on the part of the mother, which directly leads to parturition. The key innovation, necessary to evolve the extended gestation of eutherians, in spite of the invasive behavior of the conceptus, is a mechanism for controlling the damaging consequences of inflammation, in particular neutrophil invasion. This was achieved by 2 innovations, the evolutionary origin of the decidual cell, which helps control the inflammatory process and the dependency of the decidual cell on progesterone. I will discuss the implications of this model for understanding and managing pregnancy complications in eutherian mammals.

Key Words: mammalian pregnancy, implantation, inflammation

102 Immune recognition of the periattachment conceptus. T. L. Ott*, *Pennsylvania State University, University Park, PA.*

Infertility and subfertility reduce the economic viability of dairy production. Early pregnancy involves transient secretion of a unique type I interferon, interferon tau (IFNT). Our working hypothesis is that pregnancy induces changes in immune cells that are biased toward tolerance, tissue remodeling and angiogenesis. Early pregnancy is accompanied by a marked increase in the proportion of endometrial CD45+ cells expressing markers for natural killer (NK) cells and cytotoxic T cells (CD8) and an increase in MHCII+ cells, particularly around shallow glands of the endometrium of pregnant heifers at Day 20. This is accompanied by increased abundance of mRNA for interleukin (IL)-15, an NK growth factor, and IL-10, a tolerogenic cytokine, in the endometrium during early pregnancy. Furthermore, expression of indoleamine 2,3 dioxygenase (IDO) was 15-fold greater in pregnant compared with cyclic heifers at Day 17, but then declined by Day 20 of pregnancy to amounts similar to Day 17 cyclic heifers. IDO converts tryptophan to kynurenine, which is thought to alter immune function by activation of the aryl hydrocarbon receptor (AHR) and induction of tolerogenic mediators. Interestingly, AHR protein expression is greater in the endometrium during this same period. Pregnancy is also associated with increased expression of inhibitory proteins programed death ligand-1, lymphocyte activation gene-3 and cytotoxic T-lymphocyte associated protein-4. These molecules interact with receptors on antigen-presenting cells and induce lymphocyte tolerance. Expression of IDO, IL10 and other immune cell regulators were greater in pregnant compared with cyclic heifers but then decreased between Day 17 and 20 of pregnancy. We interpret this to indicate that early pregnancy signaling in dairy heifers involves immune cell activation including increased lymphoid and myeloid lineage cells as well as induction of molecules known to mediate tolerance. Early pregnancy is accompanied by induction and then repression of mediators of immune function, which may serve as a developmental switch to promote immune privileged niche and tissue remodeling in the endometrium. *Supported in part by Agriculture and Food Research Initiative Competitive Grant 2017-67015-26455 from the USDA National Institute of Food and Agriculture.*

Key Words: uterus, immune, pregnancy

103 Mechanisms for disruption of fertility by infectious diseases of the reproductive tract. R. Gilbert*, *Ross University School of Veterinary Medicine, Basseterre, St. Kitts and Nevis.*

Reproductive tract infections are common and impede reproduction by reducing per cycle fertilization, increasing pregnancy loss, and impairing neonatal survival. They therefore increase risk of culling and are expensive. Infection may interfere with reproduction by directly causing anatomical damage that prevents normal reproduction. Most effects are mediated by components or products of infectious organisms (such as lipopolysaccharide - LPS, endotoxin), or indirectly by inflammatory mediators, such as cytokines, eicosanoids, nitric oxide and oxidative stress affecting gamete production and function, follicular function, ovulation, fertilization, embryonic development, implantation, or survival of the conceptus. For example, bacteria and leukocytes in semen damage sperm cell membranes, mitochondrial function, sperm motility and even DNA integrity. Bacterial infection impairs ovarian follicular function, both directly and by effects on the hypothalamo-pituitary-gonadal axis, prolonging anovulation and leading to decreased concentrations of steroid hormones. Inflammation affects microtubule assembly and chromosomal structure in oocytes, preventing fertilization. Zygotes resulting from fertilization of oocytes with sperm subjected to oxidative stress are less likely to develop to the blastocyst stage. Inflammation affects endometrial adhesion molecule expression, resulting in implantation failure and inflammation may result in pregnancy failure. Extragenital inflammation (e.g., mastitis) may also impair reproductive functions and outcomes. These observations make it clear that direct and indirect effects of infection and inflammation, even in locations remote from the reproductive tract, can interrupt successful reproduction at several crucial stages.

Key Words: infection, inflammation, reproduction

104 A role for seminal fluid in promoting optimal pregnancy outcomes. J. J. Bromfield*, *University of Florida, Gainesville, FL.*

Seminal plasma is the cell-free, fluid fraction of the ejaculate which is significantly diluted during semen preparation for use in artificial insemination (AI) programs. Seminal plasma contains several protein complexes that stimulate strong changes within the lining of the uterus. In dairy cows, the infusion of seminal plasma at the time of AI has been shown to increase pregnancy rates by 4.6%. While these studies were underpowered, the studies reveal that infusion of seminal plasma at AI improved pregnancy outcomes in herds with poor pregnancy rates. The supplementation of seminal plasma at insemination in other species, including mice, horse, swine, and humans improves pregnancy outcomes. In rodents, we have observed significant seminal plasma induced changes in expression of genes which promote early embryo development, modulate endometrial tissue remodeling and immune adaptation required for pregnancy success. One seminal plasma protein that facilitates positive pregnancy changes in the uterus of other species is transforming growth factor-β. The role of specific seminal plasma proteins in modulating the maternal environment is currently lacking in the cow. Our work has begun to unravel the importance of seminal plasma in modulating the endometrial environment of the cow. The studies will help lead to the development of new AI protocols to improve the fertility of lactating dairy cows and aid in the use of sexed semen, where fertility is further compromised. *This work is supported by Select Sires and the Southeast Milk checkoff.*

Key Words: seminal plasma, endometrium, embryo development

105 Saturated fat supplemented in the form of triglycerides decreased digestibility and reduced performance of dairy cows as compared to calcium salt of fatty acids. A. Oyebade*[1,2], L. Lifshitz[1], H. Lehrer[1], S. Jacoby[1], Y. Portnick[1], and U. Moallem[1], [1]*Department of Ruminant Science, ARO, Volcani Center, Rishon LeZion, Israel,* [2]*Department of Animal Science, University of Jerusalem, Rehovot, Israel.*

Several forms of protected fats are common in dairy cow rations. The objectives of this study were to determine the effects of supplementation of saturated fatty acid (SFA; 70–80% C16 and 5–10% C18) in the form of triglycerides (TG), as compared with calcium salt of free fatty acids (CSFA; contained 44% C16, 40% C18:1 and 9.5% C18:2), on milk and milk solids yields, efficiency and digestibility. Twenty-eight multiparous cows were fed a basal diet supplemented either with (on DM basis): (i) SFA - 1.2% SFA (~350 g/cow/d), or (ii) CSFA - 1.4% CSFA (~440 g/cow/d). The supplements were balanced according to fat content (SFA – 99% fat vs. CSFA – 84% fat). Data were analyzed using the PROC MIXED model of SAS. Milk yield of the SFA cows was 3% lower (46.2 vs. 47.6 kg/d; $P < 0.001$), and fat corrected milk (4%) was 4.5% lower than in CSFA cows. No difference in milk-fat content was observed, but milk-protein content was higher in the SFA cows. No differences in DMI or efficiency calculations between groups were observed. The ruminal pH and ammonia concentrations were similar between groups, while acetate concentrations and acetate:propionate ratio were greater for the CSFA cows. The apparent total track digestibility of all dietary components was lower in the SFA than CSFA cows: DM - 55.8% and 62.0% ($P < 0.001$); fat - 58.1% and 70.5% ($P < 0.001$); NDF - 43.0% and 47.8% ($P < 0.02$), respectively. SFA did not depress milk protein, which would seem an important advantage of the SFA over CSFA. Because supplements in the diet were balanced for their fat content, the lower yields observed in the SFA cows could be due to probable overestimation of the energy value of the SFA supplement>, which resulted in lower inclusion level of fat (and energy) in the SFA than CSFA diet. The lower digestibility of all dietary components as observed for the SFA, is probably due to the effects of the degree of saturation and the form of the SFA (highly saturated TG). This leads to the conclusion that although the SFA supplement in the form of TG contains 99% fat comparing to 84% in the CSFA, the net energy for lactation value for SFA might be similar or lower than that of CSFA, mainly because of lower digestibility.

Key Words: calcium salt of free fatty acids (CSFA), saturated fat

106 Effect of supplementation of pasture-based diet on ω-3 and ω-6 fatty acid profile of sheep milk. A. Cabiddu*, A. Margherita, M. Decandia, and G. Molle, *Agris, Loc. Bonassai, Olmedo, Sassari Italy.*

The aim of this study was to evaluate the effect of supplements on ω-3 and ω-6 (omega-3 and omega-6) fatty acid profile of grazing ewes. Forty-eight dairy sheep (DIM 88 + 10, BCS 2.42 + 0.24, BW 42.9 + 4.73 kg, and milk yield 1624 + 235 mL) were randomly assigned to the following dietary groups: only pasture (PAS), nonfat enriched supplementation (NFS, sheep, supplemented with 900 g of cereal based concentrate with 3.01% EE DM basis), moderate linolenic acid (ALA) supplementation (C183M, sheep supplemented with 900 g of linseed based concentrate with 6.01% EE DM basis) and high ALA supplementation (C183H, supplemented with 900 g of linseed based concentrate with 10.35% EE DM basis). All sheep grazed an Italian ryegrass paddock with an allocation of 22 h/d (PAS) or 3 h/d (all supplemented groups). Fatty acid methyl esters from milk fat were measured on one occasion using base-catalyzed methanolysis. ANCOVA model with orthogonal contrast was used to test the effect of dietary treatment (Treat). Contrasts were performed to test the effect of supplementation level (SUPP), FAT enrichment and ALA level in the fat enriched concentrate (ALAL) on ω-3 and ω-6 milk FA profile. Overall treatment affected total ω-3, EPA, DHA, and total ω-6 content ($P < 0.05$). Pasture supplementation affected ($P < 0.05$) total ω-3 and LNA content without effects on EPA and DHA

Table 1 (Abstr. 106). Adjusted least squares means of ω-3 and ω-6 fatty acids

	Treatment					Contrast		
Fatty acid	PAS	NFS	C183M	C183H	Treat	Supp	Fat	ALAL
Tot ω-3 (mg/g fat)	20.1	16.7	28.3	27.9	*	*	*	NS
C18:3c9c12c15 (% ω-3)	47.6	42.3	45.6	48.9	NS	NS	NS	NS
C18:2t11c15 (% ω-3)	43.8	48.4	47.2	43.0	NS	NS	NS	NS
DPA (% ω-3)	4.7	4.6	3.6	3.9	*	NS	*	NS
C18:2c9c15 (% ω-3)	1.5	2.0	1.9	1.8	NS	NS	NS	NS
EPA (% ω-3)	1.2	1.0	0.7	0.7	*	NS	*	NS
DHA (% ω-3)	1.1	1.5	1.0	1.4	*	NS	NS	*
Tot ω6 (mg/g fat)	12.9	22.0	23.8	25.6	*	NS	*	NS
C18:2c9c12 (% ω-6)	53.2	80.6	75.0	81.1	*	*	*	NS
C18:2c9t12t8c12 (% ω-6)	23.1	9.2	10.9	10.2	NS	NS	*	NS
CLA t10c12 (% ω-6)	6.0	1.8	2.0	2.1	*	NS	*	NS
C20:3c8c11c14 (% ω-6)	5.1	2.5	0.6	0.6	*	NS	*	NS
C18:2t9c12 (% ω-6)	3.9	1.8	3.9	2.4	*	*	NS	*
C20:4 c5c8c11c14 (% ω-6)	3.5	3.2	2.9	2.7	NS	NS	NS	NS
C18:2t9t12 (% ω-6)	1.6	2.3	4.9	2.3	*	NS	NS	*

*$P \leq 0.05$; NS = $P > 0.05$.

(Table 1). The ω-3 FA profile was mainly represented by ALA whereas in ω-6 the main components were LNA and C18:2c9t12t8c12.

Key Words: dairy sheep, grazing, milk

107 Milk production responses to altering the dietary ratio of palmitic and oleic acids varies with production level in dairy cattle. M. M. Western*, J. de Souza, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of altering the dietary ratio of palmitic (C16:0) and oleic (*cis*-9 C18:1) acids on production responses of cows with a wide range of milk production (32 to 65 kg/d) in a crossover design experiment with a covariate period. Thirty-two multiparous Holstein cows (144 ± 94 DIM) were assigned randomly within level of milk yield to treatment sequence. Treatments were diets supplemented with FA blends (1.5% of diet DM) that provided 80% C16:0 + 10% C18:1 (PA) and 60% C16:0 + 30% C18:1 (PA+OA). The corn silage and alfalfa-based diets contained 20.0% forage NDF, 28.5% starch and 17.1% CP. Treatment periods were 21 d with the final 5 d used for data and sample collection. The statistical model included the random effect of cow, the fixed effect of treatment, period, preliminary milk yield (PMY), and 2-way interactions. Linear effects for the interaction between PMY and treatments were added to evaluate responses to treatment by level of milk yield. There were no effects of treatments on DMI ($P = 0.34$), milk yield ($P = 0.38$), ECM ($P = 0.35$), BW ($P = 0.74$), or BW change ($P = 0.54$). Compared with PA+OA, PA increased fat yield (1.92 vs. 1.83 kg/d, $P < 0.01$) and protein yield (1.61 vs. 1.55 kg/d, $P = 0.03$). PA also increased the yield of de novo (448 vs. 428 g/d, $P < 0.05$) and mixed (749 vs. 669 g/d, $P < 0.01$) milk FA and decreased the yield of preformed FA (605 vs. 627 g/d, $P < 0.05$) compared with PA+OA. Interactions were detected between treatment and PMY for DMI and ECM (linear interaction both $P < 0.05$), and a tendency for milk yield (linear interaction $P = 0.12$); lower-producing cows (less than 45 kg/d) had increased DMI and ECM on the PA diet whereas higher-producing cows (over 55 kg/d) had increased DMI and ECM on PA+OA. A linear interaction was also detected between treatment and PMY for mixed milk FA yield (linear interaction both $P < 0.10$) and a tendency for de novo milk FA yield (linear interaction $P < 0.15$). Our results demonstrate that production responses (DMI, milk yield, and ECM) of high-producing cows was better with a fat supplement containing more C18:1, while lower-producing cows responded better to a supplement containing more C16:0.

Key Words: production level, palmitic acid, oleic acid

108 Effects of altering the ratio of stearic and oleic acids in supplemental fat blends on fatty acid digestibility and production responses of dairy cows. C. M. Prom* and A. L. Lock, *Michigan State University, East Lansing, MI.*

The objective of our study was to determine the effects of altering the ratio of stearic (SA) and oleic (OA) acids in supplement fat blends on fatty acid (FA) digestibility and production responses of dairy cows. Eight multiparous Holstein cows (157 DIM ± 33) were randomly assigned to treatment sequence in a replicated 4 × 4 Latin square design with 14-d periods. The treatments were a non-FA supplemented control diet (CON), and 3 diets incorporating 1.5% DM FA supplement blends containing 50% SA and 10% OA (50:10), 40% SA and 20% OA (40:20), or 30% SA and 30% OA (30:30). FA blends were balanced to contain ~30% palmitic, 5% linoleic, and < 0.5% linolenic acids. FA supplements replaced soyhulls in the CON diet. The statistical model contained the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Pre-planned contrasts included CON vs. fat supplementation and the linear and quadratic effects of increasing OA. Results in the text are presented in the following order: CON, 50:10, 40:20, and 30:30. There was no effect of treatment on DMI ($P = 0.91$). Compared with CON, FA treatments increased milk yield (43.0, 44.7, 45.7, 45.2 kg/d; $P < 0.01$), ECM (43.4, 45.5, 45.8, 44.9 kg/d; $P = 0.02$), and fat yield (1.49, 1.58, 1.58, 1.55 kg/d; $P = 0.01$) and tended to increase milk protein yield ($P = 0.10$). Compared with CON, FA treatments had no effect on the yield of de novo milk FA ($P = 0.35$), decreased mixed milk FA ($P = 0.04$), and increased preformed milk FA ($P < 0.01$). The increase in preformed FA yield was predominantly due to FA treatments increasing the yield of OA in milk (218, 250, 264, 264 g/d; $P < 0.01$) compared with CON. Increasing OA did not affect milk yield ($P = 0.63$), milk fat yield ($P = 0.33$), or milk protein yield ($P = 0.61$). Increasing OA in FA treatments linearly decreased the yield of de novo (416, 420, 411, 391 g/d; $P = 0.03$) and mixed (534, 573, 560, 544; $P = 0.05$) milk FA, but did not affect the yield of preformed FA (440, 493, 510, 514; $P = 0.12$). In summary, feeding FA supplements containing SA and OA increased milk yield, ECM, fat yield, and preformed FA in milk compared with a no added fat control diet.

Key Words: fat supplementation, oleic acid, stearic acid

109 Effect of dietary supplementation of acetate on milk fat synthesis in lactating dairy cows. N. L. Urrutia*[1,2], R. Bomberger[1], and K. J. Harvatine[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Instituto de Investigaciones Agropecuarias, Osorno, Region de Los Lagos, Chile.*

Acetate is a major source of energy and substrate for milk fat synthesis in the dairy cow. We recently reported a linear increase in milk fat synthesis and greater than a 30% net transfer of acetate to milk fat with ruminal infusion of neutralized acetate. The objective of the current study was to investigate the ability of acetate mixed in a TMR to increase milk fat synthesis. Additionally, infusion of acetate results in an increase in plasma β-hydroxybutyrate so the effect of butyrate on milk fat was also investigated. Twelve multiparous lactating Holstein cows were randomly assigned to treatments in a 3 × 3 Latin square design with 14 d periods including 7 d of treatment and 7 d washout. Cows were fed ad libitum with a low risk diet for milk fat depression (33% NDF, 24% starch, 4.5% ether extract, 17.3% CP) and treatments were mixed into the basal diet. Treatments were on a dry matter basis: 3.1% NaHCO3 (control), 2.7% sodium acetate, and 2.4% calcium butyrate (carbon equivalent to acetate treatment). Data were analyzed by repeated measures and the model included the random effect of cow, period and sequence and the fixed effect of a covariate (d 0 of each period), treatment, time, and their interaction. Treatments were compared using a protected LSD. Feeding sodium acetate increased DMI by 2.7 kg ($P < 0.05$), had no effect on milk yield, and increased milk fat yield by 4.7% ($P < 0.05$) and concentration by 4.4% ($P < 0.05$) compared with control. Calcium butyrate decreased DMI by 2.3 kg, milk yield by 5%, milk fat yield by 8.8% and milk protein yield by 8.7% and concentration by 2.2%, compared with control (all $P < 0.05$). Sodium acetate increased concentration of 16 carbon mixed source fatty acids (FA) and myristic and palmitic acid ($P < 0.05$), while decreasing preformed FA, compared with control. Calcium butyrate had no effect on concentration of milk FA by source, but increased concentration of trans-10 C18:1 in milk by 18% ($P < 0.05$), indicating a shift in rumen biohydrogenation pathways. Our data demonstrates that milk fat yield can be increased

by feeding sodium acetate and that butyrate does not increase milk fat at equivalent levels.

Key Words: acetate, butyrate, milk fat synthesis.

110 Ceramide inhibits insulin sensitivity in primary bovine adipocytes. J. E. Rico*[1,2], W. A. Myers[1,2], D. J. Laub[2], A. N. Davis[1,2], Q. Zeng[2], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV.*

In non-ruminants, the sphingolipid ceramide reduces insulin sensitivity by inactivating protein kinase B (AKT) within the insulin signaling pathway. We have established that ceramide accumulation develops with impaired systemic insulin action in ruminants during the transition from gestation to lactation, dietary palmitic acid supplementation, controlled nutrient restriction, or intravenous triacylglycerol infusion. We hypothesized that ceramide promotes AKT inactivation and antagonizes insulin sensitivity in primary bovine adipocytes. Stromal-vascular cells were grown from bovine subcutaneous adipose tissue explants and cultured in differentiation media. To modify ceramide supply, we treated differentiated adipocytes with an inhibitor of de novo ceramide synthesis (10 μ*M* myriocin) or cell-permeable C2:0-ceramide (100 μ*M*) for 18 or 2 h, respectively. Untreated controls were included for comparison. Insulin-stimulated AKT activation (i.e., Ser-473 phosphorylation) and 2-deoxy-D-[3H]-glucose (2DOG) uptake were measured using immunoblotting and radioactivity assays, respectively. Adipocyte ceramide concentrations were measured using LC/MS. Data were analyzed under a mixed model including the fixed effect of treatment and the random effect of experiment and replicate within treatment. Relative to undifferentiated adipocytes, triacylglycerol accumulation was ~7-fold greater post differentiation with visible lipid droplet formation ($P < 0.01$). Pronounced reductions in total ceramide, monohexosylceramide, and lactosylceramide concentrations were observed in differentiated adipocytes treated with myriocin ($P < 0.01$). For example, myriocin decreased C22:0 and C24:0 ceramide by ~77% ($P < 0.01$). The insulin-stimulated ratio of phosphorylated AKT to total AKT increased with myriocin by 190% (β-actin normalized; $P < 0.05$), whereas the ratio of phosphorylated AKT to total AKT decreased by 76% with C2:0-ceramide ($P < 0.05$). Moreover, adipocyte insulin-stimulated 2DOG uptake was decreased with C2:0-ceramide and increased with myriocin ($P < 0.05$). We conclude that ceramide inhibits insulin stimulated glucose uptake by downregulating AKT activation in primary bovine adipocytes.

Key Words: adipocyte, ceramide, insulin signaling

111 Effects of commercially available palmitic and stearic acid-enriched supplements on nutrient digestibility and production responses of lactating dairy cows. M. M. Western*, J. de Souza, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of commercially available fatty acid (FA) supplements enriched with palmitic (C16:0) or stearic acid (C18:0) on nutrient digestibility and production responses of dairy cows. Thirty-six multiparous Holstein cows (146 ± 84 DIM) were used in a truncated Latin square arrangement of treatments with 2 consecutive 35-d periods, with the final 5 d used for sample and data collection. Treatments were (1) control (CON; diet containing no supplemental FA); (2) C16:0-supplement (PA; 84% C16:0, 4% C18:0, 9% C18:1); and (3) C16:0 and C18:0-supplement (SA; 33% C16:0, 53% C18:0, 5% C18:1). Supplements were fed at 1.5% DM and replaced soyhulls in CON. The statistical model included the random effect of cow nested within square and the fixed effects of treatment, period, square, and their interactions. Contrasts were (1) overall effect of FA treatments [CON vs. FAT; 1/2 (PA + SA)]; and (2) effect of FA supplement (PA vs. SA). Results are presented in the following sequence: CON, PA, SA. There were no effects of treatments on DMI, BW, or BW change. Compared with CON, FAT treatments decreased total FA (76.7, 76.3, 67.6%, $P < 0.01$), 16-carbon FA (74.3, 69.0, 68.0%, $P < 0.01$), and 18-carbon FA (78.3, 82.1, 67.2%, $P < 0.01$) digestibility. Compared with SA, PA increased DM and NDF digestibility by 3.6 and 4.8% units, respectively ($P < 0.01$). PA also increased total FA and 18-carbon FA digestibility ($P < 0.01$) but did not alter 16-carbon FA digestibility ($P = 0.55$). Using a Lucas test, apparent digestibility coefficients were 0.73 and 0.62 for the PA and SA supplements, respectively. Compared with CON, FAT increased milk yield (43.1, 45.7, 44.8 kg/d, $P = 0.01$), tended to increase ECM (44.8, 46.4, 44.5 kg/d, $P = 0.08$), but did not affect yield of milk fat (1.55, 1.65, 1.52 kg/d $P = 0.19$) or milk protein (1.43, 1.44, 1.46 kg/d, $P = 0.32$). Compared with SA, PA increased ECM ($P = 0.03$) and milk fat yield, ($P < 0.01$) but had no effect on milk protein yield ($P = 0.47$). Our results indicate that high producing dairy cows respond better to a FA supplement enriched in C16:0 compared with a supplement enriched in C18:0, which is likely due in part to PA increasing FA and NDF digestibility compared with SA.

Key Words: digestibility, palmitic acid, stearic acid

112 Impact of abomasal infusion of oleic acid on fatty acid digestibility and milk production of dairy cows. C. M. Prom*[1], J. Newbold[2], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Volac International Ltd., Orwell, Royston, United Kingdom.*

Our objective was to determine the impact of abomasal infusion of increasing doses of oleic acid (OA; *cis*-9 C18:1) on fatty acid (FA) digestibility and production responses of lactating dairy cows. Eight rumen-cannulated multiparous Holstein cows (138 ± 71 DIM) were randomly assigned to treatment sequence in a replicated 4 × 4 Latin square design with 18-d periods consisting of 7 d of washout and 11 d of infusion. Animals received the same diet, which contained (%DM) 27.8% NDF, 17.0% CP, 27% starch, and 3.3% FA (1.8% DM from a saturated FA supplement containing 31% C16:0 and 54% C18:0). Treatments were 0, 20, 40, or 60 g/d of OA delivered at 6-h intervals. Production and digestibility data were collected during the last 4 d of each infusion period. The statistical model contained the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Results in the text are presented in the following order: 0, 20, 40, and 60 g/d. OA infusion did not affect dry matter intake ($P = 0.31$) or NDF digestibility ($P = 0.54$). OA linearly increased digestibility of total FA (61.1, 66.7, 65.8, and 67.4; $P < 0.01$), 16-carbon FA (60.4, 66.7, 66.4, and 68.0; $P < 0.01$), and 18-carbon FA (61.3, 66.7, 65.5, and 67.1; $P < 0.01$). Therefore, OA linearly increased absorbed total FA (638, 705, 681, and 724; $P = 0.04$), 16-carbon FA (141, 157, 153, and 163; $P = 0.02$), and 18-carbon FA (457, 504, 484, and 516; $P = 0.05$). OA tended to linearly increase milk yield ($P = 0.09$), 3.5% fat-corrected milk ($P = 0.08$), and energy-corrected milk ($P = 0.09$). OA did not affect milk fat yield but tended to increase milk fat concentration (3.35, 3.36, 3.42, and 3.21%; quadratic $P = 0.06$). OA did not affect the yield of de novo or mixed milk FA but linearly increased yield of preformed FA (578, 587, 599, and 623 g/d; $P = 0.04$), predominantly through increased yield of OA (linear $P < 0.01$). OA also tended to linearly increase C4:0 yield ($P = 0.09$) in milk. OA increased plasma insulin concentration from 0.80 mg/L to 0.98 mg/L, but did not differ by dose ($P < 0.01$). In conclu

sion, OA infusion increased FA digestibility, preformed milk FA yield, and circulating insulin without negatively affecting dry matter intake.

Key Words: digestibility, milk production, insulin

113 Long-term effects of olive oil and hydrogenated vegetable oil supplementation on the expression of genes related to fatty acid metabolism in adipose tissue of dairy cows. E. Vargas-Bello-Pérez*[1], N. Cancino-Padilla[1], P. Sciarresi-Arechabala[2], M. S. Morales[2], J. Romero[3], M. Bionaz[4], and J. J. Loor[5], [1]*Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Universidad de Chile, Santiago, Chile,* [3]*Instituto de Nutrición y Tecnología de los Alimentos, Santiago, Chile,* [4]*Oregon State University, Corvallis, OR,* [5]*University of Illinois, Urbana, IL.*

The objective of this study was to characterize the mRNA expression in subcutaneous adipose tissue (AT) via RTqPCR of genes related to fatty acid (FA) synthesis and desaturation (*ACACA*, *FADS2*, *FASN*, *SCD1*); regulation of transcription (*INSIG1*, *SCAP*, *SREBF1*, *THRSP*, *PPARGC1A*); lipid droplet formation (*PLIN2*, *SLC27A6*); triacylglycerol synthesis (*DGAT1*, *DGAT2*, *LPIN1*); FA import into cells (*LPL*, *VLDLR*); and intracellular transport (*ACSL1*, *ACSL2*, *FABP3*, *FABP4*) in dairy cows supplemented with unsaturated (olive oil; OO) and saturated (hydrogenated vegetable oil; HVO) lipids. Fifteen cows averaging 189 ± 28 d in milk (average ± SD) at the beginning of the study were randomly assigned to treatment groups. During 9 wk animals were fed a control diet with no added lipid (n = 5 cows; basal diet), and fat-supplemented diets containing OO (n = 5 cows; 30 g/kg DM) and HVO (n = 5 cows; 30 g/kg DM). AT was obtained from the tail-head area at the onset of the study (P0) and after 9 wk of supplementation. Compared with control and HVO, OO increased ($P < 0.05$) milk yield, and reduced ($P < 0.05$) milk fat yield and milk somatic cell counts. Relative expression was determined using P0 as a reference condition. Relative mRNA expression was determined using the Pair Wise Fixed Reallocation Randomization Test built in REST (2008) using 5000 iterations. Correction and normalization to the reference genes (*GAPDH*, *UXH*, *EIF3K*) were calculated using the Ct values for each sample as the input variable. OO upregulated ($P < 0.05$) the expression of *ACACA*, *PLIN2*, *THRSP*, *DGAT1*, *LPL*, and *FABP4*. HVO upregulated ($P < 0.05$) the expression of SLC27A6. Overall, OO upregulated some genes related to FA metabolism in adipose tissue whereas HVO induced upregulation on a gene related to FA import. Our results suggest that unsaturated lipid sources may have stronger lipogenic effects in bovine AT than saturated sources in long-term supplementation. This study provides further knowledge on FA metabolism in adipose tissue and data can be used to develop new strategies for a better nutritional management in dairy cows. *This study was sponsored by a research grant from FONDECYT 1170400, Chile.*

Key Words: fatty acid metabolism, gene expression, olive oil

114 Altering the ratio of dietary palmitic and oleic acids impacts production and metabolic responses during the immediate postpartum and carryover period in dairy cows. J. de Souza*, C. Prom, and A. L. Lock, *Department of Animal Science, Michigan State University, East Lansing, MI.*

Fifty-six multiparous cows were used in a randomized complete block design to determine the effects of altering the ratio of dietary palmitic and oleic acids on production and metabolic responses. The treatments fed from 1 to 24 DIM were a control diet (CON; non-FA supplemented diet) and diets supplemented at 1.5% DM with FA supplements differing in the ratio of palmitic (C16:0) and oleic (C18:1) acids. FA treatment diets were 80:10 (80% C16:0+10% C18:1); 70:20 (70% C16:0+20% C18:1); and 60:30 (60% C16:0+30% C18:1). From d 25 to 60 postpartum (carryover period), all cows were offered a common diet to evaluate carryover effects. The statistical model included the random effect of block and cow, and the fixed effect of treatment, time, and its interaction. Results are presented in the following sequence: CON, 80:10, 70:20, and 60:30. During the fresh period, FA-supplemented diets increased milk yield (46.5, 48.6, 48.8 and 49.7 kg/d; $P < 0.05$), ECM (50.2, 54.8, 53.5 and 54.3 kg/d; $P < 0.01$), and milk fat yield (1.90, 2.15, 2.08 and 2.09 kg/d; $P < 0.01$) compared with CON. Increasing C18:1 in FA treatments decreased plasma NEFA (0.72, 0.84, 0.75, and 0.67 mEq/L; quadratic, $P < 0.05$) and BW loss (−1.55, −2.54, −1.63, and −1.48 kg/d; quadratic, $P < 0.05$), and tended to increase DMI (20.3, 20.7, 20.9, 21.8 kg/d; linear, $P < 0.1$) and plasma insulin (0.26, 0.27, 0.31, and 0.31 ug/L; quadratic, $P < 0.1$). Increasing C18:1 in FA treatments did not affect milk yield, ECM, and the yields of milk fat and protein. During the carryover period, cows that received FA-supplemented diets during the fresh period increased ECM (55.6, 59.5, 58.7, and 60.3 kg/d; $P < 0.05$), and milk fat yield (1.91, 2.06, 2.11, and 2.13 kg/d; $P < 0.05$) compared with CON. Our results indicate that feeding FA supplements containing C16:0 and C18:1 during the immediate postpartum period increased milk yield and ECM compared with a nonfat control diet. Increasing C18:1 in the FA supplement reduced BW loss and plasma NEFA and tended to increase DMI. Also, the diets fed during the immediate postpartum period had a tremendous carryover effect during early lactation, when cows were fed a common diet.

Key Words: body condition, fat supplementation, postpartum

115 Changes in the omasal flow of long-chain fatty acids alters the yield of de novo and preformed milk fatty acids. J. de Souza*[1], H. Leskinen[2], K. J. Shingfield[4,2], A. L. Lock[1], and P. Huhtanen[3], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Animal Genomics, Green Technology, Natural Resources Institute Finland (Luke), Jokioinen, Finland,* [3]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden,* [4]*Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom.*

We determined milk fatty acid (FA) yield response in relation to omasal flow of long-chain FA. Our analysis used individual observations (n = 132) in lactating Nordic Red dairy cows from 9 Latin square or switchback design studies. The yield of milk FA (g/d) was classified as: < 16C (summation of FA lower than 16-carbon, de novo FA), 16C (summation of 16-carbon FA, mixed FA), > 16C (summation of FA greater than 16-carbon, preformed FA). Mixed model regressions were developed between variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors. Increasing palmitic acid (16:0) omasal flow (g/d) linearly increased the yield of < 16C FA [158 ± 26.5 + 1.41 ± 0.25 × 16:0 flow, $P < 0.001$, $R^2 = 0.68$], 16C FA [207 ± 36.1 + 1.60 ± 0.35 × 16:0 flow, $P < 0.001$, $R^2 = 0.73$], > 16C FA [333 ± 51.9 + 1.41 ± 0.25 × 16:0 flow, $P = 0.02$, $R^2 = 0.29$], and total FA [740 ± 80.5 + 3.59 ± 0.74 × 16:0 flow, $P < 0.001$, $R^2 = 0.72$] in milk. In contrast, increasing stearic acid (18:0) omasal flow (g/d) did not affect the yield of < 16C and 16C ($P > 0.05$), but quadratically increased the yield of > 16C [233 ± 40.3 + 0.66 ± 0.14 × 18:0 flow - 0.003 ± 0.0001 × 18:0 flow^2, $P < 0.001$, $R^2 = 0.84$] and total FA [795 ± 75.8 + 0.87 ± 0.27 × 18:0 flow - 0.0005 ± 0.0002 × 18:0 flow^2, $P < 0.01$, $R^2 = 0.48$] in milk. For the flow of 18:0, maximum > 16C and total FA yields were achieved when 18:0 flow was 1065 and 943 g/d, respectively. Increasing oleic acid (*cis*-9 18:1) omasal flow

(g/d) linearly increased the yield of < 16C [246 ± 18.5 + 1.24 ± 0.41 × 18:1 flow, $P < 0.01$, $R^2 = 0.37$] and total FA [965 ± 55.9 + 2.98 ± 1.16 × 18:1 flow, $P < 0.01$, $R^2 = 0.37$] in milk, and did not affect 16C and > 16C ($P > 0.05$). Increasing linoleic acid (18:2n-6) omasal flow (g/d) linearly increased the yield of < 16C [206 ± 17.2 + 4.46 ± 0.79 × 18:2n-6 flow, $P < 0.001$, $R^2 = 0.96$], 16C [278 ± 25.2 + 3.85 ± 1.13 × 18:2n-6 flow, $P < 0.001$, $R^2 = 0.63$], and total FA [888 ± 62.0 + 9.33 ± 0.25 × 18:2n-6 flow, $P < 0.001$, $R^2 = 0.65$] in milk. Our results highlight the interdependence between de novo synthesized and preformed milk FA and demonstrates that effects on de novo, mixed, and preformed milk FA synthesis are dependent upon the amount and profile of absorbed FA.

Key Words: fatty acids, milk fat, meta-analysis

116 Comparison of fat supplements containing palmitic or stearic acid on intake and production in lactating dairy cows. R. Shepardson* and K. Harvatine, *Penn State University, University Park, PA.*

Fatty acid (FA) supplements based on palmitic acid (PA) and stearic acid (SA) are commonly used to increase dietary energy density for lactating dairy cows. There is limited literature directly comparing supplements enriched in PA and SA to a blend of these FA. Our hypothesis was that PA would increase milk fat and decrease intake while SA would have no effect on intake. Treatments were (1) Control (CON; no additional fat), (2) high palmitic supplement (HP; 90.7% C16:0, 2.4% C18:0, and 5.8% *cis*-9 C18:1), (3) high stearic supplement (HS; 1.7% C16:0, 90.5% C18:0, and 5.9% *cis*-9 C18:1), and (4) a blend of PA and SA (INT; 45.7% C16:0, 46.4% C18:0, and 5.7% *cis*-9 C18:1). Twelve multiparous, post-peak (138.5 ± 21.0 DIM) Holstein cows were arranged in a 4 × 4 Latin square design with 21-d periods. Samples were collected the last 3 d of each period. The statistical model included the random effect of cow and period and the fixed effect of treatment. There were no differences between treatments for DMI (25.03 ± 2.29 kg), milk yield (39.19 ± 7.31 kg), milk protein concentration and yield, or MUN. Milk fat percent was increased 0.44 and 0.14 percentage points by HP compared with CON and SA, respectively ($P = 0.03$). Milk fat yield was increased by 110 g/d by HP and 140 g/d by INT compared with CON ($P < 0.01$). Although HP increased milk fat concentration 0.34 percentage points compared with INT, fat yield did not differ. Milk fat concentration and yield were not different between CON and SA. As expected, HP and INT increased C16 FA in milk fat and decreased both de novo and preformed FA. Preformed FA concentration was the highest in SA (both $P < 0.0001$). There was a trend for HP to decrease de novo FA yield ($P = 0.09$). The INT treatment increased preformed FA yield 60.5 and 50.4 g/d compared with CON and HP ($P = 0.02$). The HP and INT treatment increased yield of C16 FA 154.1 and 90 g/d compared with CON, respectively. On the basis of fat yield, the 2 supplements that had greater amounts of PA had a greater response.

Key Words: fatty acid supplement, milk fatty acid profile

Ruminant Nutrition Symposium: Management and Nutrition of Dairy Cattle in the New Era of Automation

117 What have we learned about automated milk feeders? M. Endres*, *University of Minnesota, St. Paul, MN.*

The majority of dairy farms in the United States house preweaned calves individually. However, group housing is growing in popularity and many farms choose to install automated milk feeders for raising these calves. We conducted an 18-mo cross-sectional longitudinal study on 38 farms with autofeeders to learn about best management practices for achieving good calf health when using autofeeders. A single observer scored a total of 10,185 calves for health using a modified health scoring system on all 38 farms and we also collected treatment and mortality rates based on farm records (from a subset of 26 farms). We found that the following factors were associated ($P < 0.05$) with better calf health scores and/or lower mortality/or treatment rate in autofeeder farms: reduced time to reach peak milk allowance (minimum peak allowance suggested is 8 L/d); feeding milk/milk replacer with low bacterial count (SPC less than 100,000 cfu/ml); use of positive pressure ventilation tubes in the calf barn; adequate amount of space/calf in the resting area (minimum suggested is 3.7 m^2); small number of calves per group (suggested less than 15 calves); adequate farm average serum total protein concentration (an indicator of passive immune transfer); use of drinking speed as a warning signal to identify potentially sick calves; practicing navel and pen disinfection between calf groups consistently; and having narrow age range within calf groups. We also observed that winter was the season with worst health scores and highest treatment rates. It appeared that cleaning of the autofeeder and its various components was one of the most important keys to making these systems work successfully. Other studies have shown similar results. Housing calves in groups allows calves to express natural behaviors that cannot be expressed when housed individually, but it can be more difficult to achieve good health. Studies indicate that good calf health is achievable when using autofeeders to raise preweaned calves in groups as long as appropriate management practices and maintenance of the feeding equipment are emphasized and implemented. *The MN project was funded by USDA-AFRI-NIFA competitive grant no. 2012–67021–19280.*

Key Words: automated milk feeder, preweaned calf, group housing

118 Economics considerations for automatic milking systems (AMS). L. Tranel*, *Iowa State University Extension and Outreach Dairy Team, Ames, IA.*

A partial budget tool was developed to compare a producer's present milking system with an automatic milking system (AMS). One AMS can handle an estimated 55 to 65 milking cows or approximately a 72-cow herd. The milk price projected over the life of the AMS is an important variable as is the estimated cost per AMS and the expected years of useful life of the AMS, often ranging 10 to 15 years. The value of AMS after its useful life is not well defined at this time but an important consideration. Labor cost and availability tends to be the leading reason for adopting AMS. Current hours of milking needs to be compared with the anticipated hours of milking labor after the AMS is installed. The AMS herd management software often includes rumination, milk conductivity, and cow activity. This information can lead to labor and herd health savings from heat and mastitis detection and faster identification of sick cows. There will likely be an increase in records management with the AMS. In adopting AMS, producers may experience losses in milk production 6 to 9% from 3× milking. From 2× milking, one could confidently expect a 3 to 5% increase in milk production. However, this milk production response varies widely, depending on present system and has increased as high as 25% on certain herds. Somatic cell counts (SCC) and bacteria counts tend to increase in the first few months after adoption to the AMS but tend to drop to initial levels or even lower after the adoption period. Feed costs per pound of dry matter and feed intake level changes can also be significant. Use of pelleted feeds and more individually fed cows will alter feed costs. Most producers report little change in culling percent but reasons for culling may change. High repair costs tend to be the biggest concern of AMS owners. AMS systems may increase electrical and water usage along with chemical and supply costs. Teat dip costs have been reported to increase dramatically but dependent on the previous system used. Overall, AMS can be an economic improvement in many herds relative to present milking systems but not true for all who have adopted AMS technology.

Key Words: automatic milking system (AMS), dairy economics

119 Successful feeding and nutrition in robotic herds. M. Brouk*, *Kansas State University, Manhattan, KS.*

Nutritional management of robotically milked dairy herds has presented the dairy nutrition world with a new challenge. For the last several decades, nutritionists have concentrated on feeding total mixed rations (TMR) and managing cows in groups primarily based on parity, nutritional requirements, and reproductive status. With the adoption of automatic milking systems (AMS), cows are now generally allotted to a group for the entire lactation. Parity may still be considered in some herds. Balancing nutrient intake from the partial mixed ration (PMR) and the pellet or concentrates fed in the AMS becomes an added challenge for the nutritional professional. In some cases, the AMS can dispense multiple feeds to individual cows. This can further complicate the equation, but also provided greater targeted nutrition for individual animals. This can create opportunities to feed certain groups of cows (early lactation) differently from other cows housed and managed within the same group. It could also provide a method of different feeding programs for cows differing in parity yet housed within the same group. An additional complicating factor is differences in designed cow traffic of the facility, free-flow or guided-flow. Goals for cow traffic (daily visits) may be associated with feed intake in the milking center and thus change the nutritional goals of the PMR. With all the choices and various feed settings available with AMS, often other items like forage quality, foot health, training and cow comfort are often forgotten. These factors have a major effect on cow movement and can alter the use of the AMS, negatively affecting the amount of feed obtained from the robot, forcing the animal to depend more on the PMR. This is especially critical to the proper nutrition of early lactation cows. Balancing the ration for the robotically milked herd is very similar to conventional milked cattle from a nutritional aspect. However, understanding and predicting how nutrient intake is altered by split feeding between the PMR and the robot feed or feeds is the challenge. Balancing nutrition becomes more complicated because with AMS, cow behavior (visits to the milking machine) become an important part of the nutrition equation.

Key Words: nutrition, management, milking

120 Incorporating technologies in nutrition and transition management. E. A. Eckelkamp*[1] and J. M. Bewley[2], [1]*University of Tennessee Institute of Agriculture, Knoxville, TN,* [2]*CowFocused Housing,, Bardstown, KY.*

Precision dairy technology is being incorporated in several areas related to nutrition, ration management, and even health disorders. Through automation, feeding consistency can be assured through feed kitchens, in-line ration analysis, and individualized feeding programs. Continuous access to feed can be accomplished with automated feed pushers. Fresh, individualized group rations can also be provided through monitoring the feed-way. Automated milking systems (AMS) have incorporated the benefits of electronic stand-alone feeders with milking information. This allows producers to manage based on production, ramping cows up at the start of lactation and down toward dry-off. In the future, producers may be able to incorporate other information, such as automated body condition scoring, to better inform ration decisions. Incorporation of all of these technologies could result in a completely autonomous system. The result being a single producer could coordinate rations specialized for several groups. Some technology companies are already embracing coordinated technology. A producer can purchase a technology with a TMR mixer on top with a feed pusher on the bottom. The technology also incorporates a laser-sight to determine when new feed is needed, and an interface with a feed arm for forages incorporated into a PMR. The forage fed complements the pellet fed through either an AMS or standalone electronic feeder. Automated milking systems may incorporate electroconductivity and SCC. Some AMS also incorporate β-hydroxybutyrate for in-line hyperketonemia monitoring. Wearable technologies can currently measure rumination time and eating time, allowing producers to monitor individual cows. Wearable technologies can also identify changes in behavior to detect or predict hyperketonemia, hypocalcemia, and metritis. Future directions may include incorporating machine-learning techniques to narrow the window of detection to 24 h before an event and create disease-specific alerts. The future is full of possibility for nutrition and disease management.

Key Words: precision dairy technology, precision farming, automated systems

121 Opportunities and limitations in farm data integration and analytics for strategic decision-making. M. J. Jerred*, G. F. Schroeder, R. A. Daura, and C. Van Der Meijde, *Cargill Animal Nutrition, Minnetonka, MN.*

Data analytics has become a key driver for decision making in many businesses. Although the dairy industry is typically collecting enough real time data, it lags other industries in the ability to access, integrate, and utilize that data for making strategic decisions that will optimize the business to improve animal productivity and return on investment. It is easy to envision the opportunities that exist if we apply modern data analytics and visualization tools to farm data. For instance, an integrated data platform connecting animal performance, feed management data, diet formulation systems, and animal sensors (e.g., feeding time, rumination, activity, rumen pH) will create new opportunities to optimize farm diets, provide field data to validate technologies and decisions, and create farm-specific prediction models. During the development of an integrated data platform, we identified many data access and collection issues that must be overcome. Farm-level challenges include obsolete hardware, out of date software and operating systems, and inconsistent data collection. In addition, there is a lack of integration with off-farm data such as ration information, lab analysis, farm advisor data collection, and milk sales. Analytics opportunities include the creation of integrated reporting and dashboards using data visualization tools, alert systems to indicate management intervention opportunities and "what if" decision and management recommendation support. Dashboards and reports can present high value information but most farm managers have limited time to integrate it into strategic decision-making. One approach is to use the data platform and analytics to enhance the farm manager – dairy advisor relationship. As we move to a data driven approach new skills will be required. Farm managers need to ensure data collection on farm is organized, consistent, and accurate. Dairy advisors need to understand how to integrate ever-increasing amounts of information into strategic recommendations. This will improve the quality and timeliness of nutrition and management decisions.

Key Words: data, analytics, decision-making

122 Technology, automation, and dairy industry: How far can we go? A. D. Aguiar* and N. Charlton, *DeLaval, Bannockburn, IL.*

Automation on dairy farms began in the late 19th century. Gustaf de Laval invented the cream separator to aid in the process of collecting cream for dairy products. Refrigeration followed in the same period and refined the dairy industry. Recently, several devices have become available to producers, from vacuum systems that support stable milk extraction, automatic take-offs and heat detection systems, automatic calf feeders, rotary parlors, and, more recently, robots. Automation can be used in daily activities to increase productivity, food safety, time management, and animal welfare. Automation collects numbers, which are converted to data that has the potential to help producers make better decisions. Data-acquiring technologies are still in their infancy. Companies are using more scientific research to create reports to analyze the data and turn it into information. Software is one of the most powerful tools. Reports can help farmers make decisions to act on cows that need attention. In addition to change management to improve health, production and reproduction. Dairy farmers and advisors rarely utilize software to its full potential. Support and training from software companies are essential to ensure the full understating of the system. Moreover, the dairy community and scientific community need to work closer to develop benchmarks and key performance indicators (KPI). Indicators should be available to dairy farmers, and advisors to improve the overall health and time management for each dairy farm. Each farm should have their own goals, and a plan to get there. KPIs need to be applicable to each dairy farm to create a plan to what needs to be monitored. Recently 3D image technology has been used to identify and manage animals. Technology is here to stay; the scientific and dairy community needs to provide data back to producers and advisors to provide information that leads to actions. Cows and calves must benefit from the information. Technology needs to be reliable so the dairyman can have conviction in the results as false positives waste people's time and efforts. Technology will never replace the skills of a very good herdsperson but is a tool in the toolbox to add to the success of the dairy farm.

Key Words: technology, automatization, management

Small Ruminant Platform Session: Addressing Management Challenges and Improving Performance in Small Ruminants

123 Review of old and new approaches to evaluate milking impact and milking ability in goats. P.-G. Marnet*[1], A. Dzidic[2], L. Le Caro[3,1], and A. Hubert[4], [1]*Agrocampus Ouest, Department of Animal Sciences, Agri-food and Human Nutrition, Rennes, France,* [2]*Faculty of Agriculture, University of Zagreb, Zagreb, Croatia,* [3]*Chamber of agriculture d'ille et Vilaine, Rennes, France,* [4]*French Livestock Institute, animal health and milk products quality, Le Rheu, France.*

French goat milk has shown a constant and significant increase in SCC since 1995. This increment could be explained by numerous factors, including increasing adoption of simplified management methods (e.g., once a day milking and extended lactation); insufficient genetic selection of goats for important traits (e.g., mastitis resistance, milk flow rate, udder and teat morphology); and the fact that milking equipment and settings (e.g., automatic cluster remover feature and teat cup liners) are not well designed to fit teat morphology in goats. To help address these problems, our studies focused on the animal-machine interaction, especially at the teat level. Milk flow kinetic recording (as milkability indicator) and teat-cup vacuum stability at different levels (by small devices such as VaDia) are basic techniques to evaluate efficiency of milking; however, these approaches do not include detailed teat characteristics. A "vacuometer" records the vacuum needed to open teat sphincters, which is highly correlated with milk flow, and could serve as a functional trait for selection. This information provides accurate use of vacuum levels to maintain teat tissue integrity. Ultrasonography is used to evaluate streak canal dimensions (associated with milk flow rate), and teat wall thickness, as well as teat congestion after milking; however, it is considered as an experimental approach. A "cutimeter" is a user-friendly caliper measuring teat tissue congestion by producers at the farm level. This method helps to evaluate machine settings impacts to better match teat traits along with liner design and its performance. Thermography is a more recent technology (initially expected to help in mastitis detection) that can be used to detect abnormal teat wall blood flow. Thermal imaging provides the possibility to evaluate teat-liner interactions at different parts of the teat. The 2D numeric imaging (typically used to evaluate udder morphological traits in small ruminant's genetic selection schemes) is now being upgraded to 3D imaging, which is more accurate for evaluating teat morphology. Application of these new tools will help in measuring new traits and in the diagnosis of milking machine effects on teats and udder in dairy goats.

Key Words: teat morphology, dairy goat, milking machine

124 Comparison of milk fat, protein, somatic cell count, and urea nitrogen concentrations between mid-infrared spectroscopy calibrated with cow milk and reference methods of dairy goat milk samples. M. Duplessis*[1], D. Cinq-Mars[2], C. Brunelle[3], R. Kouaouci[3], and D. M. Lefebvre[3], [1]*Agriculture & AgriFood Canada, Sherbrooke, QC, Canada,* [2]*Université Laval, Québec, QC, Canada,* [3]*Valacta, Ste-Anne-de-Bellevue, QC, Canada.*

Today, bulk tank goat milk samples for payment are analyzed using mid-infrared (MIR) spectroscopy based on cow milk calibrations in Québec, Canada. The aim of the study was to compare milk fat, protein, SCC, and urea nitrogen (MUN) concentrations of goat milk analyzed by reference methods and MIR using cow milk calibrations. Bulk tank milk samples were obtained from 20 goat herds 5 times between March 2016 and February 2017. Samples were analyzed by MIR based on cow milk calibrations to obtain milk fat, protein, SCC, and MUN concentrations. Gravimetric method, determination of nitrogen content with Kjeldahl method, microscopy, and continuous flow analyses were considered as reference methods for fat, protein, SCC, and MUN concentrations, respectively. Proc MIXED of SAS with repeated measures in sampling time was used to compare MIR and reference methods. No significant difference was noted for milk fat and SCC concentrations between the 2 methods (Table 1). However, MIR underestimated milk protein concentration by 0.16 percentage point compared with the reference method ($P = 0.02$). In opposition, MIR overestimated MUN concentration compared with the reference method ($P < 0.0001$). As dairy goat producers in Québec, Canada are paid based on the milk components they sell and protein is the most profitable component, specific calibrations for goat milk should be developed or an adjustment should be proposed. In summary, MIR based on cow milk calibrations should be avoided to estimate protein and MUN concentrations of goat milk.

Table 1 (Abstr. 124). Comparison of milk component concentrations between mid-infrared (MIR) spectroscopy based on cow milk calibrations and reference method

Milk components	Mean	SD	Min	Max	*P*-value
Fat, %					0.69
MIR	3.72	0.43	2.76	5.38	
Reference	3.77	0.46	2.84	5.62	
Protein, %					0.02
MIR	3.33	0.28	2.80	4.72	
Reference	3.49	0.30	2.96	5.04	
MUN, mg/dL					<0.0001
MIR	29.4	3.8	17.8	38.8	
Reference	19.0	3.8	10.3	27.0	
SCC, $\times 10^3$ cells/mL					0.93
MIR	1,381	543	337	3,152	
Reference	1,290	470	294	2,589	

Key Words: goat, mid-infrared (MIR), milk

125 Protecting dietary n-3 fatty acid and vitamin E and C in feed and its effect on the nutritional profile of goat milk. A. Discua, J. H. Lee*, and C. Jeanjulien, *Fort Valley State University, Fort Valley, GA.*

This study aimed to evaluate the effect of feeding rumen-protected dietary supplements (RPDS) containing n-3 fatty (omega-3) acids and vitamin E and C on the nutritional profile of goat milk. The RPDS were prepared with n-3 fish oil, defatted soy flour, a generally recognized as safe (GRAS) chemical and α-tocopherol (TOC) either with or without ascorbic acid (ASC). Nine Saanen lactating goats (4 yr old; BW = 42.1 ± 2.06 kg) were divided into groups of 3 and randomly assigned to 3 diets using a 3 × 3 Latin square design with 14-d periods. The 3 diets consisted of control (BD plus TOC+ASC), RPDS-TOC (75%BD plus 25%RPDS containing TOC) and RPDS-TOC+ASC (75%BD plus 25%RPDS containing TOC+ASC) diets. Furthermore, the BD (basal diet) contained alfalfa meal, yellow corn and soybean meal. Each period consisted of a 10-d adjustment to assigned diets followed by 4-d of milk

ing collection. The designated diet was provided once daily ad libitum at individual feed stations. The collected milk samples from each goat were analyzed for basic nutrient, α-tocopherol, ascorbic acid, and fatty acid composition. All the data were analyzed as a 3 × 3 Latin square design using the PROC MIXED procedures of the SAS. No significant differences were found in the basic nutrient of milk samples from the 3 different diets. Compared with lactating goats fed a control diet, goats fed both RPDS containing diets (RPDS-TOC and RPDS-TOC+ASC) had higher ($P < 0.05$) concentrations of α-tocopherol (0.87 vs 1.27 and 1.28 μg/mL) in milk. The concentration of ascorbic acid in skim milk from goats fed either a control or RPDS-TOC+ASC diet was higher ($P < 0.05$; 14.2 or 15.1 vs 11.1 μg/mL) than that from goats fed a RPDS-TOC diet. Compared with lactating goats fed control diet, goats fed either RPDS-TOC or RPDS-TOC+ASC diet had higher ($P < 0.05$) concentrations of eicosapentaenoic (C20:5n-3, 0.32 vs 0.95 or 0.92%) and docosahexaenoic (C22:6n-3, 0.28 vs 0.75 or 0.71%) acids in milk. The results indicate that feeding lactating goats with the rumen-protected dietary supplements prepared with GRAS chemicals can increase the deposition of α-tocopherols and n-3 fatty acids in their milk.

Key Words: fish oil, vitamin E and C, goat milk

126 The fatty acid profile of goat milk with supplementation of fish oil in the diet. M. Nuñez de González*, R. Attaie, A. Mora-Gutierrez, S. Woldesenbet, Y. Jung, J. Kirven, and D. Myers, *Prairie View A&M University, Prairie View, TX.*

The objective of this study was to evaluate the effect of goat diet supplementation with fish oil on the profile of milk fatty acids, particularly the polyunsaturated fatty acids (PUFA). The benefits of increased levels of PUFA in the diet of humans are numerous. A total of 10 Alpine goats were randomly selected from the dairy herd of the International Goat Research Center at Prairie View A&M University. The animals were divided into 2 groups (5 animals/group) and each group was assigned to either the control or experimental diet. The control animals received the basal diet without supplementation of fish oil, whereas the experimental group was given the same basal diet with 1.14 g of fish oil/kg of concentrate. The total feeding duration of this experiment was 8 weeks with 2 weeks of the same basal diet as the adaptation period. Milk samples were collected and analyzed for chemical composition at wk 0, 2, 4, and 6. The fatty acid profile of collected milk samples were analyzed using GC-MS. The data were analyzed using the PROC GLM procedure of SAS based on repeated measure design. The model included diet and time as the main effects and their interaction. All tests to determine statistical differences were at 5% significance level. Compared with the control group, the level of fish oil supplementation did not affect the total fat content of goat milk with diet as the main effect. However, the protein and SNF contents of milk from the experimental group were significantly higher ($P < 0.05$) than the milk of the control group. With respect to time as the main effect, significant differences ($P < 0.05$) were observed in the quantities of the following fatty acids: C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C15:0, C15:1, CLA *trans*-10,*cis*-12, C22:0, and C20:4?-6 during the study period. Based on the results obtained, the level of fish oil supplementation in the diet used in this study did not affect the total fatty acids content of goat milk, suggesting that higher levels of fish oil may be required to increase the fatty acid profile of goat milk.

Key Words: goat milk, fatty acids, fish oil

127 Thermophysiological traits within a flock of dairy ewes and variability in the response to a heat stress challenge. A. Elhadi* and G. Caja, *Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain.*

The response to a short-term heat stress (HS) challenge was studied in a flock of Manchega dairy ewes (n = 100). Ewe classes and features (n; BW; BCS) were hoggets (12; 33.9 ± 1.1 kg; 2.98 ± 0.01), dry (14; 55.1 ± 2.1 kg; 2.98 ± 0.16) and lactating (74; 75.6 ± 1.0 kg; 3.64 ± 0.04). All the ewes were open. Lactating ewes (162 ± 15 DIM; 1.07 ± 0.05 L/d) were machine milked × 1-daily. Flock was fed a TMR ad libitum. On the afternoon of the previous HS challenge day, ewes were moved in groups of 20 to 24 to a heat-temperature controlled shelter (Trivic, ES) and randomly allocated in groups of 4 ewes on sawdust-bedded pens at thermoneutral conditions (TN, 17.7°C and 71%RH; $THI_{NRC} = 63$). Feed and water were ad libitum. On the morning of the challenge day, thermophysiological traits consisting of rectal temperature (RT; AccuVet digital, TW), infrared skin temperature (ST; Optris MS Plus infrared, DE) and respiratory rate (RR, 2 operators by sight) were recorded. After fed and water removal, the shelter was switched to HS (35.6°C and 43%RH; $THI_{NRC} = 84$) in 30 min and these conditions maintained for 150 min. Thermophysio traits were again recorded after 2 h at HS. Data were treated using the PROCMIXED of SAS (v.9.4). RT varied by class, hoggets showed higher values than adult ewes under TN (39.29 ± 0.08 vs. 39.02 ± 0.04°C; $P < 0.001$) and HS (39.57 ± 0.07 vs. 39.28 ± 0.05°C; $P < 0.001$) conditions. No differences were detected in ST by class, which values depended on ambient conditions (TN vs. HS, 35.67 ± 0.08 vs. 37.73 ± 0.43°C; $P = 0.60$ to 0.12). RR was the most affected trait by class and challenge. All ewe classes showed similar RR under TN (38 ± 1 breaths/min; $P = 0.21$), but lactating ewes showed lower RR than dry and hoggets (99 ± 3 vs. 122 ± 7 breaths/min; $P < 0.001$) under HS, the last not differing between them. RR and RT responses showed normal distributions and wide ranges, suggesting interest for selection. Most sensitive classes to HS were hoggets and dry ewes, the lactating ewes being able dissipate better their body heat. *Study supported by MINECO Spain (Project RTA2015-0035-CO3-02).*

Key Words: heat stress, dairy sheep, temperature

128 On-farm welfare assessment update and its relation to productivity in dairy small ruminants. G. Caja*[1], R. González-González[2], and M. Rovai[3], [1]*Universitat Autonoma de Barcelona, Bellaterra,Barcelona, Spain,* [2]*Gestion Empresarial Ovina, Benavente, Zamora, Spain,* [3]*South Dakota State University, Brookings, SD.*

Welfare is a current hot topic for consumers and animal scientists, while at the same time it is a concern for many farmers who are worried about increasing production costs due to implementation of animal wellbeing measures. With this in mind, currently available animal-based welfare indicators were reviewed looking for valid, reliable, and on-farm feasible protocols for wellbeing assessment in dairy small ruminants. The 5 wellbeing principles, also called freedoms (freedom from hunger and thirst; from discomfiture; from pain, injury, and disease; from fear and distress; and freedom to express most normal behaviors) are internationally recognized, but they are ideal states rather than standards for acceptable welfare. Despite being usually considered together, there are remarkable differences in physiological and behavioral features of sheep and goats, as well as among their breeds which show dramatic physical differences (e.g., weight and size, wool cover, ear length), making necessary specific studies to adapt the key indicators to be used in each

case (i.e., pen and feeders size, critical temperatures, acidosis, ketosis, lameness) for the assessment protocols under on-farm conditions. A good protocol is a powerful instrument for welfare assessment but also for production and health monitoring, and should be used regularly by scientists, veterinarians, technicians, and farmers. Special interest have the 2-step AWIN welfare assessment protocols for dairy sheep and dairy goats under intensive systems, that have been currently tested in a great number of farms in the European Union. Use of new technologies (e.g., infrared cameras, activity accelerometers, rumen and body sensors for pH and temperature), most of them similar to those used in dairy cattle, are very limited in small ruminants and need further research. This review focuses on the state-of-the-art of the wellbeing assessment protocols and the measuring technologies currently available, summarizes the known impact of some management practices and the physiological particularities of dairy sheep and dairy goats, with regard to wellbeing assessment and its relationship with the main production traits in dairy small ruminants.

Key Words: welfare assessment, animal-based indicator, dairy small ruminant

129 A2 variant of β-casein: Friend or foe? L. R. Harrison*, D. R. Winston, and K. M. Daniels, *Virginia Tech, Blacksburg, VA.*

Two predominant alleles for β-casein exist in dairy cattle: A1 and A2. Cows that are homozygous for the A1 allele produce a variant of β-casein that differs by 1 amino acid from cows that are homozygous for the A2 allele. With the increasing prevalence of niche markets for dairy products, A2 milk has moved into the spotlight, bringing with it conflicting views about possible benefits. Three studies were evaluated to determine the impact of A2 milk on the dairy industry, from producers to consumers. Bugeac et al. (2015) found that Holstein-Friesian cows homozygous for the A2 allele produced 2,564 kg more milk over the lactation period compared with homozygous A1 cows. However, although A2 cows exhibited an increase in yield, Poulsen et al. (2017) examined the relationship between β-casein variants and milk coagulation properties and identified a possible negative impact for processors. Milk with the A2 variant was associated with longer rennet coagulation times and lower curd firming rates, making it a poor choice for cheese production. Finally, Jianqin et al. (2016) explored possible effects of A1/A2 versus purely A2 milk on the gastrointestinal physiology of consumers who had reported an intolerance to "traditional" (commercially available A1/A2) milk. Switching from A1/A2 to purely A2 milk was reported to reduce stomach inflammation in 36.4% of those who suffered from it, compared with only 11.1% who improved when switched from A2 milk to the A1/A2 combination. In addition, A2 milk was associated with fewer gastrointestinal symptoms and shorter transit times. Although there is great potential for additional research, these studies have indicated that while the A2 allele may reduce the efficiency of cheese production, it can provide milk yield benefits to farmers and possibly positive health effects to consumers.

Key Words: A2 variant, β-casein, protein structure

130 Comparison between non-dairy milk-like beverages and cow's milk. C. P. Cecil*, G. Mazon, and J. H. C. Costa, *University of Kentucky, Lexington, KY.*

Milk is defined as a secretion produced by the mammary glands of all female mammals after giving birth. However, many non-dairy milk-like beverages are offered to consumers as milk. Sales of fluid dairy milk to consumers have decreased 15% since 2012 and are expected to continue declining in the near future (Mintel Group Ltd., 2017). One of the many reasons for the decline in fluid dairy milk consumption is that consumers are now switching to non-dairy milk-like beverages, such as almond, soy, and cashew. From 2012 to 2016, non-dairy milk-like beverages sales increased by 53%. An online survey done with 2000 internet users over the age of 18 showed more than half purchased non-dairy milk-like beverages during the 3 mo before the survey (Mintel Group Ltd., 2017). When exploring consumer's views and attitudes concerning non-dairy milk-like beverages, a study found a very diverse opinion. Consumers' acceptance of non-dairy milk-like beverages depended on many factors, specifically taste and consistency (Kempen et al., 2017). One of the arguments often used to explain the changes in population consumption patterns is that alternative beverages are a healthier option when compared with dairy products. However, cow's milk is very nutritious, containing naturally occurring and easily absorbable macro and micronutrients. Heaney et al. (2000) concluded that calcium from calcium-fortified soy beverages was absorbed only at 75% the efficiency when compared with milk. Another study found a negative relationship between non-dairy milk-like beverages consumption and childhood height when compared with children who drank milk. (Morency et al., 2017). Children were 0.4 cm shorter for every daily cup of non-dairy milk-like beverage consumed. Milk is an excellent source of nutrients and has many benefits. The dairy industry should support the understanding of milk's nutritional content, consumers' perceptions, and should investigate how beneficial dairy products truly are to a healthy diet.

Key Words: alternative milk, soy milk, almond milk

131 Spore-forming bacteria reduce milk quality. Z. P. Itle* and D. R. Olver, *The Pennsylvania State University, University Park, PA.*

Extended shelf life (ESL) fluid milk products have made significant gains in sales across the world. Research by the National Center for Biotechnology Information indicates that psychrotolerant spore-forming bacteria (PSF) often lead to premature spoilage in these beverages. PSF may reduce pasteurized milk shelf life by several days, thus restricting a processor's ability to market the milk as ESL. Studies have shown that almost 20% of the most commonly sold HTST pasteurized milk in the United States is discarded before consumption. Much of this microbial spoilage can be attributed to PSF. Extensive studies have shown that the *Paenibacillus* strains are the predominant PSF found in fluid milk and can originate on the farm environment from sources such as fermented feeds and bedding. Additionally, udder cleanliness and milking preparation directly correlate with raw milk quality in the bulk tank. These highly heat-resistant endospores exhibit thermophilic and mesophilic properties that allow them to survive harsh environments such as UHT pasteurization. Post pasteurization contamination (PPC) has also been implicated in PSF growth. Biofilm formation in corners, cracks, crevices, and dead ends of stainless steel processing lines can result in PPC because milk is an ideal culture medium for the growth of food spoilage flora such as PSF. Effective practices to extend shelf life of milk by limiting PPC and PSF include using aseptic lines that eliminate corners, strict adherence to cleaning-in place processes (CIP), and biofilm control by detergents. Controlling PSF in the milk supply offers dairy producers and processors the opportunity to improve milk quality and profitability.

Key Words: milk processing, psychrotolerant spore-forming bacteria

132 An udder option: A2 milk. B. Davidson*, B. Dado-Senn, and J. Laporta, *University of Florida, Gainesville, FL.*

Starting 20 years ago, research has been conducted on the correlation of milk consumption and disease outbreaks around the world. Researchers found a high percentage of lactose intolerance claims which led the research into lactose intolerance causes and prevalence. It was discovered that a very small portion, about 4 to 5% of those who self-diagnosed as lactose intolerant (75 million worldwide), may actually only be milk protein intolerant. One protein of recent consideration is β-casein, the second major protein found in milk. Two variants A1 and A2 have been identified, differing only by one AA at position 67 on the 209 AA chain. People never medically tested for lactose intolerance that experience discomfort after consuming milk may turn to plant based milk alternatives and juices under the misconception that lactose was to blame. This

proved to be detrimental to the dairy industry, thus, prompting the A2 Milk Company to lead research exploring the cause of consumer milk consumption discomfort. It was determined that A1 milk, when digested, will produce β-casomorphin-7 (BCM-7) which has been linked to poor gastrointestinal function and increased inflammation in the gut. Breaking research is still being conducted to determine if A2 milk confers more health benefits than A1 milk, such as reduced allergic reactions and higher digestibility. If such benefits are found, there is an increased chance that consumers will return to consuming milk and milk products. With an increased demand for A2 milk products, the industry should focus on developing more A2 milk protein herds through, for example, focusing on breeding programs. We believe A2 milk will continue to provide a wholesome, nutrient-rich, and safe product for consumers with an added benefit of reduced digestive complications.

Key Words: A1, A2, milk

ADSA Graduate Student (MS) Production Oral Competition

133 Evaluating the duration of increased milking frequency during early lactation for increased yield through lactation. K. R. Tate*, M. L. McGilliard, A. J. Lengi, and B. A. Corl, *Virginia Tech, Blacksburg, VA.*

Increasing the milking frequency of dairy cows to 4 times a day (4×) in early lactation increases milk yield both during the increased milking frequency (IMF) treatment and after the cows are returned to 2× milking. Modifications of this approach have been examined to maximize production and determine the most efficient practice for producers. The aim of this study was to investigate the effect of duration of early lactation IMF on milk yield (MY). Twenty-one primiparous and multiparous Holstein cows were assigned to have the right udder half milked 4× (0300, 0600 1500 and 1800) for 10, 20, or 40 d (n = 7), and the left udder half 2× (0300 and 01500). Udder-half milk yields were measured at 10, 20, 40, 60, 120, 150, 180, 210, 240, and 270 d of treatment, and yield differences per day between halves were calculated. Data were processed through the GLIMMIX procedure of SAS (SAS 9.4; SAS Institute, Inc., Cary NC). For 10-, 20-, and 40-d treatments, mean udder half differences (4× − 2×) through 270 d of treatment were calculated for MY (0.87, 1.75 and 4.09 kg), fat yield (13, 61, and 136 g) and protein yield (28, 52, and 117 g). Only the 40-d treatment differed from zero for each yield, including solids-nonfat, lactose, and somatic cell count ($P <$ 0.05). Interactions of treatment by DIM and treatment by parity were not significant for udder half differences of any measures ($P = 0.36$). The MY difference was different from zero on d 20 for the 20-d treatment, and on d 10, 20, 40, 180, 210, 240 and 270 for the 40-d treatment ($P <$ 0.05). Fat yield difference was different from zero on d 10 for the 20-d treatment and d 10, 20, 60, 120, 180, 210, 240 and 270 for the 40-d treatment ($P < 0.05$). Protein yield difference was different from zero on d 10 for the 10-d treatment, on d 20 for the 20-d treatment, and on d 10, 20, 40, 180, 210, 240, and 270 for the 40-d treatment. Overall, the 40-d treatment resulted in greater milk and component yields. *Supported by Agriculture and Food Research Initiative Competitive Grant no. 2017–67015–26538 from the USDA NIFA and project 683 of the VA Agricultural Council.*

Key Words: milking frequency, early lactation, milk yield

134 Effects of differing planes of pre- and post-weaning phase nutrition on intake, growth and puberty in Holstein heifer calves. J. P. Rosadiuk*[1], F. Moslemipur[1,2], T. C. Bruinjé[1], D. J. Ambrose[1,3], and M. A. Steele[1], [1]*Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Gonbad Kavous University, Golestan, Iran,* [3]*Livestock Research Section, Alberta Agriculture and Forestry, Edmonton, AB, Canada.*

By offering an elevated plane of nutrition to heifers through the pre- and post- weaning phases, energy intake, growth, and future productivity may be increased. The objective was to determine the effects of pre- and post-weaning diets, with differing energy levels, on the intake, growth, and attainment of puberty in Holstein heifer calves. Animals were randomly assigned to either a low (n = 18) or high (n = 18) pre-weaning diet (5 L/d or 10 L/d of pasteurized whole milk, wk 1 to 8.5), and to either a low (n = 18) or high (n = 18) ad libitum post-weaning dry TMR diet [30% straw and 70% concentrate) or 15% straw and 85% concentrate, from wk 9 to 25 of age], in a 2 × 2 factorial experiment. Free choice concentrate was available to all calves in the pre-weaning phase, and free choice water was available to all calves throughout the study. Feed intake was recorded daily from birth to 6-mo of age. Body weight, hip height, withers height, and heart girth, were recorded on a weekly basis. Effects of pre- and post-weaning treatments were assessed using PROC MIXED. Mean heights of hip and withers did not differ between treatment groups at any point during the study. There was a significant treatment by week effect on body weight in the pre-weaning period from wk 2 to 9, however this interaction did not occur in the post-weaning period. Regardless of the diet received in the pre-weaning phase, heifers offered the high plane of nutrition in the post-weaning phase had significantly greater metabolizable energy intake than those receiving the low plane in wk 11 (high = 9.52 Mcal/d, low = 8.53 Mcal/d, $P = 0.003$) through 25 (high = 22.05 Mcal/d, low = 17.38 Mcal/d, $P < 0.001$). A subset of heifers from both high (n = 11) and low (n = 12) post-weaning treatments was monitored for confirmation of first ovulation via transrectal ultrasonography. Mean days to first ovulation were significantly reduced in high post-weaning calves compared with low post-weaning calves (201.73, SE = 7.76 vs. 240.75, SE = 7.36, $P =$ 0.04). Overall, early life elevated planes of nutrition increased energy intake, and decreased age at puberty without compromising growth.

Key Words: plane of nutrition, feed intake, pre- and post-weaning

135 Effects of feeding supplemental butyrate on passive transfer of immunity in Holstein calves. R. Hiltz* and A. Laarman, *University of Idaho, Moscow, ID.*

Passive transfer of immunity, a key component of animal health in early life, is primarily the result of immunoglobulin (IgG) production in the dam and IgG absorption in the calf. This study examined the effect of supplemental butyrate on serum IgG concentrations in late pregnancy dams and their calves. Twenty multiparous Holstein dams were fed a close-up TMR ad libitum and assigned either a control treatment (DAM-C) or a butyrate treatment (DAM-B). Dams in the DB treatment were fed butyrate daily at a rate of 1% of DMI, mixed into the TMR, from 3 wk prepartum until calving. Until calving, DMI was measured daily and blood serum was sampled weekly. At calving, colostrum was sampled and analyzed for IgG. At birth, calves were assigned to either a control (CALF-C) or a butyrate treatment (CALF-B). Within 2 h of calving, all calves were fed one gallon of colostrum replacer; colostrum fed to CALF-B calves was supplemented with butyrate at 2.5% wt/vol. Weekly, calves were weighed and blood was sampled for determination of average daily gain and serum IgG, respectively. Data were analyzed using PROC MIXED in SAS with the fixed effect of treatment, random effect of animal, and, where applicable, fixed effect of week as a repeated measure. In dams, there was no difference in DMI between DAM-C and DAM-B treatments (25.0 ± 3.2 vs. 24.1 ± 2.1 kg/d, respectively; $P = 0.77$). Serum IgG concentration in dams was not different between the DAM-C and DAM-B treatments (1785 ± 117 vs. 1736 ± 137 mg/dL, respectively; $P = 0.79$). Colostrum IgG levels were also unchanged between DAM-C and DAM-B treatments (160.3 ± 72.09 vs. 117.45 ± 35.09 g/L respectively; $P = 0.46$). In calves, serum IgG levels were higher in CALF-C than in CALF-B treatments (1489 ± 71 vs. 997 ± 63 mg/dL, respectively; $P < 0.01$). Calf average daily gain did not differ between CALF-C and CALF-B treatments (0.54 ± 0.05 vs. 0.49 ± 0.05 kg, respectively; $P = 0.43$). In dams, feeding supplemental butyrate did not impact DMI, serum IgG concentration, or colostrum IgG concentra

tion. In calves, supplemental butyrate had detrimental effects on serum IgG concentration, adversely affecting passive transfer of immunity.

Key Words: passive transfer, immunoglobulins, butyrate

136 Double-blind, block-randomized, placebo-controlled clinical trial on effectiveness of zinc supplementation on diarrhea and average daily gain in pre-weaned dairy calves. H. R. Feldmann*[1], D. R. Williams[1], J. D. Champagne[1], T. W. Lehenbauer[1,2], and S. S. Aly[1,2], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare, CA,* [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA.*

The objective of this clinical trial was to evaluate the effectiveness of zinc supplementation on diarrhea and average daily weight gain (ADG) in pre-weaned dairy calves. A total of 1,482 healthy Holstein heifer and bull calves from a large California dairy were studied between December 2015 and June 2016. Each calf was enrolled at 24 to 48 h of age until exit from the hutch at approximately 90 d of age. Calves were block-randomized by time to 1 of 3 treatments: (1) placebo, (2) zinc methionine (ZM), or (3) zinc sulfate (ZS) administered in milk once daily for the first 14 d. Serum total protein at enrollment and body weight at birth, end of treatment, and hutch exit were obtained. Fecal consistency was assessed daily for 28 d post-enrollment. For a random sample of 127 calves, serum zinc concentrations before and after treatment and a fecal antigen ELISA at diarrhea start and resolution using a commercial kit (Pathasure Enteritis 4; Biovet, Quebec, Canada) for *Escherichia coli* K99, rotavirus, coronavirus, *Cryptosporidium parvum* were performed. Linear regression demonstrated that bull calves treated with ZM had 22g and 27g increased ADG compared with placebo-treated ($P = 0.042$) and ZS-treated bulls ($P = 0.014$), respectively. Conversely, ZM-treated heifers had 12g decreased ADG compared with placebo-treated heifers ($P = 0.019$). There were no other significant differences in ADG. Cox Proportional Hazard regression showed that ZM and ZS-treated calves had a 14.7% ($P = 0.015$) and 13.9% ($P = 0.022$) reduced hazard of diarrhea, respectively, compared with placebo-treated calves. Additionally, 8-d-old calves treated at least the first 5 d of diarrhea with ZM and ZS had a 15.6% ($P = 0.028$) and 8.4% ($P = 0.039$) increased hazard of clinical cure, respectively, compared with placebo-treated calves. Logistic regression showed that the odds of microbiological cure at diarrhea resolution for any single fecal pathogen was not different between treatments. The current trial showed a potential role of zinc supplementation for improved weight gain and diarrhea prevention in pre-weaned dairy calves with the need for further research on sex-specific or weight-based dosing.

Key Words: zinc, dairy calf, diarrhea

137 Manipulating serotonin pathway impacts glucose metabolism in dairy calves. S. L. Field*, M. G. Marrero-Pérez, A. L. Skibiel, B. Dado-Senn, D. R. Silva, and J. Laporta, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Serotonin (5-HT) is a monoamine that play a role in the regulation of energy balance through the modulation of glucose and lipid metabolism. Here, we examined whether manipulating 5-HT pathway by administering Fluoxetine (FLX, a 5-HT reuptake inhibitor) or 5 hydroxy-L-tryptophan (5-HTP, a 5-HT precursor), would impact hepatic glucose metabolism in pre-weaned dairy calves. Bull Holstein calves (21 ± 2d old) were fed milk replacer (8 L/d) with saline (CON, 8 mL/d n = 8), FLX (40 mg/d, n = 8) or 5-HTP (90 mg/d, n = 8) for 10 consecutive d in a complete randomized block design. Blood samples were collected before (d-1), during treatment (d1–10) and after treatment withdrawal (d12, 13, 14, 16, 24) to measure glucose concentrations. Two subset of calves (n = 4/group) were euthanized on d10 or d24. Liver tissue was harvested to measure gene expression of 5-HT receptor 2 (*5-HTR2A, 2B, 2C*), 5-HT transporter (*SERT*), tryptophan hydroxylase (*TPH1*), glucose transporters (*GLUT-1* to *12*) and gluconeogenic enzymes (*PDK4, PC, G6P*) by real-time PCR. Data were analyzed using mixed models in SAS. Calves fed FLX and 5-HTP had higher circulating glucose compared with CON (110 and 105.5 vs 96 ± 3.8 mg/dl, respectively; $P < 0.001$). On d10, *TPH1* tended to be downregulated in 5-HTP treatment ($P < 0.10$) and *SERT* was downregulated in both treatments compared with CON ($P < 0.01$). On d24, *SERT* and *TPH1* were not different among treatments. On d10, *5-HTR-2A* was and *2B* tended to be upregulated in the 5-HTP treatment ($P < 0.09$) while *2C* was downregulated in the FLX treatment compared with CON ($P < 0.02$). On d24, *5-HTR-2A* and *2C* were not expressed and *2B* was not different among treatments. Gluconeogenic enzymes were not different among treatments on d10, but *PDK4* was and *G6P* tended to be downregulated and PC was upregulated in 5-HTP treatment compared with CON ($P < 0.07$) on d24. On d10, glucose transporters were not different among treatments, while on d24 *GLUT-9* tended to be upregulated in 5-HTP treatment and *GLUT-12* was downregulated in both treatments compared with CON ($P < 0.09$). Feeding FLX and 5-HTP increased circulating glucose and altered hepatic expression of gluconeogenic enzymes in dairy calves.

Key Words: glucose, serotonin, calves

138 Feeding behavior, dry matter intake and intake rate in dominant and subordinate dairy heifers allocated in competitive dyads. C. Fiol*[1], I. Donadio[1], L. Eustathiou[1], G. Matto[1], M. N. Méndez[1], V. Sánchez[1], F. Triay[1], M. Aguerre[2], M. Carriquiry[3], and R. Ungerfeld[4], [1]*Departamento de Bovinos, Facultad de Veterinaria, UdelaR, Libertad, San José, Uruguay,* [2]*Red Tecnológica Sectorial de Lechería, Montevideo, Uruguay,* [3]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, UdelaR, Montevideo, Uruguay,* [4]*Departamento de Fisiología, Facultad de Veterinaria, UdelaR, Montevideo, Uruguay.*

The objective was to evaluate feeding behavior, individual DMI and intake rate in dominant (DOM) and subordinate (SUB) pre-pubertal dairy heifers maintained under competitive situations. Holstein (n = 12) and Jersey × Holstein (n = 4) pre-pubertal heifers (250.8 ± 9.8 d old, 208.5 ± 13.9 kg; mean ± SEM) were allocated into 8 homogeneous dyads for 120 d (d 0 = beginning of measurements), and received a total mixed ration (TMR) on 1 feeder/day. The DOM and SUB heifers were determined by observation of the winner in agonistic interactions in each dyad after TMR supply. Behavioral activity of each heifer was recorded by instantaneous scan-sampling, every 10 min for 12 h (hour 0 = TMR), in 7 periods (corresponding to d 1, 21, 35, 60, 75, 100 and 120). Individual DMI was estimated with double marker technique, in 3 periods (I, II, and III corresponds to d 17–26, 78–87, and 112–120), while intake rate (DM/min) was calculated as DMI/eating time. Behavioral data were analyzed with PROC GLIMMIX, while DMI and intake rate were analyzed by PROC MIXED. During the first 5 h after feed delivery, DOM spent more time ruminating (12.2 and 8.4%, DOM and SUB; SE = 1.2; $P < 0.01$) and lying (18.6 and 17.4%; SE = 2.7; $P = 0.04$) than SUB, but SUB spent more time standing than DOM (30.0 and 34.8%, DOM and SUB; SE = 2.4; $P = 0.05$). Along the 12 h of recording, DOM spent more time eating than SUB on hour 4 (32.1 and 26.6%; SE = 3.0; $P = 0.05$) and 6 (15.9 and 10.2%, SE = 3.6; $P < 0.01$). While on hour 1 (11.3 and 16.9%, SE = 3.6), 2 (21.6 and 28.7%; SE = 3.6) and 4 (44.6 and 51.6%; SE = 3.6) SUB spent more time standing than DOM heifers ($P < 0.01$). Dry matter intake was similar for DOM and SUB (6.7 and 6.9 kg/d, DOM and SUB; SE = 0.3; $P > 0.05$), but SUB ate at a faster rate (0.044 and 0.049 kg/min, DOM and SUB; SE = 0.003; $P = 0.02$) on Period II compared with DOM heifers. In conclusion, SUB had greater intake rate, with no differences on food intake, spent less time ruminating, and showed more behaviors probably associated with greater levels of social stress than DOM heifers.

Key Words: cattle, social hierarchy, social behavior

139 Will dairy cows sort their diet in response to negative energy balance? S. M. Moore* and T. J. DeVries, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to determine how feed sorting behavior of dairy cows is altered in response negative energy balance. Holstein cows (n = 30; DIM = 59 ± 5) were followed for 2 wk where they were fed (on a DM basis) a lactating diet (NE_L = 1.66 Mcal/kg; 68% forage) and produced 44.6 ± 1.2 kg/d of milk. To induce negative energy balance, cows were then exposed for 3 wk to a TMR formulated for a 12% reduction in energy available for milk (NE_L = 1.56 Mcal/kg; 73% forage). Blood samples were taken 1x/wk during the baseline period and every 4 d while on the treatment diet and analyzed for NEFA and BHB. TMR samples (fresh and orts) were collected every 3 d and separated into: long (>19 mm), medium (<19 mm, >8 mm), short (<8 mm, >4 mm), and fine (<4 mm) particles. Feed sorting was calculated as actual intake of each particle fraction expressed as a % of predicted intake. Data were analyzed in repeated measures, mixed-effect linear regression models. During the baseline period cow NEFA and BHB averaged 0.29 ± 0.04 mmol/L and 0.71 ± 0.08 mmol/L, respectively. During the experimental period, NEFA and BHB increased ($P < 0.001$) to 0.32 mmol/L and 1.00 mmol/L, on average, with a peak of NEFA (0.59 ± 0.06 mmol/L) and BHB (1.12 ± 0.11 mmol/L) occurring 4 d after dietary change. During the baseline period, cows sorted against long particles (95.3 ± 1.4%), did not sort medium particles (99.8 ± 0.2%), and sorted for short (101.1 ± 0.8%) and fine (101.8 ± 0.6%) particles. During the experimental period, cows increased ($P = 0.008$) sorting against long particles (92.2 ± 1.3%), continued to not sort medium particles (100.0 ± 0.1%), increased ($P = 0.002$) their sorting for short particles (102.1 ± 0.8%), and continued to sort for fine (102.5 ± 0.6%) particles. In the baseline period there was no association ($P > 0.6$) of feed sorting and blood NEFA levels. In the experimental period, higher NEFA levels were associated with greater sorting against long particles (% long sorting = −15.0 × NEFA(mmol/L) + 97.0; $R^2 = 0.20$; $P = 0.01$). Further, in that period higher NEFA levels were associated with greater sorting for short particles (% short sorting = 4.8 × NEFA(mmol/L) + 100.5; $R^2 = 0.18$; $P = 0.02$). The results indicate cows alter their feed sorting behavior in response to experience of a period of negative energy balance.

Key Words: negative energy balance, sorting, behavior

140 Dynamic feed delivery times of an automatic feeding system and the effects on feeding behavior of dairy cows. R. Oberschätzl-Kopp*[1], B. Haidn[2], R. Peis[2], K. Reiter[2], and H. Bernhardt[3], [1]*Lely Germany GmbH, Waldstetten, Germany,* [2]*Bavarian State Research Center for Agriculture, Poing-Grub, Germany,* [3]*Technical University of Munich, Freising, Germany.*

Automatic feeding systems (AFS) are gaining importance due to rising demands on performance-related feeding and welfare of cows. Nevertheless, under practical conditions AFS are used with static settings for feeding times and frequencies without considering animal demands. Therefore, the objective of the study was to develop a dynamic way of feeding with an AFS concerning behavior of dairy cows and to analyze the effects of this feeding strategy on feeding behavior (frequency, duration, daily rhythm of visits at the feed bunk, meal criteria, frequency and duration of cows' meals). 80 lactating dairy cows housed in a barn with an AFS and an automatic milking system were subjected to each of 2 treatment periods with 7: static (A) and dynamic (B) feeding times per day in winter and summer time (n = 6 d/period). During period A cows were fed at 815, 1115, 1400, 1600, 1945, 2315 and 345 h. A real time location system collected individual animal behavior. Based on real time procession of positioning data regarding cows' duration of stay at the feed bunk, decisions were made about starting time of AFS feedings. A programmed algorithm using the median with boundaries time (60 s) and way (1 m) summarized positioning data. Meal criteria were calculated by fitting mixed models to observed frequency distribution of $\log_e$-interval lengths between feed bunk visits. Feedings in both trial periods in winter led to similar proportions of animals at the feed bunk with maxima of about 80% while cows left the feed bunk significantly ($P = 0.002$) earlier in the first 60 min after feeding during periods B. The daily duration of stay at the feed bunk did not differ in winter periods, whereas cows stayed there longer ($P = 0.003$) in summer

period B (5.42 ± 1.53 h/d). Meal criteria tended to be on a lower level during periods B, whereas there was a difference observed in summer investigation (39.74 ± 23.07 min vs. 32.63 ± 30.56 min, $P = 0.02$). Primiparous dairy cows showed slightly higher average duration of meals than multiparous cows during summer period B (1.03 ± 0.75 h vs. 0.93 ± 0.42 h). The results demonstrate that dynamization of feed delivery times by an AFS might be beneficial for an even distribution of feed bunk visits and meals, particularly in the summer time.

Key Words: automatic feeding system, dynamic feeding, animal behavior

142 Effect of a mechanical calf brush on the behavior and performance of recently weaned heifer calves. A. Velasquez*[1], D. Manriquez[1], S. Paudyal[1], G. Solano[1], H. Han[1], R. Callan[2], J. Velez[3], and P. Pinedo[1], [1]*Department of Animal Sciences, College of Agricultural Sciences, Colorado State University, Fort Collins, CO,* [2]*Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO,* [3]*Aurora Organic Dairy, Boulder, CO.*

Calf stress at weaning and after transfer to group pens is a concern in dairy farms. Favoring natural behaviors, such as grooming, may help to reduce this challenge. Our objective was to evaluate the effect of a mechanical brush on the performance and behavior of recently weaned calves. A paired comparison design with 2 treatment groups (control [CON, n = 81], treatment [TRT, n = 81]) was performed. Organic Holstein heifer calves (94 ± 7 d old) were each monitored for 3 wk. Four cohorts were enrolled sequentially considering one CON and one TRT group (1 brush/pen, n = 19–20 calves/pen). At enrolment, each calf was weighed and subject to a health evaluation. A 3-D accelerometer sensor was attached to the left ear. Continuous and excluding measurements (min/h) were generated for the following behaviors: Not-active, active, highly active, eating, and ruminating. Calf were weighted at the last day of the study. Data were summarized as daily averages (min/h). In addition, total monitoring time was divided in 4 periods of 5 d and analyzed as hourly averages (min). Data were examined using repeated measures analyses ANOVA, considering values by day or hour as the repeated measure. No difference was found in average daily weight gain between treatment groups. Significant differences were found for the interaction time and treatment group, indicating greater values for CON calves on the time spent not-active (22.8 ± 0.82 vs. 21.7 ± 0.82 min/h; $P = 0.014$) and lower values for the time eating (6.43 ± 0.40 vs 7.01 ± 0.40 min/h; $P = 0.01$) during the 20 d in study. Hourly not-active time was greater for CON pens for Period 1 (18.3 ± 0.78 vs 17.9 ± 0.78 min/h; $P < 0.001$), Period 2 (24.7 ± 1.08 vs 22.8 ± 1.08 min/h; $P = 0.01$). Similarly, differences in favor of TRT group were found for time with high activity in Period 2 (10.3 ± 0.57 vs 10.0 ± 0.57 min/h; $P < 0.001$), Period 3 (9.7 ± 0.58 vs 9.1 ± 0.58 min/h; $P = 0.049$), and Period 4 (9.9 ± 0.58 vs 9.6 ± 0.61 min/h; $P = 0.02$). Differences in daily eating time were found for Period 1 in favor of CTR (8.3 ± 0.51 vs 8.0 ± 0.51; $P <$ 0.001 min/h) and Period 2 in favor of TRT group (6.44 ± 0.54 vs 5.48 ± 0.54 min/h; $P = 0.003$). We conclude that the use of a mechanical brush throughout the days of group housing reduced inactive time, while increasing eating time.

Key Words: mechanical brush, grooming, behavior

143 Short-term overstocking, heat stress, or combination on the welfare of lactating dairy cows. A. R. Lee*[1], G. M. Pighetti[1], R. J. Grant[2], J. L. Edwards[1], and P. D. Krawczel[1], [1]*University of Tennessee Knoxville, Knoxville, TN,* [2]*William H. Miner Agricultural Research Institute, Chazy, NY.*

Overstocking (OS) and heat stress (HS) are 2 primary management challenges impacting dairy cow welfare. The objective was to evaluate the effects of a single stressor, OS or HS, or concurrent dual stressors (OS and HS; OSHS) on behavior and milk production of lactating dairy cows. A 4 × 4 Latin square was implemented on 64 cows (parity and days in milk (DIM) ± SEM; 1.7 ± 0.1 and 129 ± 8, respectively) housed in sand freestalls and milked 2×/d during summer 2017. Each period included a 7 d acclimation and 7 d of data collection. Respiration rate (RR) was recorded at 16:00 ± 1 h 4 ×/weekly and vaginal temperature (VT) was collected via blank CIDR and Ibutton (Embedded Data Systems, Lawrenceburg, KY) on 6 cows/pen, to determine cows' heat stress. Pen temperature humidity index (THI) was recorded during all periods. A subset of cows (n = 11/pen; parity and DIM ± SEM; 1.5 ± 0.8 and 122 ± 39, respectively) were equipped with accelerometers. The MIXED procedure (SAS 9.4, Cary NC) evaluated the fixed effects of DIM, parity, treatment, and THI on lying time, steps/d, milk production, and all 2-way interactions, blocked by pen and period. Manual backward elimination removed non-significant 2-way interactions, with all fixed effects remaining in the model, regardless of significance. Respiration rate was 14 to 16 breaths/min greater among HS and OSHS cows than non-HS cows ($P < 0.001$), and VT was equal between treatments ($P \geq 0.73$). Elevated RR suggested cows experienced mild HS without modifying VT. Primiparous OSHS cows lay 51 and 71 min/d less than OS and HS cows, respectively ($P < 0.003$). Overstocked and OSHS cows took 186 and 193 more steps/d than cows exposed to no induced stressor ($P < 0.002$), suggesting more displacements and standing time among OS cows. Heat stressed and OSHS cows produced 1.7 ± 0.8 and 2.1 ± 0.8 kg/d ($P < 0.001$) less milk than non-HS cows. Despite short duration of treatments, behavior and milk production were influenced by individual stressors, with no greater effect on cows exposed to dual stressors. We hypothesize prolonged dual stressors may be more detrimental to animal behavior and production.

Key Words: behavior, overstocking, heat stress

144 A novel approach to estimate intake of lactating dairy cows through multiple on-cow accelerometer sensors. N. A. Carpinelli*, F. Rosa, R. C. B. Grazziotin, and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Accurate prediction of intake of dairy cows will provide substantial improvements in herd health and management. Therefore, the objective was to evaluate the feasibility of using 3-dimensional accelerometer sensors for the estimation of individual intakes of lactating dairy cows. Twenty-four late-lactation Holstein dairy cows housed in a freestall barn were fitted with 3 sensors that record acceleration in the 3 axes (i.e., x, y, and z), one on the lateral side of the left hind leg and 2 attached to a halter directly superpose over the jaw and nose. Cows were assigned 2 groups, a data collection group (A; n = 12) and a validation group (B; n = 12). Cows were trained to use Calan gates during an adaptation period (7 d), and followed by 10 consecutive days of data collection of acceleration and individual intakes for both groups. Four cameras were used to continuously video record all cows, and eating times for each cow were generated. Sensors were set to record the 3D accelerations at 10-s intervals. Eating times and accelerometer data from group A was cross-reference based on date and time. Acceleration data corresponding to eating times and falling between the 1st and 3rd quartile was selected. The REG procedure of SAS 9.4 was used to regress the pre-selected acceleration data against the daily intake data for each cow to evaluate which acceleration combination could account for most of the varia

tion. The combination Y-nose + Y-jaw + Y-leg + X-leg had the highest R^2 of 40.3%. The model obtained from this accelerometer combination in group A was tested in group B, and the overall estimated DMI was 23.2 ± 1.65 kg/d, while the actual DMI of group B was 23.7 ± 4.7 kg/d. Although the overall DMI estimation is accurate, a significant amount of cow variation is not accounted for in this model, and this is reflected in the greater standard deviation in the actual intakes in group B. These results suggest that multiple accelerometer data can accurately predict DMI; however, a more robust model will be obtained using a greater sample size.

Key Words: accelerometer, intake, sensor technology

145 Validation of an ear-tag accelerometer to identify feeding and activity behaviors of tie-stall housed dairy cattle. A. Zambelis, T. Wolfe, and E. Vasseur*, *Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, QC, Canada.*

The objective of this study was to validate the CowManager SensOor ear-tag accelerometer against visual observations of feeding, rumination, resting, and active behaviors in a tie-stall dairy facility. Prior validation of the sensor has been published for free-stall and grazing dairy herds, however the vast array of behavioral differences that exist among these and a tie-stall system necessitate additional validation. Lactating Holstein cows (n = 10) in different lactation stages and parities were included in the study at the McGill University Macdonald Campus Dairy Complex. Cows were monitored both visually and with the sensor for 10 h/d for 4 consecutive days (10 cows × 10 h × 4 d = 400 h of observation total). A single trained observer classified each minute of visual observation into 1 of 13 behaviors, and then summarized them into the 4 categories of eating, rumination, not active, or active. The sensor registered ear movements continuously and based on a proprietary model, converted them into the 5 behavioral categories of eating, rumination, not active, active, and high active. Multivariate mixed models were run to obtain covariance estimates, from which correlation coefficients were computed to assess agreement between observer and sensor data. The models included the percentage of each behavioral category per day as the dependent variable, and technology (observer versus sensor) and day as fixed effects. The models also included the random effects of technology, and the repeated effects of technology and day. The correlation strength between visual observation and sensor data varied from poor to almost perfect by behavior category (eating: r = 0.27; rumination: r = 0.69; total nutrition: r = 0.83; not active: r = 0.95; and active: r = 0.89). The results suggest that the sensor can be used to accurately monitor active and not active behaviors of tie-stall housed dairy cows. The results also suggest that while the sensor shows promise for identifying feeding behaviors in general, the independent classification of rumination and eating requires additional sensitivity.

Key Words: precision dairy technology, activity monitoring, feeding behavior

146 The effect of two different indoor AMS loose-housing options and pasture-access on dairy cow step activity and time budget. E. Shepley*[1], H. Leruste[2], J. Lensink[2], and E. Vasseur[1], [1]*McGill University, Ste-Anne-de-Bellevue, QC, Canada,* [2]*Yncréa Hauts de France, ISA Lille, Lille Cedex, France.*

Dairy producers are moving away from tie-stall housing to loose-housing systems. However, gaps in knowledge on the effects of different indoor loose-housing systems exist, particularly regarding cow movement and activity opportunity. The study objectives were to determine (1) the effect of freestall vs. strawyard housing of dairy cows with AMS on step activity and time budget, and 2) whether activity was affected by pasture access. Twenty-four cows, balanced by DIM and parity, were randomly assigned to 6 groups and subjected to both a freestall (FS) and strawyard (SY) treatment for 1 wk each in a crossover design. Leg-mounted pedometers were used to obtain step activity and lying data. Time budget was assessed by instantaneous live scan sampling 2×/wk for 2 h/d. This design and procedure was repeated twice: at the end of winter housing and in the summer after 6 wk of pasturing. Correlations between step activity and pasture visits were done at the cow level and all other analyses were done at the group level using mixed models. There was no difference in step activity between housing types in winter or summer. In summer, number of visits to pasture was positively correlated with higher step activity in both the FS (r = 0.59) and SY (r = 0.59). There was no difference in lying time, but SY cows had more daily lying bouts during winter than FS cows (10.69 vs 9.23 bouts, $P \leq 0.05$). Maintenance behaviors were not affected by housing treatment, but SY cows socialized more than FS cows in winter (1.7 vs 0.9%, $P \leq 0.05$) and tended to exhibit more locomotor behaviors in summer (3.28 vs 1.58%, $P = 0.06$). Fewer environmental obstructions in the SY may have facilitated the expression of non-maintenance behaviors as well as ease of lying and rising, thus increasing lying bouts. Cows that were most active in both areas were also more likely to seek pasture access, suggesting that these cows had a greater motivation to move and may be more restricted by extensive indoor confinement. While SY had some benefits over FS housing, additional research is needed to find housing options that can meet cow movement and activity needs.

Key Words: housing, movement opportunity

147 An observational longitudinal cross-over study of the impact of an ammonium-lactate commercial product for the prevention of subclinical ketosis in postpartum lactating dairy cows. G. Poppy*[1], D. Barkey[1], and J. Pinter[2], [1]*Fermented Nutrition Corp., Luxemburg, WI,* [2]*Perdue AgriBusiness, Salisbury, MD.*

Subclinical ketosis (SCK) is an economic significant event in early lactation dairy cows. A field study was conducted to evaluate the feeding of 2 new commercial ammonium-lactate products, GlucoBoost Liquid or GlucoBoost Dry, on the prevalence rate (*PR*) of sub-clinical ketosis in postpartum, lactating dairy cows. Subclinical ketosis, defined as blood BHB ≥1.2 mmol/L, was evaluated using a Precision Xtra (Abbott Laboratories, Abbot Park, IL) handheld ketone meter. Samples were collected from postpartum 1–14 DIM primiparous and multiparous dairy cows. The test population was 51 herds, representing 84 separate trials. The herds were a convenience sample of herds that agreed to test the product and allow pre and post exposure testing. The herds were tested at a minimum of 2-week intervals for all cows in the post fresh pen 1–14 DIM up to a max of 100 cows per lactation. The herds were fed TMR diets containing either GlucoBoost Dry at 1 lb./cow/d and GlucoBoost Liquid at 2 lb./cow/day, both targeted to contained 336 g of lactate. The study included 5,972 first lactation, 4,750 second lactation, and 6,420 third or greater lactation cows. The 52 herds were analyzed for risk factors for prevalence of subclinical ketosis with SAS 9.4 (SAS Institute Inc., Cary, NC), using Proc GLIMMIX as a Poisson distribution, with herd as a repeated measure. Herds were stratified with "Low" ≤ 10% prevalence; "Mid" herds were > 10% and ≤25%, and "High" herds were > 25% starting prevalence. The final multivariable Poisson model included Product (Dry or Liquid), Strata (Low, Mid or High), and the Quarter of the year the test was conducted. The *PR* was calculated from the parameter estimate in the GlIMMIX model by taking the anti-log of the −2 Res Log Pseudo Likelihood estimate. The *PR* for feeding Dry GlucoBoost was 0.718 (CI = 0.639–0.806, $P \leq 0.001$), and feeding Liquid GlucoBoost form of ammonium lactate had a *PR* of 0.653 (CI = 0.581–0.735. $P \leq 0.001$). The intercept was 0.086. This study suggests both liquid and dry form of ammonium lactate are effective at lowering the prevalence of SCK, defined as blood BHB levels ≥1.2 mmol/L, by 30 to 35%.

Key Words: ammonium-lactate, ketosis, lactating

148 Validation of an infrared test measuring β-hydroxybutyrate on test-day milk samples to detect hyperketonemia. D. L. Renaud*, D. F. Kelton, and T. F. Duffield, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Ketosis is a common metabolic disorder affecting dairy cattle that results in a greater risk for the development of disease. The Dairy Herd Improvement Association (DHIA; CanWest DHI, Guelph, ON, Canada) have begun to utilize an infrared test (MilkoScan FT600, Foss Analytical A/S, Hillerød, Denmark) to detect milk β-hydroxybutyrate (BHB) as a herd surveillance test. However, the test has not been compared with the gold standard serum BHB as determined by a referent laboratory. The objective of this cross-sectional diagnostic accuracy study was to validate the DHIA milk BHB test to identify cows with hyperketonemia as determined by quantification of BHB in serum. A total of 320 cows from 17 dairy herds in southwestern Ontario with milk collected at routine DHIA collection and blood sampled within 24 h of the milk test. The BHB concentration in milk was determined using the DHIA milk BHB test and serum was sent to the Animal Health Laboratory (AHL). A nonparametric receiver operating characteristic (ROC) curve was generated to compare DHIA milk BHB concentrations to serum BHB concentrations determined at a laboratory. A total of 33 cows (11%) had a level of serum BHB >1.2 mmol/L. A linear regression model demonstrated that the concentration of DHIA milk BHB was moderately correlated with the concentration of serum BHB yielding an R^2 value of 0.61. The optimal cut point to determine hyperketonemia (>1.2 mmol/L) on the DHIA milk BHB test was ≥0.135 mmol/L, yielding a sensitivity of 79% and specificity of 92%. The performance of the DHIA milk BHB test varied depending on the DIM of the cows tested and the herd-level prevalence of hyperketonemia. This study demonstrates that the DHIA milk BHB test is a reliable measure to evaluate hyperketonemia when tested at routine DHIA collection and could be used as a monitoring tool for ketosis.

Key Words: ketosis, dairy cattle, β-hydroxybutyrate

149 Perilipin5 promotes hepatic steatosis in dairy cows via increasing lipid synthesis and inhibiting VLDL assembly. X. Sun, G. Liu, X. Li, and X. Li*, *College of Veterinary Medicine, Jilin University, Changchun, Jilin, China.*

Fatty liver is a common metabolic disorder in dairy cows during the transition period. Perilipin5 (Plin5), a lipid droplet coat protein, plays important roles in the development of hepatic steatosis. However, the expression of hepatic plin5 in dairy cows with fatty liver and the regulation mechanism of Plin5 in the development of hepatic steatosis of dairy cows remain unknown. Liver and blood samples were collected from healthy (n = 10) and fatty liver Holstein cows (n = 10). Hepatocytes were isolated from calves and transfected with Plin5 overexpression adenovirus and/or sterol regulatory element binding protein-1c (SREBP-1c) silencing adenovirus or treated with nonesterified fatty acids (NEFA) and/or Plin5 silencing adenovirus. In vivo studies, data from serum very low density lipoprotein (VLDL) and hepatic triacylglycerol (TAG) were analyzed with Wilcoxon-signed-rank-test; data from RT-qPCR and Western Blot of liver samples were analyzed with paired *t* tests. In vitro studies, data were analyzed with one-way ANOVA with subsequent Bonferroni correction. Immunohistochemistry, Western Blot and RT-qPCR result showed that Plin5 was highly expressed in steatotic liver of cows. Compared with healthy cows, cows with fatty liver exhibited severe hepatic TAG accumulation and increased fatty acid and TAG synthesis, but decreased VLDL assembly. Accordingly, we deduced that Plin5 could regulate the fatty acid and TAG synthesis and the assembly of VLDL. Overexpression of Plin5 in cultured bovine hepatocytes promoted fatty acid and TAG synthesis, but reduced the VLDL assembly. Knockdown of SREBP-1c reversed the effects of Plin5 overexpression on lipid synthesis and VLDL assembly. Moreover, NEFAs increased Plin5 expression, promoted lipid synthesis and inhibited VLDL assembly, which led to TAG accumulation in hepatocytes. Knockdown of Plin5 reversed the NEFAs-induced lipid synthesis and VLDL assembly. These results indicate that NEFAs activate Plin5-SREBP-1c pathway, which promotes TAG accumulation and inhibits VLDL assembly, thereby inducing the hepatic steatosis of dairy cows.

Key Words: perilipin5, fatty liver, dairy cow

150 The effect of feeding synthetic zeolite A prepartum on indices of mineral and metabolic status, milk production and reproduction in grazing dairy cows. J. R. Roche[1], A. Heiser[2], M. A. Crookenden[3], C. R. Burke[1], S.-A. Turner[1], B. Kuhn-Sherlock[1], and C. V. C. Phyn*[1], [1]*DairyNZ Ltd., Hamilton, New Zealand,* [2]*AgResearch Ltd., Palmerston North, New Zealand,* [3]*DairyNZ Ltd., Auckland, New Zealand.*

This study investigated the effect of feeding synthetic Zeolite A (sodium aluminum silicate) prepartum on blood minerals, metabolic indices, milk production, and reproduction in grazing dairy cows. Multiparous Holstein-Friesian cows were randomly allocated to either an untreated control group (n = 23) or a treatment group receiving 500 g/cow/d Zeolite A (n = 24) for 14 d before expected calving date (actual duration = 19.2 ± 3.9 d prepartum). Cows grazed pasture and were individually supplemented with 2–3 kg DM/cow/d maize silage with or without zeolite prepartum. Pastures were dusted with 90 g/cow/d magnesium oxide throughout and cows received 200 g/cow/d calcium carbonate for 4 d postpartum. Blood samples were collected pre-treatment (d −19) and at d-14, 0, +1, +2, +3, +4, +7, +14, +21, and +28 relative to calving. A subset of cows (n = 10 per group) also had blood collected daily from d −10 to +10, and again at d +18 and +24. Data were analyzed using a repeated-measures ANOVA with a mixed model (Proc Mixed, SAS 9.3) including treatment, day, and their interaction as fixed effects, cow as a random effect, and treatment duration and pre-treatment data as co-variants. Zeolite increased ($P < 0.05$) plasma calcium concentrations compared with controls from d −1 to +4 relative to calving; reducing the average prevalence of subclinical and clinical hypocalcemia (<2 and < 1.4 mmol/L plasma calcium) from 22.6 and 2.6% to 0% ($P < 0.001$ and $P = 0.07$), respectively. Plasma phosphorus were lower between d −14 to −1 prepartum ($P < 0.001$) and on d 0 and +1 ($P < 0.001$) and d +2 ($P = 0.09$) postpartum, indicating hypophosphatemia. Zeolite-treated cows also had lower ($P < 0.05$) average plasma magnesium prepartum, particularly on d −1 and 0. There were no differences ($P \geq 0.35$) in milk yield, energy-corrected milk yield, or somatic cell count during the first 100 d in milk. Plasma β-hydroxybutyrate and nonesterified fatty acid concentrations, and BW and BCS did not differ between groups ($P \geq 0.39$). Zeolite cows calved 2.8 d earlier (SED 1.04; $P < 0.05$) than control cows and conceived 7.5 and 13.8 d earlier ($P = 0.09$) relative to calving and the start of seasonal mating, respectively. In conclusion, prepartum Zeolite A supplementation in grazing cows reduced hypocalcemia risk and tended to reduce days to conception, but did not alter milk production or indicators of metabolic status.

Key Words: hypocalcemia, zeolite, periparturient

151 First study into the temporal relationship between metabolic disorders and lameness events over the course of a lactation. A. Mineur*[1], C. Egger-Danner[2], J. Sölkner[3], S. Vanderick[1], H. Hammami[1], and N. Gengler[1], [1]*ULiege-GxABT, Gembloux, Belgium,* [2]*ZuchtData, Vienna, Austria,* [3]*BOKU, Vienna, Austria.*

Lameness in dairy cows is an issue that can vary greatly in severity, and is of concern for both producers and consumers. Metabolic disorders are a major problem in themselves, and, next to this, can cause lameness. Indeed, lameness is an often occurring consequence of various metabolic disorders, such as subacute ruminal acidosis (SARA), ketosis or milk fever. The caused lameness event can occur weeks to months after the metabolic disorder making the detection of causality difficult. Moreover, detection of many metabolic disorders is very challenging and not straightforward. Mid-infrared (MIR) technology is already used for the prediction of major milk components, such as fat or protein, during routine milk recording and for milk payment. It was recently shown that this technology can also be used to predict novel components, linked to metabolic disorders of cows, such as ketone bodies, citrate and minerals. In the context of limiting the occurrence and severity of lameness, early prediction of lameness could help indicate the need to adapt the management and the environment of a cow at risk of lameness. Therefore, the aim of this study was to analyze the temporal link between metabolic disorders and lameness events, using locomotion scores of the cow and MIR based milk biomarkers for different metabolic disorders of her milk from previous test days. Data recorded between July 2014 and December 2014 consisted of 9,324 records, from 3,895 cows and 122 farms. Correct definition of the response variable is an important aspect as extremes in lameness severity, expressed on lameness scales, were more easily predictable. First results were obtained using covariance functions on correlations computed between averaged metabolites and lameness scores, per animal, across the lactation stage classes. Correlations tended to be higher when comparing predicted metabolites with lameness in the 3 following months, rather than the same one, hinting at a temporal relationship.

Key Words: mid-infrared spectroscopy, covariance functions, milk composition

152 An evaluation of the relationship between hyperketonemia and pre- and post-calving hoof lesions in dairy cattle. E. M. Wynands* and G. Cramer, *College of Veterinary Medicine, University of Minnesota, St. Paul, MN.*

Recent research has suggested changes related to fat mobilization are a risk factor for lameness development. The objective of this study was to determine the relationship between β-hydroxybutyrate (BHB) blood concentration of cows post-calving and hoof lesions during the first 150 d in milk. The study was an observational cohort with cows enrolled from 5 free stall dairy herds in Minnesota. Primiparous and multiparous cows were enrolled at the time of their pre-calving hoof trim, at the end of their lactation. Enrolled cows were hoof trimmed twice: pre-calving within 90 d before parturition, and post-calving within 150 d following parturition. Commercial hoof trimmers trained in lesion identification trimmed cows and determined the lesion type. After parturition, cows between 3 and 16 d in milk were tested once weekly for hyperketonemia using a hand-held ketone meter. Cows were classified as hyperketonemic if they had a blood BHB ≥1.2 mmol/L. In total, 517 cows had both a pre- and post-calving hoof trim and at least one BHB measurement. At the pre-calving trim, the lesion prevalence was 12% (62/517). The lesions present were corkscrew claw (32%), digital dermatitis (30%), thin soles (11%), white line (8%), sole ulcer (6%), and other (11%). The incidence of hyperketonemia was 30% (118/517). At the post-calving trim 26% (136/517) of cows had a lesion. The lesions present were corkscrew claw (44%), digital dermatitis (27%), white line (9%), sole ulcer (5%), and other (15%). The incidence of newly developed lesions was 22% (100/455). A logistic regression model was constructed for the odds of having a lesion at the post-calving hoof trim (n = 517). The odds ratio for the association between post-calving lesion status and hyperketonemia was 0.82 CI: 0.48 – 1.4 ($P = 0.45$). Cows with a lesion pre-calving had higher odds (OR: 4.5 CI: 2.5 – 8.1) of having a lesion post-calving. The precision of the estimate was low due to sample size. There was no interaction between pre-calving lesion status and hyperketonemia ($P = 0.53$). In conclusion, hyperketonemia does not appear to be a risk factor for future hoof lesions.

Key Words: hoof lesions, lameness

153 Using once per day milking as an adjunct treatment of hyperketonemia. M. E. Williamson*, T. F. Duffield, S. LeBlanc, T. DeVries, and B. W. McBride, *University of Guelph, Guelph, ON, Canada.*

Subclinical ketosis affects approximately 40% of dairy cows in North America. Current recommended treatments for ketosis focus on providing cows with more energy. However, the most effective treatments, such as oral propylene glycol, only resolve ketosis approximately 50% of the time. The objective of this research was to evaluate the effect of decreasing milking frequency from 2 milkings/day, to 1 milking/day for 2 wk in conjunction with a standard treatment of 5 d of oral propylene glycol in cows diagnosed with ketosis. Ketosis was defined as a blood β-hydroxybutyrate concentration of ≥1.2mmol/L. From November 2016 to September 2017, 104 ketotic cows from the University of Guelph Livestock Research Innovation Centre were studied. 55 cows were randomly allocated to the once/day milking group, and 49 were enrolled in the twice/day milking group. All cows inhabited the same pen during their time on trial, and all cows were milked in a DeLaval VMS robot equipped with Herd Navigator. Blood, milk, and urine samples were collected in a 21-d period to analyze ketones over time in the cows. Disease occurrence was recorded up to 60 DIM and reproductive performance, examining days to first breeding, and pregnancy on the first breeding were explored. CanWest DHI herd recording data were collected weekly, for a period of 15 wk, examining milk production, milk component data, and SCC. Results indicate that once per day milking reduced the risk of subsequent ketosis, this was determined through the use of a logistic regression model in SAS. For cows in lactation 1, the odds of ketosis for once/day milking treatment was reduced by 99% compared with those in the twice/day milking treatment group on d 18 of trial ($P = 0.0005$, CI: 3.38×10^{-6}–0.0258). For cows in lactation 2+, the odds of ketosis for once/day milking treatment group is 0.18 times the odds of ketosis in the twice/day milking treatment group on d 18 of trial ($P = 0.0002$, CI: 0.0729–0.426).

Key Words: ketosis, milking frequency, dairy cow health

154 Calves born from cows fed with alfalfa enriched with selenium have higher Se in blood and higher phagocytosis. M. Mezzetti*[2,1], S. Jaaf[1], S. Busato[1], M. Premi[2,1], E. Trevisi[2], G. Bobe[1], and M. Bionaz[1], [1]*Oregon State University, Corvallis, OR,* [2]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Supplementation of dairy cows with selenized yeast during the last 8 weeks before parturition has been shown to improve selenium and IgG status of their offspring. Agricultural biofortification is an efficient, cost-effective method for selenium supplementation of cows and their offspring. In this study, we evaluated the effect of feeding sodium-selenate fertilized alfalfa to dairy cows during the close-up period on selenium status, immune function, and performance of their offspring. After blocking for breed, 12 dairy heifers (6 Holsteins and 6 Jerseys) were randomly assigned to 2 groups. One group received 1 kg DM alfalfa enriched with Se (3.2 mg/kg DM)/100 kg of BW mixed with TMR or the same amount of alfalfa with a standard Se level (0.4 mg/kg DM) for 40 d before parturition. Calves received their mother's colostrum and were monitored for BW at calving, ADG and health status. Whole blood samples from calves collected at 1 and 24 d of life were used to assess Se, Co, Zn, and Cu level using ICP-MS and white blood cells count (WBCC), leukocytes differentials (LD), and phagocytosis. Data were analyzed using proc GLIMMIX of SAS with treatment, breed, and time and their interactions as main effects. Feeding selenium enriched alfalfa vs. low selenium alfalfa to pregnant heifers increased ($P < 0.05$) Se (149.1 vs. 207.3 ng/mL), Co (0.34 vs. 0.54 ng/mL), and Cu (872 vs. 948 ng/mL) in whole blood of their offspring, indicating a synergistic effect of Se on utilization of other trace minerals. Overall, the Se was higher in Jersey vs. Holstein calves (203.87 vs. 147.5 ng/mL, $P < 0.05$). BW, ADG, health status, WBCC and LD were not affected in calves by the treatment of their dams. Higher rate of phagocytosis ($P < 0.05$) was detected in calves born from cows supplemented with high selenium vs. low selenium, indicating a better immune system in the former vs. the latter. Our data indicate that agricultural Se biofortification of dairy cows during the close-up period is an effective and cost-efficient management tool to improve the level of Se and other trace minerals in whole blood and the immune capacity in their offspring without adversely affecting their performance.

Key Words: agricultural biofortification, immune response, calves

Animal Health: Joint ADSA-National Mastitis Council Platform Session: Milk Quality and the Dairy Industry Today

156 Milk quality challenges and opportunities in robotic milking systems. D. Kelton*, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Milking robots were first introduced in Europe in 1990 and in Canada in 1999. Currently it is estimated that about 15% of dairy farms across Canada utilize this technology. While some systems have been installed in retrofitted barns, others have had new housing systems designed to maximize their potential for improved animal and labor efficiency. Advocates of robotic milking point to labor savings of up to 30%, the ability to easily segregate milk from cows with non-marketable milk, and the welfare advantages of allowing the cows to determine their own milking frequency. Detractors suggest that the milk from robot milked herds is of inferior quality (higher SCC and bacteria counts) and may contain more free fatty acids due to more aggressive milk handling than milk from farms using traditional milking systems. A recent review of milk quality data from over 3,600 dairy farms in Ontario, Canada, where almost 11% of farms milked with robotic milking systems were compared with parlor and pipeline milked herds, suggested that at least some of these milk quality concerns may be warranted. While raw milk quality penalties for SCC did not differ among the systems, robotic herds had more penalties for elevated bacteria counts, freezing point, and inhibitors. A more detailed analysis is currently underway to better describe and interpret these milk quality differences and to determine if there are important differences in free fatty acid levels in milk from robot milked herds.

Key Words: milk quality, robotic milking, SCC

157 Precision dairy technology-generated health alert accuracy and disease prediction. E. A. Eckelkamp*[1] and J. M. Bewley[2], [1]*University of Tennessee Institute of Agriculture, Knoxville, TN,* [2]*CowFocused Housing, Bardstown, KY.*

Precision dairy monitoring technologies provide real-time monitoring of cow health, reproduction, and management. The objective of this study was to assess disease detection accuracy by precision dairy technology health alerts and machine-learning techniques. We hypothesized the technology would detect health events, machine-learning would improve disease detection, and incorporating cow history with behavior changes would improve disease detection. The study occurred from October 2015 to October 2016 on 4 Kentucky dairy farms (1,374 cows). Health alerts were generated based on changes in eating (h/d), lying (h/d), standing (h/d), walking (h/d), and activity (steps/d). The FREQ procedure of SAS 9.4 was used to identify true positives, true negatives, false positives, and false negatives based on technology-generated cow alerts and recorded disease events by the lead researcher and dairy producers. Sensitivity, specificity, accuracy, and balanced accuracy were also calculated. Daily information collected by the technology (eating time, lying time, standing time, walking time, and activity) and cow history were incorporated into machine-learning prediction models. Technology-generated health alert sensitivity remained between 13 and 48% with a 91 to 97% specificity. Maximum balanced accuracy achieved with technology-generated health alerts was 59% at the widest time-windows of 5 d before to 2 d after and 3 d before to 3 d after the day of disease detection. The greatest balanced accuracy occurred when all behavior changes were considered in combination predicting any possible disease instead of a specific disease. All machine-learning analyses performed similarly and improved sensitivity and balanced accuracy compared with the technology-generated health alerts. Sensitivity ranged from 67 to 90%, specificity from 42 to 87%, accuracy 67 to 99%, and balanced accuracy from 66 to 87% across time-windows, disease categories, and behavior combinations. Unlike the technology-generated alerts, machine-learning predictions were best during the 24 h before the day of disease identification and when individual diseases were predicted.

Key Words: precision dairy technology, herd health, machine-learning

158 Intramammary casein hydrolysate alone or combined with other treatments when drying off dairy cows. J. E. Britten*, D. J. Wilson, and K. A. Rood, *Utah State University, Logan, UT.*

Alternatives to treatment of all quarters with antibiotics at dryoff are of interest in the dairy industry. The objective was to evaluate intramammary casein hydrolysate (CH), alone or in combination with antibiotic dry cow treatment (DCT) and/or teat sealant (TS) at dryoff (FDA and animal use committee sanctioned). Dairy cows (n = 32) from 6 commercial farms comprised 2 study groups: 16 pregnant cows estimated 60 d prepartum and 16 non-pregnant cows > 300 d in milk (DIM). A split udder design was used, with each udder half assigned a treatment and the contralateral half administered DCT + TS as a control. There were 4 treatments: 1. CH, 2. CH + DCT, 3. CH + TS, 4. CH + DCT + TS. Pregnant cows had quarter milk samples collected, with time "0" the time of dryoff: 0 d, 2 d, 4 d, 7 d, 10 d dry for SCC and biomarkers of involution pH, lactoferrin and serum albumin. Milk culture was at −4 d, 0 d, and 1–7, 8–14, and 15–21 DIM. Milk production was measured in udder halves before dryoff and again 3 DIM for proportion of total-cow milk production by each half. Cull cows were euthanized 2 d (n = 8) or 7 d (n = 8) after dry treatment and mammary tissue was sampled from ventral, mid, and dorsal regions of each quarter. Histopathology and tissue staining for degree of involution were performed. All 32 cows had no clinical signs or milk leakage at dryoff, and all 64 quarters of pregnant cows resumed milk production after calving. All milk was Delvotest SP-NT-negative for antimicrobial residues at 3 DIM. Treated and control halves' proportion of milk production were 47%:53% at dryoff, 51%:49% at 3 DIM ($P = 0.38$, 0.39, t-test). Involution was more evident histologically in all CH treated quarters than in controls; biometry measurements of % involuted tissue are in progress. Bacterial cure rates and new infection (NI) rates during dry period by treatment were: 1. 0%, 75%; 2. 100%, 0%; 3. 0%, 25%; 4. No infections to cure, 75%; controls 0%, 56% ($P = 0.66$ for cures, $P = 0.61$ for NI, chi-squared). Casein hydrolysate may be useful as an adjunct to other treatments, or possibly as a non-antibiotic alternative at dryoff of dairy cows.

Key Words: casein hydrolysate, dry treatment, mastitis

159 Selective versus blanket dry cow therapy. A. Lago*, *Dairy-Experts Inc., Tulare, CA.*

There is a pressing need for the judicious use of antibiotics in food animals to reduce the risk of antimicrobial resistance in pathogens of importance to human health. Selective dry cow therapy (SDCT) refers to the treatment with long-acting antimicrobials of only cows or quarters identified with or at risk of having an intramammary infection at dry-off,

or at risk of acquiring one during the dry period. Conversely, blanket dry cow therapy (BDCT) consists in the treatment of every quarter of every cow at dry off. BDCT has been widely adopted in the last decades and led to an important success in the reduction of contagious mastitis. However, recent studies report a low prevalence of intramammary infections at dry-off in many herds. This, in addition to the recent introduction of rapid on-farm diagnostic tests, and the availability of teat sealants, may allow development of successful SDCT strategies. Although the number of clinical trials with adequate internal validity evaluating the efficacy of SDCT is limited; it appears that farm selection is key for the success of SDCT strategies. Bulk tank SCC, as well as intramammary infection prevalence and etiology at dry off have been used to select herds benefiting from SDCT. Thereafter, the accurate identification of cows or quarters benefiting from antimicrobial treatment is the cornerstone for the implementation of SDCT. Strategies followed vary from use of cow records (e.g., SCC records, clinical mastitis history), culture results, cow-side diagnostic test results (California Mastitis Test, milk leukocyte differential count, etc), or a combination of them. The economic returns of SDCT include savings on antibiotics and milk withholding after parturition. However, even with no differences in health or performance for not treated cows or quarters, there are associated diagnostics and labor costs. In conclusion, the current epidemiology of mastitis in addition to the availability of new technologies make SDCT a logical step to reduce antibiotic use in dairy cows. However, there is still a need to large multi-herd randomized controlled studies to investigate whether SDCT programs will be effective under diverse management conditions.

Key Words: selective dry cow therapy, mastitis, antibiotics

160 Selective dry cow therapy to control mastitis and reduce antimicrobial use. S. McParland*[1], J. Flynn[1], N. Ryan[2], and P. Dillon[1], [1]*Animal and Grassland Research and Innovation Centre, Teagasc, Fermoy, Co. Cork, Ireland,* [2]*Department of Agriculture, Food and the Marine, Dublin, Ireland.*

Blanket dry cow therapy is a common preventative measure of mastitis control. However, concerns over antimicrobial resistance, may lead to concerns over blanket therapy as an unnecessary use of antimicrobials. Selective dry cow therapy involves only administering antimicrobials to those cows that require them at dry-off to cure existing infections. This research was conducted at 3 Irish research herds between 2015 and 2017. Prior to dry-off, weekly milk recording data were used to identify cows which had not exceeded 200,000 somatic cells at any point in lactation and to randomly assign them to 1 of 2 treatments: (1) antibiotic plus teat seal (AB&TS), or (2) teat seal only (TS). SCC was analyzed as (1) average, (2) minimum, and (3) maximum animal SCC across lactation, and as (4) test-day SCC. Analyses were conducted using records from the first 3 wk and first 120 d of lactation. The effect of treatment was quantified using a repeatability model accounting for concurrent experiment treatment level, breed (proportion of Holstein, Jersey, or Norwegian Red), heterosis, recombination, month of calving, parity (n = 4; 1, 2, 3, 4+), year (n = 3). For SCC traits with a single lactation value animal was repeated across years of the study. Animal was repeated across lactation week when the dependent variable was test-day SCC. The likelihood of having an SCC reading ≥ 200,000 was quantified using logistic regression adjusted for the same fixed effects as the linear model. Up to 56% of cows in the research herds (n = 364 lactations) were eligible for inclusion in the analyses. The minimum, maximum, and test-day SCC of TS only cows was greater than those cows that received AB&TS both in the first 3 wk and 120 d of lactation ($P < 0.05$). TS only cows were 2.9 times more likely to have an SCC reading > 200,000 within the first 120 d of lactation. However, the majority of cows (>80%) in both treatments maintained SCC < 200,000. Administering TS only to cows that have not had high SCC throughout lactation may offer a viable method to reduce on-farm anti-microbial use while not affecting herd-level SCC.

Key Words: selective dry cow therapy, antimicrobial use

161 Assessment of acoustic pulse therapy (APT), a non-antibiotic treatment for mastitis in dairy cows. G. Leitner*[1], D. Zilberman[2], E. Papirov[3], and S. Shefy[3], [1]*National Mastitis Reference Center, Kimron Veterinary Institute, Bet Dagan, Israel,* [2]*Department of Agricultural and Resource Economics, University of California, Berkeley, California,* [3]*HI-Impacts, Petach Tikva, Israel.*

A new acoustic pulse therapy (APT) device developed specifically for treating dairy cows produces high power, deep penetration acoustic pulses distributed over a large treatment area. We show findings from a clinical assessment of the technology for treating dairy cows with (1) subclinical and (2) clinical mastitis. In study 1, overall, 116 cows of 3 herds were identified with subclinical intramammary infection and enrolled in the study with 78 cows assigned to the treatment group and 38 cows to the control. In the treatment group 70% of the cows returned to normal milk production compared with 18.4% of the control. Daily milk yield of treated cows increased significantly ($P < 0.05$) and the percentage of cows with SCC $<300 \times 10^3$ cells/mL was significantly higher ($P < 0.001$). Milk of the infected quarters appeared normal with lactose greater than 4.8%, but with no significant difference. Of the treated cows with identified bacteria, 52.6% of quarters were cured while in the control only 25% ($P < 0.001$). Specifically, all cows identified with *Escherichia coli* in the treatment group were cured, with only 60% cure with no intervention in the control. Spontaneous cure of glands infected with coagulase-negative staphylococci (CNS) and streptococci was low, while treatment successfully increased cure of CNS from 13% to 50% and that of streptococci from 18% to 36%. Of the 4 cows identified with *Staphylococcus aureus*, 3 were cured. In study 2, 29 cows identified with clinical mastitis were submitted either to antibiotic (n = 16) or APT treatment (n = 13). The antibiotic treatment cured 18.7% of the cows where SCC lowered to $< 300 \times 10^3$ cell/mL and 52.6% were culled. The APT treatment cured 76.9% of cows with only a single cull (7.7%). Both studies were analyzed by SAS mixed procedure and GLM. Acoustic pulse therapy was found more effective than antibiotics or no-intervention in treating clinical and subclinical mastitis in dairy cows. In contrast to current treatment options of subclinical mastitis, which require early detection, APT is an easy to use confined treatment of cow's udders. It does not require bacterial identification or discarding of milk after treatment.

Key Words: clinical mastitis, subclinical mastitis, acoustic pulse therapy

162 Diffusion of antimicrobial resistance across management niches on dairy farms. W. M. Sischo*, J. A. Afema, M. A. Davis, and D. S. Kinder, *Washington State University, Pullman, WA.*

Background: A challenge in efforts to mitigate the global impact of farm origin antibiotic resistance is a failure to understand dissemination patterns of resistance within the farm via animals or environmental flow. This study focuses on diversity of phenotypic antibiotic resistance within niches on the dairy. The goals were to identify niches where diversity is generated and maintained, identify niches connected by phenotype similarity, and identify niches that narrow diversity. The premise was that diversity is generated in niches with high antibiotic use and disseminate

through the dairy system with animal movement or environmental flow. Twelve commercial dairy herds were sampled. Farm niches were defined by housing and function. Housing niches included areas with pre-weaned heifers, weaned and bred heifers, early lactation cows, lactating cows, pregnant nonlactating cows, antibiotic treated cows, and cows leaving the herd. Function niches include milk, waste, water, and feeds. For each sampling time, approximately 100 samples were collected across the niches. We used *E. coli* as our model and antimicrobial susceptibility tested 4 isolates from each sample against 13 antimicrobials. Latent class analysis (LCA) was used to organize isolate-based resistance patterns and clustered using K-means. Risk factors were assessed using multinomial logistic regression. Each farm was visited 3 times. More than 13,000 *E. coli* isolates were tested for antimicrobial susceptibility patterns. Based on LCA, 24 different patterns were observed. The most common pattern was pan-susceptible (68%) followed by a pattern with only tetracycline resistance (7%). The greatest diversity was observed in pre-weaned and weaned calves followed by treated animals. *E. coli* from adult cows were pan-susceptible (approaching 90% of isolates). *E. coli* from soil samples were pan-susceptible except for samples in pre-weaned housing areas. There were farm-specific patterns and between farm differences in diversity. There are niche-specific patterns of resistance that suggest there is little to no dissemination across niches. The between-farm patterns also suggest that resistance traits are farm-specific and driven more by local features rather than shared across spatially distinct farms.

Key Words: antibiotic use

163 Antimicrobial resistance in non-*aureus* staphylococci isolated from milk is associated with systemic but not intramammary administration of antimicrobials in dairy cattle. D. B. Nobrega*, J. De Buck, and H. W. Barkema, *University of Calgary, Calgary, AB, Canada.*

Emergence and spread of antimicrobial resistance (AMR) are major concerns for human and animal health worldwide. Consequently, there is increasing pressure to reduce antimicrobial use (AMU) in food-producing animals. In this project, we studied the association between AMU and AMR in non-*aureus* staphylococci (NAS) isolated from Canadian dairy herds. Distinct types of dry cow therapy (DCT), antimicrobials, and routes of administration were compared. The AMR profile was determined using a micro-broth dilution method against a panel of 23 antimicrobials for 1,702 NAS isolates obtained from 89 herds. A subset of these isolates was submitted to whole-genome sequencing and presence of AMR genes was determined using data from 4 databases. Antimicrobial use was determined for all herds using an inventory of empty drug containers and quantified for each antimicrobial as number of antimicrobial daily doses (ADD) administered. Data on management practices, including type of DCT, were obtained using questionnaires. Only systemic AMU was associated with prevalence of AMR in NAS, whereas intramammary or intrauterine use were not. Three drug classes, all of high or very high importance for human medicine, were associated with drug-specific AMR when administered systemically: penicillins, 3rd generation cephalosporins, and macrolides. Prevalence of *tet*, *erm* and bla_{ARL} genes in NAS was higher in herds that used more tetracyclines, macrolides, and 3rd generation cephalosporins, respectively. Mean AMU of antimicrobials administered as DCT was lower in herds using selective DCT in comparison to herds using blanket DCT. However, use of selective DCT was not associated with prevalence of AMR in NAS. The either weak or non-existent association between AMR and antimicrobials administered intramammarily suggest that a decrease in AMR of NAS following implementation of selective DCT would be minimal in comparison to reduced use of systemic antimicrobials

Key Words: antimicrobial use, antimicrobial resistance, non-*aureus* staphylococci

164 Antimicrobial resistance patterns of bacterial isolates from cases of mastitis in dairy cows. R. D. Abdi*, B. E. Gillespie, S. Headrick, G. M. Pighetti, R. A. Almeida, S. P. Oliver, and O. K. Dego, *Department of Animal Science, The University of Tennessee, Knoxville, TN.*

Mastitis is the most prevalent disease of dairy cows mainly caused by bacteria. Antimicrobials are largely used in dairy farmers for prophylactic control of mastitis as dry cow therapy. The use of antimicrobials in dairy cows for treatment of mastitis has improved their health and productivity, but prophylactic usage is under scrutiny due to the risk of antimicrobial resistance (AMR). AMR is a threat to animal and human health due to rising reservoir of resistant population of pathogenic bacteria. The aim of this study was to determine sensitivity of bacterial isolates from cases of bovine mastitis to commonly used antimicrobials. In total, 174-quarter milk samples from 148 cows, 3 composite milk samples from 3 cows and 1 bedding sample were collected from 35 dairy farms in Tennessee, Kentucky, and Mississippi. We tested isolates for sensitivity with a panel of 10 antimicrobials by MIC using the Sensititer system. We used descriptive statistics and logistic regression for data analysis. Overall, we isolated 197 isolates, comprising of 34% *Staphylococcus aureus*, 21.3% *Streptococcus uberis,* 18.3% *Streptococcus dysgalactiae*, 17.8% *Escherichia coli*, 6.6% *Klebsiella pneumoniae* and 2% *Klebsiella oxytoca*. The most prevalent pathogen at cow level was *S. aureus* followed by *Str. uberis, Str. dysgalactiae, E. coli*, *K. pneumoniae* and *K. oxytoca*, whereas at the farm level *Str. uberis* was the most prevalent followed by *Str. dysgalactiae*, *S. aureus*, *E. coli*, *K. pneumoniae* and *K. oxytoca.* Cows infected with *E. coli* mainly had clinical mastitis, whereas those infected with *S. aureus* had subclinical mastitis ($P < 0.05$). Ceftiofur showed the highest efficacy on the most isolates followed by cephalothin, but *K. pneumoniae* isolates were resistant to most of the antimicrobials tested. Isolates from subclinical mastitis had higher MIC ($P > 0.05$) compared with isolates from clinical mastitis for some antimicrobials. The 197 isolates showed 32 different AMR patterns, which varied with farms and states. Compared with *S. aureus* and *Str. dysgalactiae*, *E. coli* and *Klebsiella* spp. had widespread resistance to pirlmycin and higher MIC for most of the drugs tested. These results suggested that confirmatory diagnosis and subsequent sensitivity testing would be a prerequisite to treat these mastitis pathogens effectively.

Key Words: antimicrobial resistance, dairy cow, mastitis pathogen

Breeding and Genetics II: Methodologies, Inbreeding, and Breeding Strategies

165 Managing population diversity through genomic optimal contribution selection. C. Maltecca*[1], G. Gebregiwergis[3], J. T. Howard[4], C. F. Baes[2], and F. Tiezzi[1], [1]*North Carolina State University, Raleigh, NC,* [2]*University of Guelph, Guelph, ON, Canada,* [3]*Norwegian University of Life Sciences, Ås, Norway,* [4]*University of Nebraska-Lincoln, Lincoln, NE.*

Managing population diversity has been traditionally accomplished through pedigree information and various systems aimed at constraining the accumulation of inbreeding in mating populations. Optimal contribution selection (OCS) has been a popular method to guarantee long-term gains without compromising variability. Little information is available on the impact of using genomic information in conjunction with OCS. In this research, we investigated the use of alternative metrics of ancestry in OCS in simulated scenarios using genomic information. One production trait and one fitness trait were generated with the GenoDiver software following a typical dairy population structure. For the production trait, a polygenic trait (h^2 0.5, 1000 QTL) was simulated. For the fitness trait, partial dominance was simulated with varying proportions of lethal and sub-lethal fitness trait loci (FTL) ranging from 0 to 5% of the total FTL number. OCS was simulated for 30 generations. At each generation, genomic information was used to obtain breeding values of individuals, while different relationship measures (pedigree, genomic, and ROH (10 MB and 20Mb) were employed for optimal contribution. Selection was performed only on the production trait. Genetic progress for both production and fitness in all scenarios was measured at generation 30. Diversity measures analyzed included homozygosity, lethal equivalents, fitness, segregating (sub-)lethals, QTL and FTL lost. All methods were compared with a baseline of no OCS. In all cases, OCS maintained greater genomic variability in respect to the baseline. ROH-based methods achieved larger genetic gains compared with other methods. Pedigree-based methods maintained the largest variability with the lowest genetic gain. Genomic-based OCS was best at constraining homozygosity at lethal loci, while ROH-based methods were more effective for constraining sublethal loci. Both ROH- and genomic-based OCS were effective, with the ROH resulting in a good compromise between short-term genetic response and long-term fitness management.

Key Words: optimal contribution, genomic selection, runs of homozygosity (ROH)

166 Characterizing runs of homozygosity in Ayrshire, Brown Swiss, and Guernsey populations using varying sample sizes. C. Vogelzang*[1], F. Miglior[1,2], N. Melzer[3], M. Sargolzaei[1,4], C. Maltecca[5], G. Marras[1], B. Makanjuola[1], A. Fleming[1], F. Schenkel[1], and C. F. Baes[1], [1]*CGIL, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Canadian Dairy Network, Guelph, ON, Canada,* [3]*Leibniz Institute for Farm Animal Biology, Institute of Genetics and Biometry, Dummerstorf, MVP, Germany,* [4]*The Semex Alliance, Guelph, ON, Canada,* [5]*Department of Animal Sciences, North Carolina State University, Raleigh, NC.*

The accumulation of deleterious alleles can compromise the fitness of an animal. Genomic data provides a more accurate measure of homozygosity within an individual. Runs of homozygosity (ROH) are homozygous regions in the genome that occur when closely related parents transmit identical haplotypes to offspring. As such, ROH can be used to quantify realized inbreeding. Differences in the length, location, and number of ROH between different breeds can be observed, however population sizes of genotyped animals differ. To make breed comparison possible, it is of interest to characterize and compare ROH in different dairy breeds while taking sample size into account. The objective of this study was to investigate how sample population size affects estimation of inbreeding in a multi-breed study using ROH. A total of 13,274 genotyped animals in 3 breeds (6,560 Ayrshire, 5,667 Brown Swiss, and 1,047 Guernsey) were available. In a first analysis, ROH-based inbreeding was calculated for 500 randomly selected animals of each breed (5-fold cross-validation). In a second analysis, the same approach was used for 1,000 animals randomly selected from each population. A third analysis considered 500 and 1000 animals from each breed individually, and a fourth analysis considered all animals together. Statistical analyses were performed using the R package "*detectRUNS"* to determine if there is a significant difference in the calculation of inbreeding between different population sizes in a multi-breed study. The results of this study will help us to better understand how to approach multi-breed studies in the future, and to quantify potential breed biases when dissimilar breed population sizes are available.

Key Words: inbreeding, runs of homozygosity, dairy cattle

167 Indirect predictions based on SNP effects from single-step GBLUP in large genotyped populations. D. Lourenco*[1], A. Legarra[2], S. Tsuruta[1], D. Moser[3], S. Miller[3], and I. Misztal[1], [1]*Department of Animal and Dairy Science, University of Georgia, Athens, GA,* [2]*Institut National de la Recherche Agronomique, UMR*1388 *GenPhySE, Castanet Tolosan, France,* [3]*Angus Genetics Inc., St. Joseph, MO.*

The objectives of this study were to investigate whether SNP effects can be accurately estimated when the algorithm for proven and young (APY) is used in single-step GBLUP (ssGBLUP), and how close indirect predictions, based on SNP effects, are to genomic EBV (GEBV) from regular ssGBLUP. Tests involved an American Angus data set with 8,221,346 animals phenotyped for birth weight (6.2M), weaning weight (6.8M), and post-weaning gain (3.4M). Genotypes for 80,993 animals were used. Among the genotyped animals, the youngest 15,040, born from 2013 to 2014, were used for validation. The reduced data set had genotypes and phenotypes up to 2012; the complete data set had genotypes up to 2014. Based on the reduced data set, GEBV were calculated using regular ssGBLUP with direct inversion of G ($\mathbf{G}^{-1}$), and APY ssGBLUP ($\mathbf{G}^{-1}_{APY}$) with 11,000 core animals. The SNP effects were calculated based on a) $\mathbf{G}^{-1}$, b) $\mathbf{G}^{-1}_{APY}$, c) inverse of the core portion of $\mathbf{G}$ ($\mathbf{G}^{-1}_{core}$). Direct genomic values (DGV) for validation animals were obtained as the sum of SNP effects weighted by the genotype content, and the difference between pedigree and genomic base was added to obtain indirect predictions. The benchmark GEBV was obtained using regular ssGBLUP with complete data. Correlation between SNP effects obtained with $\mathbf{G}^{-1}$ and $\mathbf{G}^{-1}_{APY}$ was >0.99; lower correlation (0.93) was observed when using $\mathbf{G}^{-1}_{core}$. However, correlations between the benchmark GEBV and DGV from $\mathbf{G}^{-1}$, $\mathbf{G}^{-1}_{APY}$, and $\mathbf{G}^{-1}_{core}$ were all 0.99. The average difference between benchmark GEBV and DGV was 113.95, indicating a large bias. Indirect predictions that included DGV and the difference between pedigree and genomic base were unbiased. Accurate indirect predictions can be obtained when APY ssGBLUP is used. In addition, indirect predictions are comparable to GEBV after

adjustments for a genomic base. Backsolving genomic predictions to SNP effects may require only a group of genotyped animals representing the dimensionality of the genomic information. The results obtained in this study are applicable to large genotyped populations.

Key Words: algorithm for proven and young, direct genomic value, interim evaluations

168 Potential benefits from using a new reference map in genomic prediction. D. J. Null*[1], P. M. VanRaden[1], D. M. Bickhart[2], J. B. Cole[1], J. R. O'Connell[3], and B. D. Rosen[1], [1]*USDA Animal Genomics and Improvement Laboratory, Beltsville, MD,* [2]*USDA Dairy Forage Research Center, Madison, WI,* [3]*University of Maryland-Baltimore, Baltimore, MD.*

Many genomic studies in cattle have used the 2009 reference assembly from the University of Maryland (UMD3.1). A new USDA Agricultural Research Service-University of California, Davis (ARS-UCD) assembly based on longer DNA reads from the same cow (Dominette) should improve sequence alignment, imputation, and genomic prediction. To test imputation, markers were converted from their previous map locations to new map locations in a process called liftover. Flanking sequences from array manifests or from the UMD3.1 map were remapped to the new assembly. For the 60,671 markers currently used in US genomic evaluations, > 99% aligned in forward direction to the same chromosome, but a few previously used markers were no longer usable. The new locations were then used to impute the 60,671 markers from genotype subsets available on most arrays for 1,748,453 Holsteins (HO), 215,800 Jerseys (JE), 32,724 Brown Swiss (BS), 4,834 Ayrshires (AY), and 3,517 Guernseys (GU). Average numbers of distinct haplotypes per segment decreased 5% for HO and from 1 to 30% for other breeds. Many previous problem areas no longer have excess numbers of haplotypes, particularly on the X chromosome and the pseudoautosomal region of X. Truly lethal haplotypes were more cleanly separated from false candidate haplotypes. Percentage of haplotypes with parent-progeny noninheritance dropped from 3.7 to 3.1 for HO, 4.4 to 3.9 for JE, 1.4 to 1.2 for BS, 2.5 to 1.5 for AY, and 1.6 to 1.4 for GU. Percentage of inherited haplotypes with 1 mistake dropped from 4.9 to 3.7 for HO, 4.3 to 3.6 for JE, 2.9 to 2.8 for BS, 3.5 to 2.8 for AY, and 3.0 to 2.7 for GU. Only a few segments such as on the left end of chromosome 8 had poorer properties for all breeds. Several regions of UMD3.1 were previously known to be on incorrect chromosomes and were excluded from use but can now be used with ARS-UCD. To test sequence alignment, paired-end reads from a HO bull were aligned to both maps, and 2.3% more paired reads aligned in the correct orientation within 5,000 base pairs. The new map improves genotype imputation, sequence alignment, and marker locations.

Key Words: reference assembly, genomic prediction, liftover

169 Implications of limited dimensionality of genomic information on persistency of genomic predictions and GWAS. I. Misztal*, I. Pocrnic, and D. Lourenco, *University of Georgia, Athens GA.*

The purpose of this study was finding possible explanation on peculiarities of dimensionality (M) of genomic information. The gene content matrix derived from 35 to 60k SNP chips has a limited M as determined by singular value decomposition; identical results are obtained with eigenvalues of genomic relationship matrix. Even with a very large number of animals, M ranges from about 4,000 for commercial pigs and broiler chicken, to about 15,000 in Holsteins. This number is normally attributed to the expected number of chromosome junctions as derived by Stam: M = 4NeL, where Ne is effective population size and L is genome size. However, approximation of realized accuracies assuming M for animals with same information is not accurate. Accuracies of genomic prediction assuming M/4 animals in genomic recursions and the APY algorithm are >90% of those assuming full dimensionality. These recursions also suggest that predictions based on M animals with very high reliability should be both very accurate and persistent, and predictions from large national evaluations in Holsteins could converge. However, the real accuracies seem lower than expected. The genome in a population can be visualized in 2 ways. First, as Ne haplotypes within each 1/4 Morgan segment. Second, as 4NeL sequential segments. Eigenvalues analyses of the genomic information shows that popular segments cluster along the genome. Subsequently the number of segments can be higher than determined by singular values. In particular, M/4 clusters could account for 90% of segments. SNP selection decreases the dimensionality; the minimum is the number of causative SNP. SNP selection can eliminate clusters without substantial variation but point to clusters with high variation, potentially creating high GWAS signals not related to QTL. Some ideas in this study were derived from simulated populations assuming complete genome coverage and an additive model. It remains to be seen whether accuracy predictions in real populations are affected by additional factors such as incomplete genome coverage and non-additive effects. Singular value analysis of gene content (or eigenvalue analysis of genomic relationship matrix) helps understand the complexity of genomic selection.

Key Words: genomic selection, APY algorithm, dimensionality

170 Modelling uncertain paternity to address differential pedigree accuracy. H. L. Bradford*[2,1], Y. Masuda[1], J. B. Cole[2], I. Misztal[1], and P. M. VanRaden[2], [1]*University of Georgia, Athens, GA,* [2]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.*

The objective was to implement uncertain parentage models to account for differences in daughter pedigree accuracy. Elite sires have nearly all daughters genotyped resulting in correct paternity assignment. Bulls of lesser genetic merit have fewer daughters genotyped creating the possibility for more paternity errors in their daughters. Data were simulated with QMSim for a moderately (0.3) heritable, sex-limited trait. We created missing pedigrees by removing 8% of sires and 18% of dams. In total, 15 bulls were selected each generation, and the daughters of the best 5 bulls had accurate pedigrees. Daughters of the remaining 10 bulls had 9% sire and 3% dam pedigree errors. Data included 164,500 pedigree animals, 90,000 phenotypes, and 18,000 genotypes and were modeled with an overall mean, additive genetic, and residual effects using single-step genomic BLUP (ssGBLUP) with unknown parent groups. The uncertain parentage model partitioned contributions in A^{-1} to the parent on record (90 to 100%) and to the appropriate unknown parent group (0 to 10%) depending on the type of animal. We validated predictions based on the youngest animals (n = 14,950) without phenotypes. Accuracy was the correlation between true and estimated breeding values. Accuracies (SE) were nearly identical with 0.65 (0.01) for ssGBLUP and 0.64 (0.01) for uncertain parentage. Dispersion was the regression of true on estimated breeding values, and no differences existed between the models with dispersion (SE) of 0.83 (0.01) for ssGBLUP and 0.84 (0.01) for uncertain parentage. Bias was the difference between true and estimated breeding value and was scaled by the genetic standard deviation. Both models had bias (SE) of 0.24 (0.01). Similarly, bias differences were small when evaluating subsets

of animals based on pedigree accuracy. Dairy data sets are complex, especially with regard to differences in daughter parentage accuracies across bulls. This complexity is difficult to simulate, and uncertain parentage models should be tested because of the potential to mitigate bias differences across bulls.

Key Words: genomic, simulation, single-step genomic BLUP

171 Genomic predictability of single-step GBLUP for production traits in US Holstein. Y. Masuda*[1], I. Misztal[1], P. VanRaden[2], and T. Lawlor[3], [1]*University of Georgia, Athens, GA,* [2]*USDA AGIL, Beltsville, MD,* [3]*Holstein Association USA Inc., Brattleboro, VT.*

The objective of this study was to validate genomic predictability of single-step genomic BLUP for 305-d protein yield for US Holsteins. The genomic relationship matrix was created with the Algorithm of Proven and Young (APY) with 18,359 core animals. The full data set consisted of phenotypes collected from 1989 through 2015 and pedigrees limited to 3 generations back from phenotyped or genotyped animals. The predictor data set was created by cutting off the phenotypes, pedigree animals, and genotypes in the last 4 years from the full data set. Genomic predictions (GPTA2011) were calculated for predicted bulls that had no recorded-daughters in 2011 but had at least 50 such daughters in 2015. We calculated the daughter yield deviations with the full data (DYD2015) for the predicted bulls (n = 3,797). We also used the official GPTA published in 2011 with a multi-step method as a comparison, although the official methods have changed since then. Coefficient of determination (R^2) and slope (b_1) were calculated from a linear regression of DYD2015 on GPTA2011. We investigated the effect of different unknown parent groups (UPGs) and a weight (ω) on the inverse of the pedigree relationship matrix for genotyped animals ($\mathbf{A}_{22}^{-1}$) to compensate incomplete pedigree. When applying QP-transformation to $\mathbf{A}^{-1}$, the R^2 was 0.52 with ω = 1 compared with 0.51 from the official GPTA. The b_1 was similar (0.78) to 0.81 from the official GPTA. Using ω = 0.90, the R^2 was still similar (0.50) but the b_1 was greatly improved (0.96). With QP-transformation in $\mathbf{H}^{-1}$, the R^2 was less than 0.4 and the b_1 was smaller regardless of ω. Without any UPGs, the predictability and the inflation showed the same level as the official GPTA. The GPTA of a young animal is equivalent to the direct genomic value when many genotypes are included in the evaluation. Fixed UPGs in $\mathbf{H}^{-1}$ added an extra value to GPTA of young animal but this addition is likely redundant in genomic prediction. We should exclude the UPG contributions from GPTA of young genotyped animals when $\mathbf{H}^{-1}$ is QP-transformed.

Key Words: genomic evaluation, incomplete pedigree, Holstein

172 Implementing SNP-level multiple-trait across country genomic evaluation without genotype sharing. B. Fragomeni*, D. Lourenco, Y. Masuda, and I. Misztal, *The University of Georgia, Athens, GA.*

There is a growing interest of Interbull in releasing a multiple across country genomic evaluation. However, most countries are not able to provide genotypes, and an alternative methodology is required. One strategy called SNP MACE posits a multiple-trait SNP BLUP based on left- and right-hand sides of national SNP BLUP. However, different countries use different sets of SNPs and multiple-trait computations with SNP may be difficult. We propose an alternative model based on reconstructing phenotypes for an independent genotyped population. Each country would submit only SNP effects, the number of reference animals, and average reliabilities of GEBV. This information can be used to create a pseudo-population with pseudo-observations. The combined data can be analyzed by multi-trait GBLUP. Conversion of GEBV would provide SNP effects in scale of every country. Simulations included 30k animals resembling the US Holstein population, with effective population size of 120. Chromosome number and size mimicked the cattle genome. The population was then divided in 3: 2 countries and 1 test population with 10k genotyped animals in each, and a different trait was assigned to each country. For the genotyped animals in the 2 countries, DYD were generated with an average reliability of 0.8. SNP effects were calculated with GBLUP in each one of the 2 countries. With SNP effects from the 2 countries, phenotypes were reconstructed for the test population. A bivariate GBLUP was then fitted, and GEBV/DGV were calculate for the test population for both countries. Accuracies were calculated for the validation population on the scale of 2 countries. When SNP effects of one country were used, the realized accuracy was 0.94 for the same population and 0.69 for the second country. When SNP effects of both countries were used, the accuracy for any country was 0.95. With the use of the APY algorithm, the procedure is computationally viable for any population size and any number of countries. An important issue is creation of pseudo-population that holds the same genomic information as the national population.

Key Words: SNP-MACE, genomic MACE, SNP effect, Interbull

173 Lifetime Net Merit versus annualized net present value as measures of profitability of selection. M. R. Schmitt*[1], P. M. VanRaden[2], and A. De Vries[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*USDA-AGIL, Beltsville, MD.*

Current USDA linear selection indexes such as Lifetime Net Merit (NM) estimate lifetime profit given a combination of 13 traits. In these indexes, every animal gets credit for 2.78 lactations of the traits expressed per lactation, independent of its productive life (PL). Selection among animals with different PL is an example of investment in mutually exclusive projects that have unequal duration. Such projects are best compared with the annualized net present value (ANPV) technique. The objective of this study was to compare the ranking and value differences between NM and ANPV for the top 1,539 Holstein sires for NM available in the December 2017 genetic evaluation from the Council on Dairy Cattle Breeding. To calculate the ANPV, economic weights from USDA estimates were multiplied by the PTA of single event traits. Heifer conception rate was recognized at first calving and livability at the end of life. The economic weight of PL was converted from a marginal value of $21 per lactating month depreciated over the standard length of 2.78 lactations, to a replacement cost (−$1500) at the beginning and a salvage value ($800) at the end of life. All other traits were considered lactation dependent, and the economic weights were multiplied by the number of expected lactations (2.78 + PTA PL/10). The values for all 13 traits were discounted and converted to ANPV to compare animals with different investment horizons on the same common horizon. Correlation and rank correlation between NM and ANPV was 0.993 for the group of 1,539 bulls. However, 32% of bulls with the same ANPV had NM deviations greater than $9.90 from the expected NM. Within the highest 300 NM bulls, correlation and rank correlation between NM and ANPV was 0.964 and 0.943, respectively, and the largest changes in ANPV rank from NM rank were −96 and +117. Bulls with a combination of low lactation traits and high PL resulted in the greatest decrease of ANPV rank compared with NM rank. In conclusion, the re-ranking of bulls based on 2 different measures of profitability suggests that further discussion is warranted about construction of selection indexes for genetic selection.

Key Words: investment, profit, genetics

174 Integrating genomic information and large-scale FTIR-based phenotyping for the genetic improvement of cheese-making traits in Brown Swiss cattle. F. Tiezzi*[1], C. Maltecca[1], H. T. Alvarado[3], A. Rossoni[2], E. Santus[2], G. Bittante[3], and A. Cecchinato[3], [1]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [2]*Italian Brown Swiss Breeders' Association, Bussolengo, Italy,* [3]*Department of Agronomy, Food, Natural resources, Animals and Environment, Legnaro, Padova, Italy.*

The objective of this study was to evaluate genotyping and phenotyping strategies for the improvement of cheesemaking traits in Italian Brown cattle. Phenotyping was considered as model-cheese manufacturing based (high-cost, low-throughput) or Fourier-transform infrared spectroscopy enabled (low cost, high throughput). Data were from 1,011 cows phenotyped using the model-cheese manufacturing method (considered as LAB in the present study) and included 3 cheese yield traits (%CY: fresh curd, curd total solids, and curd water as a percent of the weight of the processed milk), 4 curd nutrient recovery traits (REC: fat, protein, total solids, and the energy of the curd as a percent of the same nutrient in the processed milk). The FIELD data set consisted of ~660,000 FTIR predictions for the same traits on ~35,000 cows. Pool of genotyped individuals consisted of: 1,011 LAB cows, 1,493 of the FIELD cows, 181 sires with LAB and FIELD daughters (siresA), 540 sires with FIELD daughters (siresB). Individuals were genotyped with different SNP panels and imputed to 50k. A 4-fold cross-validation was used to assess predictive ability of models, meant as the ability to predict masked LAB records from daughters of progeny testing bulls. The correlation between observed and predicted LAB measures in validation was averaged over the 4 training-validation sets. Sets of phenotypic information were so defined: M1, LAB cows from the training set; M2, LAB+FIELD cows from the training set; M3, LAB cows in training and all FIELD cows. M2 and M3 considered LAB and FIELD cows as distinct traits. As for the genomic information, sets were defined as: no individuals genotyped; SiresA; SiresA+LAB; SiresA+LAB+SiresB; SiresA+LAB+SiresB+FIELD. For each trait, a total of $3 \times 5 = 15$ models were implemented. Predictions were obtained using the Single-Step GBLUP method. Results show that the use of genomic information does not provide any advantage in predictive ability. Prediction models that included FIELD records showed an advantage for the traits REC_{energy}, $REC_{protein}$, REC_{solids} and CY_{solids} over the models that included LAB records only. For REC_{fat}, CY_{water} and CY_{curd}, this advantage was negligible.

Key Words: genomic selection, FTIR phenotyping, cheese yield

175 Fusion of casein-based gel particles by means of extrusion. C. Kern* and J. Hinrichs, *University of Hohenheim, Stuttgart, Germany.*

Extrusion is a continuous, thermo-mechanical process that is common in food industries. In most applications, the feed material is dry particles. Thermal energy and mechanical strain is applied upon extrusion enabling the specific structural transformation of the feed material; for example, texturization by pasting or puffing of starch. Extrusion and transformation of high moisture (≈50%) gel particles like casein-based gels to isotropic or anisotropic structures by, for example, 3-D print, is a major challenge of plant engineering (hygienic design, CIP) and processing. Extrusion instabilities as melt fracture or phase separation are prone to occur when exceeding a certain strain during extrusion of high moisture particles. Hence, extrusion of directly acidified casein-based gel particles was researched in small scale experiments to generate specific semi-hard isotropic or anisotropic matrices. Standardized cheese milk (3.4% protein, 3% fat) was directly acidified by addition of organic acidulants to different pH values (5.5 – 6.3) to vary calcium levels. Casein-based gel particles with different calcium levels (15.8 ± 0.9 to 31 ± 2 mg Ca g^{-1} protein) were produced by rennet-induced coagulation to investigate thermo-rheological properties and shear behavior at large strain to design hot-melt extrusion. The gel-sol transition temperature (51.7 ± 0.2 to 60 ± 2°C) significantly ($P < 0.001$) decreased with declining calcium levels. Hence, extrusion die temperature was set to 60°C in capillary rheometer experiments to mimic hot-melt extrusion. The extrudate strands were evaluated visually and recorded pressure profiles were analyzed to determine critical shear rates. It was demonstrated that critical shear rate (10 to 30 s^{-1}) increased significantly ($P < 0.001$) with decreasing calcium levels. By exceeding the critical shear rate, extrusion instabilities occurred indicating limitation of extrusion. Hence, optimal extrusion process parameters were determined and should be considered to design hot melt extrusion of high moisture gel particles.

Key Words: extrusion, casein-based gel, oscillatory rheology

176 Influence of pH on whey expulsion from curd made from recombined concentrated milk. K. Bulbul* and D. J. McMahon, *Western Dairy Center, Utah State University, Logan, UT.*

Whey expulsion from cheese curd is influenced by temperature, pH, cut size and cooking. Our objective was to determine the extent to which pH drop before draining influences curd syneresis, curd moisture before draining and final cheese moisture when using concentrated milk. Recombined milk (7.5 kg) was prepared by mixing micellar casein concentrate (~9% casein), cream and skim milk to 4% casein and casein-to-fat ratio of 0.68. Four levels (0.5×, 1×, 2× and 4×) of a pH-controlled bulk starter culture were used to obtain different rates of pH change. Cheesemaking involved a typical cheddar make procedure with setting at 31°C, cutting after 30 min, cooking to 38°C with 95-min set-to-drain time followed by cheddaring with 85-min drain-to-mill time. Prior to cutting, the curd was overlaid with 2 kg of ultrafiltered milk permeate to minimize curd breakage upon stirring. Initially there was rapid moisture loss from curd after cutting, followed by a linear pattern ($R^2 > 0.95$) until whey drainage. A faster drop in pH increased whey expulsion from the curd ($P = 0.0002$). With initial curd moisture levels at 5 min of 83.7 and 83.3% using 0.5× and 4× culture, respectively, curd moisture level of 75.0 and 72.8%, respectively, were obtained at 50 min (during cooking), and 65.5 and 58.5%, respectively, at 95 min (at draining). As cheese make times were fixed, curd pH at draining was lower with faster acidification, the values being 6.5, 6.4, 6.1, and 5.8 for culture additions of 0.5×, 1×, 2× and 4×, respectively. Cheese pH after 14 d of refrigerated storage was likewise affected with pH of 5.4, 5.3, 5.2 and 5.1, respectively. Mean cheese moisture contents were 37.8, 37.1, 37.2, and 35.2%, respectively. Cheese moisture and pH were correlated with drain pH ($R^2 = 0.48$ and 0.71, respectively). To account for the increased buffering capacity of concentrated milk containing 4% casein, a drain pH of 5.9 to 6.0 would be required to obtain a cheese with d 14 pH of ~5.1. To conclude, the pH drop that occurs during cheesemaking increases rate and extent of whey expulsion and will produce cheese with lower moisture.

Key Words: micellar casein concentrate, curd syneresis, acidification

177 Effects of microfiltered milk with different casein:true protein ratios on the quality of Cheddar cheese. E. M. Reale*[1], J. A. Lucey[1,2], R. Govindasamy-Lucey[2], M. E. Johnson[2], J. Jaeggi[2], Y. Lu[2], and M. M. Molitor[2], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Center for Dairy Research, Madison, WI.*

In recent years, research has explored use of microfiltered (MF) milk for cheesemaking. The goal of this study was to determine if the amount of whey protein (WP) depleted from cheesemilk via MF affects the amount of residual WP in cheese as well as functionality and quality. Casein content for all milks was kept at 2.5% to eliminate the confounding factor of casein concentration. Three standardized cheesemilks were made with varied casein:true protein (CN:TP) ratios: (a) control with a CN:TP ratio of 0.83, (b) 35% WP depletion, 0.89 CN:TP, and (c) 70% WP depletion, 0.95 CN:TP. Standardized cheesemilks were analyzed for chemical composition. Cheddar cheeses were aged for 9 mo and composition and functionality were evaluated at 4 d, 2 wks, 1 mo, 3, 6, and 9 mo. Cheese yield, solids recovery, and nitrogen recovery were highest in the 70% WP depleted cheese followed by the 35% WP depleted and control, respectively. Because WP are mostly lost in whey drainage steps of cheesemaking, depletion of WP from the starting cheesemilk improves cheesemaking efficiency (i.e., less of the proteins/solids in cheesemilk are lost). Rheological properties of cheeses were monitored by small-strain oscillatory testing while heating to 85°C. Residual WP content of cheese decreased with increasing MF depletion displaying the effectiveness of MF treatment. The maximum loss tangent (LT_{max}), an index of meltability, was highest at all time points in the 70% WP depleted cheese followed by 35% WP depleted and control, respectively. LT_{max} also increased with ripening for all cheeses. At 1 mo, the temperature at which the LT_{max} occurred was highest in the control cheese followed by 35 and 70% WP depleted samples, respectively. The LT_{max} values increased with age for all cheeses while the LT_{max} temperature decreased. It is possible that residual WP maybe inhibiting proteolysis during ripening. Hardness decreased with age for all cheeses due to proteolytic activity. MF treatment did not affect sensory attributes. Depletion of WP in cheesemilk by MF did not negatively affects quality but resulted in improved cheese efficiency.

Key Words: microfiltration, whey protein depletion, functionality

178 Characterization and presumptive identification of surface crystals on smear-ripened cheese by polarized light microscopy. P. J. Polowsky, G. F. Tansman, P. S. Kindstedt*, and J. M. Hughes, *University of Vermont, Burlington, VT.*

Crystals of ikaite, struvite, calcite, and brushite have been identified in cheese smears by powder X-ray diffractometry (PXRD), and ikaite and struvite exist as single crystals. Polarized light microscopy (PLM) is a well-established method in geology to identify single crystals. However, use of PLM to identify cheese crystals has not been reported previously. Specific objectives of this research were (1) to presumptively identify crystals in cheese smears using selected PLM criteria; (2) to compare identification by PLM against PXRD; and (3) to evaluate a novel treatment for smear material to improve crystal analyses by PLM and PXRD. Duplicate wheels of 4 cheeses made by different manufacturers were obtained from retail sources. Scrapings of surface smears were analyzed by PLM and PXRD using previously described methods. Crystals were categorized by PLM based on angle of extinction (AE), birefringence behavior under crossed polarizers and quartz filters, and size and shape (circularity) by image analysis. Crystals observed by PLM fell almost exclusively into 2 readily differentiated groups based on birefringence behavior and estimated angle of extinction: group 1 (n = 18) were highly birefringent with AE = 88–92°; group 2 (n = 28) had no birefringence with AE = 13–26°. Group 2 crystals were significantly larger and more circular than group 1 crystals. Group 1 and 2 were presumptively identified as struvite and ikaite, respectively, based on known birefringence and AE characteristics. Struvite was identified in all 4 cheeses by PLM but in only 3 cheeses by PXRD. Ikaite was identified in 3 cheeses by PLM but in only 2 cheeses by PXRD. These discrepancies occurred because the smear scrapings from one cheese contained excessive amorphous matter that caused extreme background noise, potentially obscuring diffractogram peaks that may have been present. To minimize noise, smear scrapings were dispersed in aqueous NaOH (pH 10) before analyses, which resulted in consistent results by PXRD and PLM. The method also rendered high quality images by PLM. Data suggest that PLM may offer a simple and inexpensive means to identify and characterize struvite, ikaite and potentially other single crystals in cheese smears.

Key Words: cheese, crystal, microscopy

179 Identification and classification of crystals in cheese by powder X-ray diffractometry. P. J. Polowsky, P. S. Kindstedt*, and J. M. Hughes, *University of Vermont, Burlington, VT.*

Growing interest in artisan and specialty cheeses has resulted in proliferation of cheeses that contain crystals. Until recently, many of these crystals were rarely encountered in the American marketplace. Crystals are natural products of ripening, and it is important to educate consumers about them so that artisan cheeses can be appreciated to the fullest. Powder X-ray diffractometry (PXRD) was used previously to identify crystals in several cheese varieties. Objectives of this research were (1) to identify crystals in cheeses that have not been studied previously using PXRD; and (2) to combine results from this and previous PXRD studies to develop a guide for cheesemakers, retailers, and consumers. Imported Emmental, Comté, Appenzeller, Sbrinz, Mimolette, Pecorino Sardo, Manchego, and Roquefort cheeses, and domestically produced blue mold cheese that displayed visible crystals were obtained from local sources. Crystals were harvested, prepped, and analyzed by PXRD using previously described methods. Dense, hard crystals embedded in the bodies of Emmental, Comté, and Sbrinz cheeses were identified as L-tyrosine. Sbrinz also contained embedded crystals of brushite (calcium phosphate dihydrate), and Emmental contained crystals of L-tyrosine and brushite that deposited prominently along the surfaces of eyes within the cheese. Appenzeller developed crystals of calcite (calcium carbonate), ikaite (calcium carbonate hexahydrate) and struvite (magnesium ammonium phosphate hexahydrate) in the smear at the rind surface. Mimolette contained profuse crystallization along the surface of internal eyes, which took the form of L-leucine sheets. Pecorino Sardo contained discrete internally visible crystals of D/L-calcium lactate pentahydrate, as well as traces of brushite. Manchego contained discrete internally visible crystals consisting of leucine along with brushite. Roquefort cheese contained visible internal crystals near regions where mold grew profusely, which consisted of L-tyrosine, L-leucine and brushite. Domestic blue mold cheese that displayed surface smear growth also contained crystals of ikaite and struvite in the surface smear. Results were compiled into a guide and classification scheme to aid in navigating the world of cheese crystals.

Key Words: cheese, crystals, X-ray diffractometry

180 Influence of Mozzarella and Cheddar cheese blending on quality of pizza Cheese. A. Sameen*, N. Gulzar, N. Huma, A. Sahar, and M. I. Khan, *National Institute of Food Science and Technology, University of Agriculture Faisalabad, Faisalabad, Pakistan.*

The demand for pizza cheese is growing in Pakistan. Therefore, the present investigation was planned with the objectives to evaluate the effect of amalgamation of Mozzarella and Cheddar cheese at different levels on various quality attributes of pizza cheese. The total 7 process pizza cheeses were manufactured and designated as PC_0 (100% Mozzarella), (2 mo old Cheddar cheese) PC_1 (75:25), PC_2 (50:50), PC_3 (25:75), (4 mo old Cheddar cheese)PC_4 (75:25), PC_5 (50:50) and PC_6 (25:75) containing different levels of Mozzarella and Cheddar cheeses respectively. Pizza cheeses were analyzed for physicochemical, texture profile, meltability, stretchability, proteolysis, and microstructure. Sensory analysis was conducted to inspect the behavior of pizza cheeses during cooking (microwave and conventional oven cooking) on pizza topping. Considerably highest moisture and acidity was observed in PC_0 while their percentages decreased as the level of Cheddar cheese increased. Functional characteristics i.e., meltability and stretchability of pizza cheeses were significantly affected by the amalgamation of Mozzarella and Cheddar cheese. The results demonstrated that control sample exhibited lowest (10.17cm) meltability and highest (34.50cm) stretchability. When incorporation of Cheddar increased in the pizza cheeses the meltability increase and stretchability decreased. The pH 4.6 soluble and TCA soluble N increased significantly with the concentration and ripening months of Cheddar cheese. Electrophoresis and reverse phase HPLC results indicated that level of intact casein decreased with increasing the level and age of Cheddar cheese in amalgamated pizza cheese. Micrograph obtained from electron microscopy indicated that the size of serum channels reduced with the amalgamation of higher level of aged Cheddar cheese. Microwave oven cooking proved better for texture and flavor development. From PC_1 to PC_3 pizza cheese performed better regarding different textural attributes and stretchability, while PC_4 to PC_6 behaved well for imparting flavor, meltability, and appearance. Overall baking performance of PC_1 and PC_2 was preferred by the panelist. Based on different characteristics Pizza cheese manufacturer are recommended to use Mozzarella and 2-mo-aged Cheddar cheese in proportion of 75 and 25% respectively.

Key Words: Mozzarella, Cheddar, pizza cheese

181 Impact of high pressure and different storage temperatures on the properties of Gouda cheese. L. A. Jiménez-Maroto*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], M. E. Johnson[2], and J. A. Lucey[1,2], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

To extend the performance shelf-life of Gouda cheese intended for export, we studied the impact of high-pressure processing (HPP) and different storage temperatures on the flavor and functionality of reduced-sodium stirred curd (SC) and milled curd (MC) Gouda cheese. Batches (n = 4) of SC Gouda (37.0 ± 0.8% moisture, 33.2 ± 0.9% fat, 25.7 ± 0.3% protein, 1.2 ± 0.1% salt) and MC Gouda (37.5 ± 0.5% moisture, 32.9 ± 0.8% fat, 25.3 ± 0.4% protein, 1.2 ± 0.1% salt) were produced using a reduced proteolytic coagulant. After 1 mo of ripening, cheeses were divided into 2 groups: control (non-HPP) and HPP (600 MPa, 3 min), and stored at 3 different storage temperatures (ST) (−20, 0, or 4°C). Analyses were performed after 4, 8, and 12 mo of storage. Cheese functionality was assessed using texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology. Sensory Spectrum® and quantitative descriptive analysis were conducted with 12 trained panelists to evaluate flavor, texture, and shred attributes using a 15-point scale. TPA hardness was influenced by ST, but not HPP treatment; cheeses stored at 0°C retained their initial hardness values, while it decreased over time in cheeses stored at 4 or −20°C. Rheological maximum loss tangent (LTmax) was affected by curd type, ST, and HPP treatment; MC had higher values than SC cheeses, HPP increased the values, cheeses stored at −20°C retained their initial LTmax values, but it increased in the cheeses stored at 0 and 4°C after 4 mo and declined after 8 mo. Sensory cohesiveness scores were lower in SC than MC Gouda and decreased in non-HPP samples stored at −20°C. During storage, acid, milky, and buttery scores decreased in all cheeses, except those stored at −20°C. Strand length of shreds was influenced by ST and HPP treatment, decreasing over time except in HPP cheeses stored at 0°C, regardless of curd type. The best option for maintaining flavor over time came from storing samples at −20°C; however, performance was best maintained up to 12 mo through a combination of 0°C storage and HPP.

Key Words: Gouda, high-pressure processing, superchilling

182 Characterization of semi-hard and hard artisanal cheeses from small-scale producers in the Western Cape Province of South Africa. F. Nyamakwere*[1], G. Esposito[1], N. Muller[2], E. Moelich[2], P. Gouws[2], F. Masucci[3], and E. Raffrenato[1], [1]*Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa,* [2]*Department of Food Science, Stellenbosch, South Africa,* [3]*Department of Agricultural Science, Università degli Studi di Napoli Federico II, Naples, Italy.*

A survey was conducted to characterize artisanal cheeses from small-scale producers of the Western Cape, South Africa. Forty artisanal cheeses were identified and classified based on type (Cheddar, Gouda, others), age (1, 3, 6 mo) and milk (raw and pasteurized). The chemical, microbiological, and sensory properties of the cheeses were investigated. Rapid sensory characterization was done using the sorting technique followed by a descriptive step. Sixteen panel members were instructed to sort samples based on similarity of flavor and texture attributes. Sensory data were subjected to DISTATIS and correspondence analysis. Chemical and microbiological data were analyzed by univariate statistical methods with main factors age, cheese and milk. Raw milk cheeses had higher ash and fat content ($P < 0.05$) compared with pasteurized milk cheeses. Ash content was also higher in 3 mo cheeses compared with 6 mo. However, a wide range in fat composition was observed, ranging from 23% to over 40% and generally positively correlated with moisture content. The "others" group had the highest protein content (30%) and higher pH compared with Cheddar ($P < 0.05$). Fifteen percent of the cheeses did not fall within standards for coliforms and 10% tested positive for *E. coli*. Aging affected aerobic plate counts (higher at 1 mo compared with other months; 5.6 Log cfu/g) which was negatively correlated with the lactic acid % ($P < 0.05$). Results for sensory characterization showed a clear differentiation between mature and young cheeses. As expected, raw milk cheeses and those from the same producers fell within same groups suggesting similar sensory characteristics. The latter was not observed for cheeses belonging to the same type. Therefore, although cheeses from small-scale producers can be classified under the same broad categories such as Cheddar or Gouda, they can still express unique physiochemical and sensory properties. Understanding physiochemical and sensory properties of artisanal cheeses will help evaluate their composition and eating quality to help expand small-scale producers marketing shares.

Key Words: Cheddar, Gouda, coliforms

183 Determining the prevalence of failure of passive transfer in heifer and bull calves on Michigan dairy farms. F. Cullens* and M. Weber Nielsen, *Michigan State University, East Lansing, MI.*

The overall aims of this extension research are to determine the prevalence of failure of passive transfer (FPT) in heifer and bull calves, identify the best management practices that result in a reduced FPT in dairy calves on Michigan farms, and determine if a hand-held refractometer is a reliable measure of FPT in healthy calves. Fifty dairy farms in Michigan were included in the study. A blood sample was collected from 12 heifers and 12 bulls between 2 to 7 d of age on larger farms (over 250 milking cows, n = 38) and 6 heifers and 6 bulls on smaller farms (n = 12). Dehydration of calves was scored visually, and selected management practices for the farm were recorded following interview with the calf manager. Serum Total Protein (STP) was determined using a Misco handheld refractometer with STP <5.2 considered to be FPT. Immunoglobulin G (IGG) concentrations were determined on a subset of 343 serum samples by Saskatoon Colostrum Company, with IGG <10.0 recorded as FPT. Only 2 samples failed according to STP but passed IGG, while 13 samples passed STP and failed IGG. All 15 calves with discrepancies showed signs of dehydration, indicating that in healthy calves a refractometer can be a reliable measure of STP. When fit to the block design model, sex was not significant although numerically bulls had higher prevalence of FPT (26%) than heifers (18.5%). There was an interaction of sex and farm size; heifers showed FTP more frequently on small farms than on large farms ($P = 0.0001$). Five farms had zero cases of FPT, while 6 farms had 50% or greater FPT. Overall 18/50 of study farms met the industry goal of less than 10% FPT. Farms with high priority on colostrum feeding had lower FPT. Farms that had best management practices resulting in lower FPT had a goal of feeding 3 to 4 quarts of colostrum within 1 to 3 h of birth, followed by a second feeding, had colostrum in storage or colostrum replacer on hand, did not allow nursing of the cow, and used a Brix refractometer to test colostrum quality. A benchmarking report was shared with all 50 farms and included customized guidelines for improving management practices. Results were widely shared at extension programming.

Key Words: calves, passive transfer

184 Using whole-farm analysis based on Holos to reduce net greenhouse gas emissions: Examples from dairy systems. S. M. Little*[1], C. Benchaar[2], H. H. Janzen[1], R. Kröbel[1], E. J. McGeough[3], A. McPherson[1], and K. A. Beauchemin[1], [1]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, Canada,* [2]*Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada,* [3]*Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada.*

Before recommending a feeding or management strategy for greenhouse gas (GHG) mitigation, it is important to conduct a holistic assessment of all related emissions, including those arising from feed production, digestion of these feeds, managing manure, and other on-farm production processes and inputs. The cumulating and cascading effects of management practices on emissions from agricultural systems can rarely be measured directly, so whole-farm emissions are normally estimated using mathematical models. An example of such a model is Holos, a whole-farm model and software tool developed by Agriculture and Agri-Food Canada to estimate GHG emissions from Canadian farm systems (www.agr.gc.ca/holos-ghg). Using a whole-systems approach, the Holos model, and experimentally measured data, we compared the effects of alfalfa silage-, barley silage-, and corn silage-based diets on GHG estimates in simulated Canadian dairy production systems. Previous studies showed the impact of silage choice on enteric methane production but the impact of diet choice on GHG emissions from the complete farm system had not been explored. Utilizing the functional units of milk, meat, and total energy in food products generated by the system, the comparison demonstrated very little difference between alfalfa and corn silage production systems in terms of GHGs. In general, GHG emissions associated with barley silage-based diets were higher than those for corn silage-based diets. This demonstrates that reported GHG reduction factors cannot be simply combined additively because the interwoven effects of management choices cascade through the entire farm system, sometimes with counter-intuitive outcomes. Whole-farm analysis of GHG emissions from dairy production and exploration of mitigation strategies is facilitated by the Holos software tool.

Key Words: carbon footprint, greenhouse gas emissions, computer software

185 Producer perceptions of the National Dairy Farmers Assuring Responsible Management (FARM) Animal Care Program. K. A. Rink*[1], P. J. Turk[2], S. L. Archibeque-Engle[3], J. K. Ahola[1], J. C. Hadrich[4], and I. N. Román-Muñiz[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Department of Statistics, Colorado State University, Fort Collins, CO,* [3]*Colorado State University, Fort Collins, CO,* [4]*Department of Applied Economics, University of Minnesota, St. Paul, MN.*

The National Dairy Farmers Assuring Responsible Management (FARM) Animal Care Program provides guidelines for farms producing 98% of the US milk supply. Producers who sell milk to co-ops or processors participating in FARM must follow animal care standards defined by the program's technical writing group. Objectives of this study were to assess producers' perceptions about knowledge, experience, value, and reasons for considering FARM important, and to determine if perceptions differ based on producer demographics. Producers from collaborating dairy co-ops and processors were recruited via electronic and postal mail. A total of 487 respondents from 40 states completed the survey. Of respondents, 50.0% identified dairy co-ops or processors as the main source of information about FARM, and 73.6% reported being knowledgeable about FARM. More formal education and larger herd size were correlated with greater producer knowledge ($P < 0.01$ and $P = 0.04$, respectively). More producer input in the revisions of FARM was identified as a need by 83.3% of respondents. While 89.3% of respondents reported positive experiences with evaluations and relationships with evaluators, 45.6% did not think that FARM has value overall. Females had a neutral impression of the value of the FARM program and males had a negative impression ($P = 0.02$). Greater respondent age was predictive of greater perceived value of FARM ($P < 0.01$). Age was significant in determining the reasons for considering FARM important ($P < 0.01$). Odds that respondents described FARM as important because it improved animal health and wellbeing over describing FARM as not

important increased 7.2% (OR = 1.072; 95% OR CI: 1.024, 1.122) with each year of age. As age increased, respondents were more likely to describe FARM as important because it unified the dairy industry on animal welfare over describing FARM as not important (OR = 1.095; 95% OR CI: 1.029, 1.164). These results indicate that to increase buy-in and positive perceptions of producers, future versions of FARM should solicit producer input and target specific producer demographics for training and program promotion.

Key Words: animal care, dairy producer, FARM

Forages and Pastures Symposium: Fiber Digestibility—From Cell Wall Composition to Forage Utilization

186 A holistic vision of cell wall organization and its impact on cell wall digestibility. R. Hatfield*, *USDA-ARS, Madison, WI.*

Cell walls of forages contain a vast reservoir of potential energy that could be available to ruminants to meet their energy requirements. Unfortunately, ruminants are not able to capture all of this potential energy due to the way forages grow and develop. Cell wall formation during the growth and development of the plants is a dynamic process that changes with the stages of development. One can think about the assembly of cell walls much in the same way that a house or building is constructed. There are major scaffolding components—in this case, cellulose fibrils—that set the overall structural dimensions or size and shape of the plant. Other components, hemicellulose, pectin, and proteins, fill in around the fibrils creating and matrix that is flexible and permeable until lignification occurs. Lignin and other phenolics are hydrophobic components that stiffen the wall by driving water out and increasing hydrogen bonding resulting in a rigid less penetrable wall. Incorporation of all components that make up the wall dictates the final form and function of the "fiber" and how it may be used by ruminants.

Key Words: cell walls, cellulose, hemicellulose

187 Environmental factors affecting plant cell wall structure and cell wall and forage digestibility. K. J. Boote*[1], L. E. Sollenberger[1], and D. N. L. Pequeno[2], [1]*University of Florida, Gainesville, FL,* [2]*International Maize and Wheat Improvement Center, Texcoco, Mexico.*

In this paper, we describe the effects of environment (temperature, drought, heat stress) and management (harvest timing and N fertility) on plant cell wall structure and the digestibility of cell wall and forage, and second propose approaches to model forage digestibility as a function of environmental and management conditions. Forage scientists have equated fiber to the cell wall component (CWC), which is typically measured by the neutral detergent fiber (NDF) method. However, this method does not define the digestibility of the NDF because of varying degrees of lignification of the cell wall fraction. Furthermore, NDF is poorly correlated with total forage digestibility. Rising temperature is known to increase the cell wall fraction and reduce cell wall digestibility of recently expanded leaves and stems across a range of species, whether tropical or cool-season types. Species differ in the baseline beginning CWC and digestibility, but seem to be affected similarly by temperature, and all show an aging/maturation effect on top of the temperature effect. These temperature effects also exist in annual crops such as maize. For every 1°C increase, forage digestibility decreases 0.3 to 0.7 percentage units. Effects of water deficit are small, and often increase digestibility because of slowed maturation, lower NDF, and accumulation of soluble assimilate. Elevated CO_2 has little effect on NDF and digestibility. Stems have higher CWC and lower digestibility than leaves; therefore, leaf-stem ratio caused by differences in species and crop maturation is important. Existing forage crop models such as the CROPGRO-Perennial Forage model can predict non-structural carbohydrates, CP, leaf-stem ratio, and tissue age of the herbage produced. Prediction of forage digestibility is lacking, but present outputs may be useful in predicting digestibility. In this talk, we present conceptual approaches and plans to model effects of temperature, drought, tissue maturation, and harvest management on cell wall and tissue digestibility, based on literature information and on inverse solving against data sets on CP and in vitro digestibility. Our goal is to achieve a forage modeling tool that predicts production as well as nutritive value.

Key Words: forage, digestibility, environment

188 Utilization of fiber analysis for ration formulation. R. J. Grant*[1] and D. R. Mertens[2], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*Mertens Innovation and Research LLC, Belleville, WI.*

Effectively feeding forage and nonforage fiber to dairy cattle requires assessment of chemical and physical properties that determine ruminal fiber digestion and passage and dry matter intake. We will focus on the practical application of fiber digestibility in ration formulation and feeding management. Two historical transformative advances in fiber analysis and utilization were (1) the detergent system and summative approach to partition forages into fiber and nonfiber fractions, and (2) the concept that potentially digestible fiber follows first-order digestion kinetics. Dynamic nutrition models require kinetic measures of digestion and passage, and their required inputs will be an important driver of future fiber analyses. Research has focused on measuring multiple fiber digestion pools (i.e., fast, slow, and indigestible neutral detergent fiber; iNDF). Presumably, this 3-pool model better reflects biological reality of forage fiber digestion, but its practical significance in ration formulation has not been demonstrated. Of equal or greater importance is an understanding of multiple particle pools for passage, and forage factors such as particle size, density, and digestibility that influence passage rate. Estimation of iNDF by measurement of undigested fiber (uNDF) at long fermentation times is preferable to prediction from lignin because of the observed variation in the relationship between lignin and uNDF. Direct measures of uNDF in feed and feces will result in more accurate ration formulation and fiber utilization. Complementary analyses of nonfiber carbohydrates and fatty acids will be required to predict the impact of negative associative effects on NDF digestion. The nutritional value of cell wall monomer measurement is presently unknown. Laboratory analyses should assess the potential nutritive value of forage, but poor feeding management can reduce this potential. Future transformative analytical breakthroughs may connect plant anatomy and structure with laboratory measurements. Going forward, we need to better describe forage biology affecting ruminal digestion and passage with an analytical focus on fiber characteristics that allow formulation of rations with minimal, maximal, or optimal NDF content.

Key Words: forages, fiber digestibility, ration formulation

189 Technologies for improving fiber utilization. A. Adesogan*, *University of Florida, Gainesville, FL.*

The forage lignocellulosic complex is one of the greatest limitations to utilization of nutrients in fiber. Consequently, several technologies have been developed to increase forage fiber utilization by dairy cows. Physical or mechanical processing techniques reduce forage particle size and gut fill and thereby increase intake. Such techniques increase the surface area for microbial colonization and may thus increase fiber utilization. Genetic technologies such as brown-midrib mutants with less lignin have been among the most repeatable and practical strategies to increase fiber utilization, even though some brown midrib hybrids

are prone to lodging. Several alkalis have been effective at increasing fiber digestibility. Among these, ammoniation has the added benefit of increasing the nitrogen concentration of the forage. However, none of these has been widely adopted due to the cost and the caustic nature of the chemicals. Urea treatment is more benign but it requires sufficient urease and moisture for efficacy. Ammonia fiber expansion (AFEX) technology uses high moisture and pressure to depolymerize lignocellulose to a greater extent than ammoniation alone, but it occurs in reactors and is therefore not usable on farms. Promising biological technologies for increasing fiber utilization such as application of exogenous fibrolytic enzymes, live yeasts and yeast culture have had equivocal effects on forage fiber digestion though recent meta analyses suggest their overall effects are positive. Non-hydrolytic expansin-like proteins have can synergistically increase fiber digestion by cellulase due to their ability to expand cellulose microfibrils. White-rot fungi are perhaps the biological agents with the greatest potential for lignocellulose deconstruction but several species also degrade easily digestible carbohydrates. Less ruminant nutrition research has been conducted on brown rot fungi that can deconstruct lignocellulose by generating highly destructive hydroxyl radicals. More research is needed to increase the repeatability, efficacy, cost-effectiveness, and on-farm application of technologies for increasing fiber utilization by dairy cows.

Key Words: fiber, digestibility, utilization

Joint MILK and Lactation Biology Symposium: Milk Globules, Vesicles, and Exosomes—Update, Origin, Structure, and Function

190 Intravital imaging of the lactating mammary gland in live mice reveals novel aspects of milk-lipid secretion. I. H. Mather*[1,2], A. Masedunskas[2,3], Y. Chen[4], and R. Weigert[2,3], [1]*University of Maryland, College Park, MD,* [2]*National Cancer Institute, NIH, Bethesda, MD,* [3]*National Institute of Craniofacial and Dental Research, NIH, Bethesda, MD,* [4]*Johns Hopkins University, Baltimore, MD.*

Milk fat comprises membrane-coated droplets of neutral lipid, which constitute the sole source of all lipids for survival of the suckling neonate. From the perspective of the dairy industry, they are the basis for the manufacture of butter, and essential ingredients in the production of cheese, yogurt and specialty dairy produce. To provide mechanistic insight into the assembly and secretion of lipid droplets during lactation, we developed novel intravital imaging techniques using transgenic mice, which express fluorescently tagged marker proteins. The number 4 mammary glands were surgically prepared under a deep plane of anesthesia and the exposed glands positioned as a skin flap with intact vascular supply on the stage of a laser-scanning confocal microscope. Lipid droplets were stained by prior exposure of the glands to hydrophobic fluorescent BODIPY dyes and their formation and secretion monitored by time-lapse subcellular microscopy over periods of 1–2 h. Droplets arose from basal regions of the cell and were transported to the cell apex by directed (superdiffusive) motion. Transit was relatively slow (0–2 μm/min) and intermittent. Droplets grew by numerous fusion events regardless of size both during transport and in apical regions. On occasion, droplets caught up with and fused with droplets further along the same track or pathway. Lipid expansion was most pronounced in apical nucleation centers and continued as the droplets were budding from the cell enveloped by apical membranes. Surprisingly, droplet secretion was not constitutive but required an injection of oxytocin (i.p.) to induce contraction of the myoepithelium with subsequent release of droplets into luminal spaces. This regulated mechanism may constitute an important means for controlling the size of secreted droplets because they will continue to expand at the apical surface unless they are ejected by oxytocin released from the pituitary gland. Application of intravital imaging techniques should provide novel insights into the secretion of other milk components, including proteins and constituents transported across the mammary epithelium from the systemic circulation.

Key Words: milk lipid globules, oxytocin-mediated lipid secretion, intravital microscopy

191 Bioavailability, distribution, and phenotypes of bovine milk exosomes in non-bovine species. J. Zempleni*, *University of Nebraska-Lincoln, Lincoln, NE.*

Virtually every living cell produces and secretes nanoparticles called exosomes into the extracellular space. Exosomes play a crucial role in cell-to-cell communication, facilitated by the transfer of regulatory molecules such as microRNAs inside exosomes from donor cells to adjacent or distant recipient cells. Our laboratory was first to demonstrate that exosomes and their RNA cargos are not exclusively derived from endogenous synthesis but may also be obtained from dietary bovine milk. This presentation will discuss (1) novel protocols used to assess the bioavailability and distribution of bovine milk exosomes and their RNA cargos in humans and mice, (2) interactions between exosomes and microbiome in mice, and (3) phenotypes of dietary depletion of bovine milk exosomes in infants and mice. For example, the speaker will provide evidence that milk exosomes accumulate primarily in liver and brain, whereas distinct microRNAs have unique distribution profiles in mice. The speaker will share his findings that dietary milk exosomes alter microbial communities in the murine gut, contain and deliver immune-relevant microbial RNAs to the host, and select for single nucleotide polymorphisms and mutations in cultures of murine gut microbiota. Phenotypes of dietary exosome depletion include loss of spatial learning and memory, probably due to aberrant purine metabolism. *Supported by NIFA 2015–67017–23181 and 2016–67001–25301, NIH 1P20GM104320, Egg Nutrition Center, Gerber Foundation, USDA Hatch & Multistate W3002, and the U. Nebraska President's Office. J. Z. is a consultant for PureTech Health.*

Key Words: exosome, bioavailability, phenotype

192 Metabolic regulation of milk fat globule size. N. Argov-Argaman*, *The Faculty of Agriculture, The Hebrew University, Jerusalem, Israel.*

Milk fat globules (MFG) are secreted by mammary epithelial cells (MEC) into mammary gland alveolar lumen in a wide range of sizes, from 200 nm to over 15 μm. The significance of size derives from its tight association with MFG lipidome and proteome. Moreover, small MFG have relatively higher content of membrane compared with large globules, and this membrane exerts diverse positive health effects, as reported in human and animal studies. In addition, MFG size has industrial relevance since it affects the physicochemical and sensory characteristics of dairy products. MFG size is determined before and during its secretion from MEC, and therefore the size of their precursors, the intracellular lipid droplets, will determine the MFG diameter. Two main mechanisms regulate the size of lipid droplets; the first is based on intracellular triglyceride content, regulated by metabolic signals. In various lipogenic tissues, like adipose and liver, anabolic signals induce triglyceride storage and hence increase lipid droplet size. However, the positive association between triglyceride level and lipid droplet size in MEC and milk does not always hold. Interestingly, MFG size is usually elevated under conditions of negative energy balance, when catabolic signals reduce insulin and elevate NEFA plasma concentrations. Therefore, triglyceride synthesis and secretion capacity cannot in itself explain the differences in MFG size and a second mechanism, fusion, was proposed to contribute to MFG size regulation. Fusion between lipid droplets occur in MEC cytoplasm, as was demonstrated in murine MEC in vivo. However, the regulatory mechanisms of fusion are still illusive. We showed that membrane phospholipid composition, partially determined by mitochondria enzymes, can change membrane stability and hence control the extent of lipid droplet fusion and consequently, MFG size. This regulation is independent of the triglyceride content in the cells. The fact that mitochondria play a role in size regulation, independently of fat production, may explain the size variations occur during lactation, concomitantly with changes in metabolic status of the animal, as often demonstrated in dairy cows.

Key Words: milk fat globule, membrane, size

193 Fat globules in milk and their structural modifications during gastro-intestinal digestion. H. Singh*[1], [1]*Riddet Institute,*

Palmerston North, New Zealand, [2]Massey University, Palmerston North, New Zealand.

The fat globules in milk are unique oil droplets that are stabilized by a specific and structurally-complex membrane, the milk fat globule membrane (MFGM). In the last decade, good progress has been made in on the structure of the milk fat globules and MFGM, and how common processing treatments affect these structures to deliver dairy products with improved functional properties. However, the repackaging or structural alteration of fat globules may also affect the way they are digested, absorbed and metabolized. In addition, the surrounding structures may also play a role in the bioaccessiblity of raw or processed milk fat globules, which may affect the access of the gastrointestinal enzymes to the globules and, therefore, influence the bioavailability of lipids. Our recent work on understanding the structural changes in milk fat globules during gastro-intestinal digestion has revealed that the native fat globules remain stable under gastric conditions, although pepsin is able to hydrolyze most of the MFGM proteins. In contrast, homogenized milk fat globules, coated with caseins, aggregate readily under gastric conditions. Interestingly, it has been discovered recently that the surrounding curd structures formed by coagulation of casein in the gastric environment have a profound effect on the rate of emptying of fat globules and hence the kinetics of lipid digestion. Fat globules in natural milk show longer residence time in the stomach, as they remain entrapped in a cohesive casein network clot. The curds formed by homogenized and heat-treated milk have a more crumbled and porous structure and the fat globule are released faster into the small intestine. Under the intestinal conditions, the lipolytic products, released from the hydrolysis of the triglyceride core of the globules by the action of pancreatic lipase, lead to destabilization and coalescence of the globules. These droplets are surrounded by a liquid-crystalline lamellar phases which are then solubilized as multilamellar vesicles involving bile salts. This talk will focus on recent advances in understanding the milk globules during gastro-intestinal digestion, including the effects of processing on bioavailability of milk fat globules, and the kinetics lipid digestion.

Key Words: fat globules, digestion, milk fat globule membrane (MFGM)

194 The relevance of phospholipid and vesicles from milk in dairy foods and human nutrition. R. Jimenez-Flores*, *The Ohio State University, Columbus, OH.*

There are several challenges that the dairy industry faces today, and the image of milk is one of the biggest ones. However, in many areas of dairy foods research, there are continuous advances and discoveries that reinforce the positive aspects of milk contributions to human health and wellness, and need to be emphasized and communicated. The topic of milk fat globules, vesicles, and exosomes, which are common to the milk of all mammals, are one of the frontiers in dairy science that has great potential to show scientist, and developers of novel foods, the way to design better foods in the future. Using the natural elements in native milk fat globules and relating their composition and structure on how they are digested, is a very reasonable model from which to base the designs of structure and function of novel foods. The topic of the structure and composition of the vesicles in milk is yielding important information on its relevance in foods. We present some of the latest advances on MFGM and its structure using different methodology, also the thermodynamics of phospholipids from milk, and their physical and chemical properties in various models, including the nascent area of exosomes and their structure /function. Finally, some important observations on the possible roles of phospholipids in milk vesicles on their important role in the gut microbiome, intestinal lining health, neuromuscular development, neuronal cell culture and even some relevant reports on the importance of the lipids in milk in cognitive development in animal and human studies.

Key Words: milk fat globule membrane, milk phospholipids, biological activity of milk

195 Nutritional and greenhouse gas contributions of dairy cattle to United States agriculture. R. R. White*[1] and M. B. Hall[2], [1]*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA,* [2]*United States Dairy Forage Research Center, Madison, WI.*

The Dietary Guidelines for Americans Advisory Committee suggested that a reduction of animal-source foods would enhance sustainability and healthfulness of US diets. This work quantified contributions of domestically consumable dairy products to human-edible nutrient supply and greenhouse gas (GHG) emissions of US agriculture. Data on US dairy production were obtained from the analysis conducted by White and Hall (2017; https://doi.org/10.1073/pnas.1707322114), which utilized data from USDA databases; the US Food and Drug Administration; the US Environmental Protection Agency; the United Nations Food and Agriculture Organization; and peer-reviewed, published sources. We disaggregated the reported animal metrics to specifically assess contributions of dairy products available for domestic consumption. All assessments terminated at fluid milk production and did not consider further processing. Census Bureau data describing the US population by age and sex groups were matched to nutrient requirements (USDA and World Health Organization) to identify weighted average requirements of the US population. Dairy products were estimated to meet the average energy, protein, and calcium requirements of 71.2, 169, and 254 million people, respectively. The contributions of consumable dairy products to available domestic supply of energy (11% of total consumable supply), protein (20%), lysine (28%), methionine (24%), calcium (73%), vitamin A (39%), vitamin D (54%), riboflavin (47%), vitamin B_{12} (47%), and choline (29%) were also quantified. Dairy products available for domestic consumption account for 1.4% of US anthropogenic emissions, 16% of agricultural emissions, and 32% of emissions from animal agriculture. Although this work provides an initial snapshot of how dairy products contribute to nutrient supply and GHG emissions, future work should explore economics, land and resource use efficiency, impact of processing, and targeted assessment of alternative nutrient sources (supplements, dietary fortification, etc.) to better quantify and understand the trade-offs.

Key Words: dairy industry, greenhouse gas, nutrition

196 Genetically modified crops and no-till systems reduce production of greenhouse gases from crops used for feeds. J. L. Vicini*[1] and G. S. McNunn[2], [1]*Monsanto Company, St. Louis, MO,* [2]*EFC Systems, Ames, IA.*

Animal agriculture is challenged with societal issues such as animal welfare, antibiotic use and sustainability. Consumers are increasingly interested in sustainability of meat, milk and eggs because agriculture, both crop and animal ag, is a major contributor to greenhouse gases (GHG); however, cropping decisions can be a potential solution for storing GHG. Most farms in the US have relied in a large part on genetically modified (GM) crops and no-till (NT) planting for the last 20 years. In the United States, more than 90% of corn, soybean, and cotton are GM and livestock are major consumers of these crops. Because feed production is a significant contributor to GHG production for animal agriculture, it is important to understand how this technology affects farm sustainability. We hypothesized that GHG would be reduced by planting GM crops and adoption of NT and tested this hypothesis by developing a model-based analysis across 12 states in the US corn-belt. The analysis generated potential erosion and GHG emission estimates associated with the implementation of several practices based on public SSURGO data, NASS crop productivity data, and spatially explicit weather data. Rill and inter-rill erosion resulting from rainfall and surface runoff were modeled and sediment loss and deposition due to wind was simulated. In addition to erosion, soil organic carbon (SOC) change and nitrous oxide flux (N_2O) were modeled using the Denitrification-Decomposition model (DNDC). The model has the ability to test different factors such as type of crop, tillage, crop rotation and irrigation. Based on the analysis, average GHG emissions associated with corn production across the US Corn Belt could be reduced or mitigated with GM crops and NT. For instance, with continuous corn for 6 years, conventional corn and conventional tillage emit 461 kg CO_2e/ac/yr. In contrast, GM corn and no-till emit 151 kg CO_2e/ac/yr. These changes can help dairy farms meet sustainability goals.

Key Words: feed production, greenhouse gases

197 Manure flushing versus scraping in dairy freestall lanes reduces gaseous emissions. E. G. Ross*, C. B. Peterson, Y. J. Zhao, Y. E. Pan, and F. M. Mitloehner, *University of California Davis, Davis, CA.*

The objective of the present study was to mitigate ammonia (NH_3), methane (CH_4), and air pollutants from lactating dairy cows using different freestall waste removal techniques. Four cows per treatment (n = 4) were housed in an environmental chamber. Waste was removed by either flushing or scraping at 2 different frequencies. The 4 treatments arranged as a Latin square were (1) flushing 3 times a day (F3), (2) flushing 6 times a day (F6), (3) scraping 3 times a day (S3), and (4) scraping 6 times a day (S6). Gas concentrations were collected at the inlet and outlet air ducts of the environmental chamber and were transported to a mobile agricultural air quality lab. Gas emission rates were calculated and then analyzed using the lmerTest package in R. Ammonia and hydrogen sulfide (H_2S) emissions decreased ($P < 0.001$ and $P < 0.05$) in the flushing vs. scraping treatments, respectively. Ethanol emissions increased ($P < 0.001$) when the frequency of either scraping or flushing was increased from 3 to 6 times, but remained similar between scraping and flushing treatments. Methane emissions for the F3 were lower than all other treatments ($P < 0.001$). Removal of dairy freestall manure by flushing versus scraping has the potential to decrease gaseous emissions such as NH_3 and CH_4.

Key Words: dairy cow, ammonia emissions, freestall barn

198 A framework for conducting nonlinear meta-analysis in the dairy sciences. L. E. Moraes*, *The Ohio State University, Columbus, OH.*

A framework for conducting meta-analysis based on linear mixed-effects model has been available to the dairy science community for almost 2 decades (St-Pierre, 2001; J. Dairy Sci. 84:741–755). However, statistical methods used when nonlinear models are developed with literature data are extremely heterogeneous. Methods that ignore the underlying structure of meta-analytic databases are frequently used. For instance, ignoring study effects can result in parameter estimates that are severely biased. Further, not accounting for potential heterogeneous errors across studies violates the assumption of homogeneous error variance. Although nonlinear mixed models provide a framework

for introducing random effects into nonlinear models, meta-analytic designs are often sparse, with a narrow range of measurements for independent variables within studies. Consequently, dairy scientists have been frequently challenged with model convergence issues and have often ignored study effects or selected overly simplistic covariance structures. The objective of this study was to propose a general framework to conduct meta-analysis with nonlinear functional forms. A Bayesian hierarchical modeling approach is proposed and ideas borrowed from Empirical Bayes are utilized to increase the practicality and generality of the approach. In particular, a 2-step model fitting is proposed. Initially, weighted nonlinear least squares are used to obtain pooled estimates of model parameters. On the second step, a hierarchical model is used to introduce between study variability in the parameters of the nonlinear model. The connection between the 2 steps is that pooled estimates from the first step are used as hyperparameters for the prior distribution of the mean population parameters in the second step. Strategies to improve computational efficiency and numerical stability are also proposed within our framework, for instance, constructing the model for the between-study variability using Cholesky factors. Two examples with meta-analysis recently conducted on ruminant nutrition will be used to describe the framework, interpret results, and illustrate the use of software.

Key Words: nonlinear, meta-analysis, Bayesian

199 Effects of hormonal growth promotants on meat quality. I. J. Lean[1,2], H. M. Golder*[1], N. M. Lees[1], P. McGilchrsit[3], and J. EP Santos[4], [1]*Scibus, Camden, NSW, Australia,* [2]*Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia,* [3]*School of Environmental and Rural Science, University of New England, Armidale, NSW, Australia,* [4]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Hormonal growth promotants (HGP) improve production efficiency, profit, and reduce the environmental impact of beef cattle production. Questions remain, however, about the effects of HGP on meat quality measures of toughness such as Warner-Bratzler shear force (WBSF), and taste-panel attributes of meat (i.e., tenderness, juiciness, flavor, and content of connective tissue). This meta-analysis used 31 experiments containing 181 treatment comparisons to evaluate the effects of HGP on meat quality, almost all using *Musculus longissimus thoracis et lumborum*. Experiments used different hormonal treatments and combinations, with cattle treated once or repeated times, different breeds and sexes of cattle, with or without electrical stimulation of meat, and with different lengths of time on feed and meat aging. The effects of multiple treatment comparisons in experiments were evaluated using robust regression models and compared with Knapp-Hartung and permutation meta-analytical methods. In general, the true variance of experiments, τ^2 was low (<0.1), but heterogeneity, was high (>50%) suggesting that much of the variance was due to measurement error. Treatment with HGP increased WBSF; use of multiple HGP implants was associated with an increase in WBSF of 0.248 kg (95% CI 0.203 to 0.292), but a single implant only increased WBSF by 0.176 kg (95% CI 0.109 to 0.242). Meat aging did not alter the impact of HGP on increased WBSF (P = 0.105); however, the point direction was toward a reduced impact with aging (ES = −0.005 per d aged). Experiments using trenbolone acetate treatments did not differ in WBSF from those using other implants (P > 0.150). Treatment with HGP was not associated with reduced tenderness assessed by sensory methods (P > 0.3) and repeated treatments with HGP increased tenderness (standardized mean difference = 0.468) compared with a single implant. Juiciness, flavor, and connective tissue were not associated with HGP use; whereas, there was a 5.5-point decrease in the Meat Standards Australia meat quality 4 score, albeit with limited experiments. There is a need for more targeted studies on the role of HGP in influencing meat quality to examine the effects of different HGP treatments and aging on WBSF, tenderness, juiciness, flavor, and other sensory measures.

Key Words: hormonal growth promotants (HGP), meat quality, meta-analysis

200 Estimating the dairy farm value of infectious or non-infectious lameness prevention strategies as influenced by pre-prevention hoof disease incidence rates and prevention effectiveness. K. A. Dolecheck*[1], M. W. Overton[2], T. B. Mark[1], and J. M. Bewley[3], [1]*University of Kentucky, Lexington, KY,* [2]*Elanco Animal Health, Greenfield, IN,* [3]*CowFocused Housing, Bardstown, KY.*

The objective of this study was to adapt a farm-level stochastic simulation model to estimate the value of implementing lameness prevention on a dairy farm by calculating the change in the herd-level total cost of lameness from pre- to post- lameness prevention implementation. Two potential lameness prevention strategies were tested: strategy 1 was prevention focused on reducing the infectious hoof diseases in the model (digital dermatitis) and strategy 2 was prevention focused on reducing the non-infectious hoof diseases in the model (sole ulcer and white line disease). For each strategy, pre-prevention incidence of either infectious (strategy 1) or non-infectious (strategy 2) hoof diseases was set at 3 different levels. The effectiveness of prevention was allowed to vary from a disease incidence risk ratio of 0.0 to 1.0 in each strategy and pre-prevention disease incidence level combination. Stochastic simulation was used to account for variation within the farm model; 300 iterations were run. When implementing prevention strategy 1, the mean ± SD change in the herd-level total cost of lameness per cow-year when digital dermatitis incidence level was originally 20%, 40%, or 60% was −$6.9 ± 3.3, −$13.8 ± 6.5, and −$20.6 ± 9.8, respectively. When implementing prevention strategy 2, the mean ± SD change in the herd-level total cost of lameness per cow-year when non-infectious hoof disease incidence levels were originally 5%, 15%, or 25% was −$8.7 ± 3.9, −$26.2 ± 11.6, and −$43.6 ± 19.3, respectively. For both prevention strategies, the value of prevention increased as the effectiveness of prevention increased. However, the profitability of investing in lameness prevention would depend on the cost of the selected prevention strategy and the other benefits association with the prevention strategy. If herd-level incidence of hoof diseases and the expected effectiveness of a potential lameness prevention strategy are known, this model could be used to help identify the amount that could be paid to implement that prevention strategy.

Key Words: decision support, animal health economics, prevention investment

201 The effects of milk consumption on predicted future body weight of heifers. J. P. Boerman[1], T. S. Steckler*[1], and N. Lopez-Villalobos[2], [1]*Purdue University, West Lafayette, IN,* [2]*Massey University, Palmerston North, New Zealand.*

The objective of this study was to evaluate the long-term effects of the amount of milk consumed pre-weaning and health status on predicted BW of growing heifers. Daily milk consumption, serum total protein, pneumonia and scours incidences, and BW were recorded on a commercial dairy farm from October 29, 2015 to September 15, 2017. Calves (n = 5,234) were fed pasteurized milk with a 30% protein 5%

fat milk enhancer added at 20 g/L of milk through a Foerster-Technik automated calf feeding system (pens = 4; feeders per pen = 2) for 59.7 d ± 3.90 (mean ± SD). Calves were weighed at birth and up to 5 other times before calving. Average BW of calves at birth was 41.1 ± 4.66 kg (mean ± SD) and serum total protein was 6.4 ± 0.56 mg/dL (mean ± SD). Daily BW up to 400 d were predicted for individual animals using Legendre polynomials to best model growth data. Effects of milk consumption at 2 time points (either up to 30 d of age or total until weaning), serum total protein, and incidence of pneumonia and scours on predicted BW at 365 d were evaluated using a multiple regression model. Milk consumption up to 30 d had a linear effect on predicted BW at 365 d ($P < 0.0001$); and each additional L of milk consumed up to 30 d of age resulted in an additional 0.27 kg of predicted BW at 365 d ($P < 0.0001$). Serum total protein did not significantly affect predicted BW ($P > 0.30$). Total milk consumption had a quadratic effect on predicted BW at 365 d ($P < 0.0001$); such that after ~550 L of total milk consumption, the effect of each additional liter of milk consumed had a reduced effect on predicted BW at 365 d. The number of incidences of pneumonia significantly affected predicted BW ($P < 0.0001$). If an animal was diagnosed with pneumonia 3 or more times, the predicted BW at 365 d was 12.7 kg less than animals that never were diagnosed with pneumonia ($P < 0.0001$). Milk consumption and the incidence of pneumonia were both important factors that affected predicted BW of animals up to 1 yr of age.

Key Words: milk consumption, calf management, body weight

202 Influence of a lactic acid bacteria and yeast-based postbiotic product (Probisan) on the performance of pre-weaned newborn calves. M. Rovai*[1], L. Guifarro[1], G. Sanzol[2], E. Huarte[3], J. V. Díaz[2], J. L. Anderson[1], and A. Salama[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Pentabiol S.L., Esquiroz, Navarra, Spain,* [3]*Incyte Corp., Wilmington, DE.*

Crossbred heifer calves (n = 70) were used in a randomized complete block design to evaluate the effects of a feed supplement on growth performance in an individual outdoor hutches during winter (T = −35 to 4°C) at a commercial dairy farm in South Dakota. After birth calves were assigned to (1) Control with no supplement (CON; n = 35) and (2) supplemented with 3 g/d Probisan, a lactic acid bacteria and non-bitter fermented yeast product, added to milk from d 3 until weaning at d 56 (PRO; n = 35). Groups were balanced for birth date, weight and concentration of serum immunoglobulins. Calves in both groups were fed 2.6 L at 3x/d pasteurized milk/calf/d until wk 7. During wk 8 milk was fed once daily until weaning. A commercial nonmedicated starter (grain mix pellet) was fed ad libitum from d 14. Body weight was recorded at birth and weekly until weaning. Hip height and width were measured every other week until weaning. Individual starter DM intake was measured daily. Data were analyzed by MIXED procedures in SAS 9.4 with repeated measures. The model included the fixed effects of treatment, week, and interaction, and animal nested within treatment as random effect. No differences ($P > 0.10$) were found between treatment groups for average body weight (47 ± 0.8 kg), hip height (81 ± 0.5 cm), hip width (18.4 ± 0.2 cm), or body weight gain (0.634 ± 0.02 kg/d). Average values for body weight, hip height, hip width, and body weight gain increased ($P < 0.001$) from 32.3 ± 0.5 kg, 75 ± 0.4 cm, 17.1 ± 0.6 cm, and 0.272 ± 0.03 kg/d at wk 0 to 67.8 ± 0.8 kg, 88 ± 0.4 cm, 20.9 ± 0.1 cm, and 0.923 ± 0.03 kg/d at wk 8, respectively. For starter DM intake, both groups consumed similar amount (119 ± 19 g/d) during wk 2 and 3. However, from wk 4 until weaning PRO calves tended ($P = 0.078$) to consume less starter (CON = 574 ± 38 g/d and PRO = 477 ± 39 g/d). In conclusion, calves supplemented with Probisan consumed 17% less starter from wk 4 until weaning without reductions in body weight gain or body measurements.

Key Words: calf, growth performance, postbiotic

204 Altering the ruminal microbiota in dairy calves using rumen contents dosing. M. S. Cox*[1], P. J. Weimer[2,1], A. J. Steinberger[1], J. H. Skarlupka[1], and G. Suen[1], [1]*Department of Bacteriology, University of Wisconsin-Madison, Madison, WI,* [2]*US Dairy Forage Research Center, USDA Agricultural Research Service, Madison, WI.*

A major goal in dairy research is to improve milk production efficiency (MPE). With the advent of next-generation sequencing and advanced methods for characterizing microbial communities, efforts are underway to improve MPE by manipulating the rumen microbiota. Recent work has demonstrated that MPE is correlated and directly linked to ruminal bacterial community composition (BCC) in cows. Moreover, the adult cow rumen microbiota is highly stable and returns to a baseline community structure even after extreme perturbation. We seek to influence the rumen microbiota by early intervention in pre-weaning dairy calves. Two cannulated Holstein donors of known and disparate MPE were selected. Three cohorts of 6 bull calves each were established. Calves were dosed by oral gavage with a blended inoculum of rumen contents sourced from the high-efficiency donor (HE), the low-efficiency donor (LE), or an autoclaved 50:50 mixture from the donors as a microbe-free control (C). Dosing occurred within 3 d of birth, then every 2 wk through 6 wk of age. Feces were collected at each dosing as a proxy for gut BCC. Calves were sacrificed at 8 wk to access rumen contents and rumen wall sections were collected to assess papillation. Qualitative histological analysis suggests greater papillation in the HE and LE cohorts than in the C cohort, but subsequent quantitative measures are needed. Fecal and rumen samples from the males were subjected to 16S rRNA amplicon sequencing. We found that BCC differed by cohort in fecal and rumen samples ($P < 0.05$), with HE calf samples most similar to adult rumen samples and C calves least similar. Several bacterial OTUs were observed to have differential abundances in rumen samples at 8 wk ($P < 0.05$), including several classified to genera *Ruminococcus* and *Prevotella* and family Veillonellaceae, previously implicated in bovine feed efficiency. Pre-weaning average daily gain did not differ between cohorts (HE: 0.90 ± 0.07 kg, LE: 0.76 ± 0.07 kg, C: 0.73 ± 0.05 kg, $P = 0.186$). These data demonstrate that the microbiota can be influenced by early intervention, providing a compelling avenue for future development of whole-rumen based probiotics to the end of MPE improvement.

Key Words: microbiota, production

205 Can palmitic acid supplementation be detected by bulk tank fatty acid profile? D. E. Santschi* and D. M. Lefebvre, *Valacta, Ste-Anne-de-Bellevue, QC, Canada.*

Supplementation of palmitic acid (PA) to some or all lactating cows in a herd is a practice known to improve fat content, although questioned from a consumer health, milk processing and environmental point of view. The objective of the present study was to evaluate whether one could rely on bulk tank fatty acid (FA) profile to easily monitor the use of this feeding strategy. The database generated by a survey on the use of PA in dairy cow rations was used and matched with corresponding bulk tank FA profiles. A total of 989 herds were part of the original database. Of these, 52 herds had been feeding a source of fat rich in PA (≥85% as PA) to all lactating cows in the herd for more than 6 mo and 467 herds had never fed PA over the past 2 years. Herds feeding PA to some of the cows or feeding products containing mixed FA were excluded, leaving

519 herds for the present study. Bulk tank FA composition was analyzed by FTIR every other day from September to December 2017 and was averaged for each herd. Of the herds feeding PA, most herds (65.4%) reported feeding between 200 and 300 g/cow/d, while some herds were feeding less than 200 g/cow/d (23.1% of herds) or more than 300 g/cow/day (11.5% of herds). Supplementation of PA influenced milk and FA composition (Table 1): fat, C16:0, and C18:0 FA concentrations in milk increased, while protein, C14:0 and C18:1 FA concentration in milk remained unchanged with PA. Relative proportions (% of total FA) of de novo and mixed FA were respectively decreased and increased by PA addition to the diet. Although indicators presented in the table are influenced by PA supplementation, ranking of herds by any of these variables does not allow to confirm whether herds are supplementing PA or not. From this data set, bulk tank FA profile alone cannot serve as a tool to diagnose PA supplementation in Quebec dairy herds.

Table 1 (Abstr. 205).

Composition (g/100 g milk)	PA	No PA	SE	*P*-value
Fat	4.08	4.04	0.02	0.04
Protein	3.29	3.32	0.01	0.008
C14:0	0.445	0.445	0.004	0.96
C16:0	1.155	1.124	0.010	0.003
C18:0	0.362	0.348	0.003	≤0.001
C18:1	0.875	0.864	0.007	0.12
Composition (g/100g FA)				
De novo FA	31.22	31.60	0.15	0.02
Mixed FA	33.41	33.08	0.14	0.02
Preformed FA	35.37	35.32	0.26	0.87

Key Words: palmitic acid, FTIR, fatty acid profile

206 Effect of feeding fresh alfalfa or fresh oat/berseem clover on rumen characteristics and metabolic N of dairy cows. D. Enriquez-Hidalgo*, K. Barrera, S. Peede, and E. Vargas-Bello-Pére, *Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia, Universidad Católica de Chile, Santiago, Chile.*

Fresh forages alter rumen characteristics, but also can improve the sustainability of the system. Unlike alfalfa, berseem clover (BC) does not cause bloat and, when it is sown with grasses, grows well during the winter and improves N utilization. The study aimed to evaluate the effect of using fresh alfalfa (ALF), fresh oat/BC (MIX) and alfalfa hay/maize silage (CON) as forage basis for TMR during the winter period on dairy cows' rumen characteristics and metabolic N. The ALF diet was used to elucidate if either the fresh forage or the species affected the responses. Three nonlactating rumen-cannulated cows were allocated to each TMR for a 14-d period in a 3 × 3 Latin Square design. Cows were individually stalled and received 13 kg DM/d of the TMR (60:40 forage-to-concentrate). Forage samples were analyzed for DM, CP and NDF contents. Samples were collected on d 13 and 14 of each period: rumen fluid was analyzed for pH, VFA and NH_3 content; blood serum, urine and feces were analyzed for N content. Data were analyzed using linear models including TMR and period. Forages differed in DM (CON: 55.0; ALF: 19.3; MIX: 21.0; SEM 0.49 DM%; $P < 0.001$), CP (CON: 10.2; ALF: 23.8; MIX: 14.9; SEM 0.32 DM%; $P < 0.001$) and NDF (CON: 45.8; ALF: 34.8; MIX: 39.2; SEM 0.46 DM%; $P < 0.001$) contents. The fresh forage diets (especially ALF) had greater CP content, which increased ruminal NH_3, valeric and isoacids VFAs and ureic N serum contents (Table 1). The fresh forage diets increased the propionic and decreased the acetic acid contents. The use of fresh forages changed rumen characteristics and metabolic N, showing potential to improve production and N use efficiency. *This study was funded by project FONDECYT 11160697.*

Table 1 (Abstr. 206). Effect of different forages as basis for TMR on ruminal characteristics and metabolites

	CON	ALF	MIX	SEM	*P*-value
pH	6.34	6.49	6.39	0.176	NS
NH_3, mmol/L	6.3	14.5	7.5	0.88	<0.001
Total VFA, mmol/L	133	118	127	12.1	NS
Acetic acid, %	71.3	68.7	68.5	0.72	<0.01
Propionic acid, %	14.2	15.2	15.5	0.55	<0.01
Butyric acid, %	9.6	10.1	10.8	0.58	NS
Valeric acid, %	1.2	1.5	1.5	0.09	<0.01
Isobutyric acid, %	1.2	1.6	1.3	0.08	<0.001
Isovaleric acid, %	1.8	2.0	1.7	0.12	<0.05
Serum ureic N, mg/dL	16.2	22.0	16.5	1.29	<0.05
Urine N, mg/dl	447	295	247	60.3	<0.05
Fecal N, %	2.60	2.85	3.00	0.240	NS

Key Words: rumen function, fresh legumes, N use efficiency

207 Monitoring daily liveweight in grazing sheep using an automated walk-over-weighing system. E. González-García*, *SELMET (Systèmes d'Élevage Méditerranéens et Tropicaux), INRA, Montpellier SupAgro, CIRAD, Univ Montpellier, Montpellier, France.*

Monitoring liveweight (LW) is typical for management purposes (e.g., weight gain, body condition, slaughtering schedules). Even if time and labor consuming, measuring LW indoor is relatively easy. However, recording LW outdoor may become difficult. The aim of this project was to trial an automated small ruminant weighing prototype conceived by our team. Using the remote weighing concept of walk-over-weighing (WoW), combined with radio-frequency identification, it was designed to be light, resistant, transportable and autonomous in energy (Figure 2; González-García et al.; https://doi.org/10.1017/S1751731117002609). The LW is collected as the animal traverse freely over the WoW platform, strategically placed in an obligatory path combined to a small yard with water and mineral salts as incentives. In a series of experiments carried out in a range of sheep farming situations (i.e., indoor and outdoor) we studied the system' efficacy. Time required for achieving individual voluntary passages, the number of daily individual visits and the proportion of biologically plausible LW records were analyzed. The Lin's concordance correlation coefficient (CCC) was used to establish the repeatability of the WoW and the agreement with LW recorded in static scales. Our results showed the feasibility of achieving freely, voluntary passage of sheep and the efficiency of water and mineral salts as incentive, for encouraging the pass over the WoW. After 2–3 weeks of adaptation, 100% of animals crossed daily. Sheep misbehavior (e.g., speed of passage) can result in spurious values and accounted for many of the larger weight discrepancies (outliers; 35% of initial raw records from the database downloaded from the WoW system). The Lin's

concordance correlation coefficient (CCC) was used to establish the repeatability of the WoW and the agreement with LW recorded in static scales. Our results showed the feasibility of achieving freely, voluntary passage of sheep and the efficiency of water and mineral salts as incentive, for encouraging the pass over the WoW. After 2–3 weeks of adaptation, 100% of animals crossed daily. Sheep misbehavior (e.g., speed of passage) can result in spurious values and accounted for many of the larger weight discrepancies (outliers; 35% of initial raw records from the database downloaded from the WoW system). Once outliers removed, the prediction accuracy of the system and the CCC ranged between 0.93 and 0.99 thus showing a substantial agreement between records from the new system (WoW) and the gold standard measurement (static bascule). Using this standalone WoW system, it was possible to record daily individual LW with controlled sheep flow over the platform, allowing to prevent congestion. Our WoW prototype contributes to save labor and time, to animal welfare and to a close individual monitoring whatever the farming condition.

Key Words: autoweighing, automatic monitoring, grazing ruminant

Ruminant Nutrition II: Methane

208 Dynamics of volatile fatty acids, hydrogen, and methane in dairy cattle: A model of rumen metabolic pathways. H. J. van Lingen*[1,2], J. G. Fadel[3], L. E. Moraes[4], E. Kebreab[3], A. Bannink[2], and J. Dijkstra[2], [1]*TI Food and Nutrition, Wageningen, the Netherlands,* [2]*Wageningen University & Research, Wageningen, the Netherlands,* [3]*University of California, Davis, Davis, CA,* [4]*Ohio State University, Columbus, OH.*

Most rumen mechanistic models adopt a zero pool for hydrogen and estimate methane production based on hydrogen sources and sinks. A dynamic mechanistic model that represented substrate degradation, volatile fatty acid (VFA) production pathways, and methanogenesis in the bovine rumen was developed. This preliminary model also represented the thermodynamic control of H_2 partial pressure (pH_2) on the type of VFA formed via the NAD^+ to NADH ratio (r_{NAD}). Feed composition and intake rate (twice-daily feeding regimen) observations were used as model input. Model parameters were estimated to experimental data using a Bayesian calibration procedure, after which the uncertainty of the parameter distribution on the model output was assessed. This Bayesian mechanistic modeling effort is unique in providing a mathematical representation of diurnal dynamics of VFA, H_2 and CH_4 production in the bovine rumen, in which the type of VFA is controlled by pH_2 via r_{NAD} homeostasis, based on principles of reaction kinetics and thermodynamics. The preliminary model predicted a marked peak in pH_2 after feeding that rapidly declined in time. This peak in pH_2 caused a decrease in r_{NAD} followed by an increased propionate molar proportion at the expense of acetate molar proportion. In response to feeding, the model predicted an increase in CH_4 production that steadily decreased in time. The pattern of CH_4 emission rate followed the patterns of pH_2 and H_2 emission rate, but its magnitude of increase in response to feeding was less pronounced. A global sensitivity analysis was performed to determine the impact of parameters on daily CH_4 production. The parameter that determines the NADH oxidation rate explained 41% of the variation in predicted daily CH_4 emission. The preliminary model was evaluated using 40 measurements from 3 experiments conducted at Wageningen University. Model evaluation indicated daily CH_4 production to be under-predicted, and showed a root mean square prediction error of 15%. The present modeling effort provides the integration of more detailed knowledge than in previous rumen fermentation models and allows assessment of diurnal dynamics of rumen metabolic pathways yielding VFA, H_2 and CH_4.

Key Words: thermodynamic control, Bayesian calibration, mechanistic modeling

209 Effects of 3-nitrooxypropanol on rumen fermentation, lactational performance, and onset of ovarian activity in dairy cows. A. Melgar*[1], M. T. Harper[1], J. Oh[1], F. Giallongo[1], M. E. Fetter[1], T. L. Ott[1], S. Duval[2], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Research Centre for Animal Nutrition and Health, DSM Nutritional Products, France.*

This study investigated the effect of 3-nitrooxypropanol (3NOP), a methane (CH_4) inhibitor, on rumen fermentation, lactational performance, and reproductive function in Holstein dairy cows. The study was a 15-wk randomized complete block design with 56 multi- and primiparous cows (including 8 rumen-cannulated). Cows were blocked based on their previous lactation milk yield or predicted milk yield and within block were randomly assigned to one of 2 treatments: (1) control, no 3NOP, and (2) 3NOP applied at 60 mg/kg feed DM. Cows entered the study within 3 d after calving and remained on the study for 105 d. Enteric CH_4 emission was measured during experimental wks 2, 6, 9, and 15 using the GreenFeed system. Rumen samples for fermentation analyses were collected during wks 4, 8, and 12. Milk was sampled 3 d/wk from 2 to 8 wks after calving and assayed for progesterone concentration to determine onset of ovarian activity. Compared with the control, 3NOP decreased total VFA and acetate, increased butyrate molar proportion, ethanol, and formate ($P \leq 0.05$), and tended ($P = 0.14$) to increase dissolved hydrogen concentrations in the rumen. CH_4 emission was decreased 25% by 3NOP (337 vs. 225 g/d for the control; $P < 0.001$). 3NOP also decreased ($P < 0.001$) CH_4 emission yield (14.0 vs. 11.2 g/kg DMI) and intensity (8.3 vs. 6.3 g/kg ECM). DMI was lower for 3NOP compared with the control (23.7 vs. 24.9 kg/d; $P = 0.05$), but DMI as % of BW was not different (4.0%; $P = 0.94$) between treatments. Treatment had no effect ($P \geq 0.63$) on milk and ECM yields, averaging 44 kg and 41.7 kg/d, respectively. Feed and ECM feed efficiencies were increased ($P \leq 0.01$) by 3NOP compared with the control. Milk composition and milk fat and protein yields were not affected ($P \geq 0.39$) by treatment. There was no effect ($P \geq 0.14$) of 3NOP on return to ovarian activity, days to first and second CL, and length of the first and second luteal phases. In this experiment, 3NOP decreased enteric CH_4 emission, yield, and intensity without affecting milk yield and composition or onset of ovarian activity in early lactation dairy cows.

Key Words: 3-nitrooxypropanol, dairy cattle, reproduction

210 Dose-response effect of 3-nitrooxypropanol on enteric methane emission in dairy cows. A. Melgar*[1], K. C. Welter[2], K. Nedelkov[3], C. M. M. R. Martins[2], M. T. Harper[1], J. Oh[1], S. E. Räisänen[1], X. Chen[4], S. F. Cueva[5], S. Duval[6], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of Sao Paulo, Pirassununga, Brazil,* [3]*Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria,* [4]*College of Pastoral Agriculture Science and Technology, Lanzhou University, China,* [5]*Panamerican Agricultural University, Zamorano., Francisco Morazán, Honduras,* [6]*Research Centre for Animal Nutrition and Health, DSM Nutritional Products, France.*

This experiment was designed to test the effect of inclusion rate of 3-nitrooxypropanol (3NOP), a methane inhibitor, on enteric methane emission in dairy cows. The study was conducted with 49 multiparous cows (155 ± 7.7 DIM, 634 ± 8.1 kg BW, and 44 ± 1.1 kg/d milk yield) in a randomized complete block design. The experiment was conducted in 2 phases; phase 1 was with 21 cows and phase 2, with 28 cows. Cows were blocked based on DIM, milk yield, and enteric methane emission during a 14-d covariate period. Cows were fed ad libitum a basal TMR diet containing (%, DM basis): corn silage, 50; alfalfa haylage, 10; and concentrate feeds and mineral-vitamin premix, 40. Treatments were control (placebo, no 3NOP) and 40, 60, 80, 100, 150, and 200 mg 3NOP/kg feed DM. Following a 14-d adaptation period, enteric gaseous emissions (methane, carbon dioxide, and hydrogen) were measured in 3 d using the GreenFeed system (C-Lock Inc., Rapid City, SD) at 0900, 1500, and 2100 h (d 1), 0300, 1200, and 1700 h (d 2), and 0000 and 0500 h (d 3). Dry matter intake (24.6 kg/d; SEM = 1.12), milk yield (44.0 kg/d; SEM = 1.15), and cow BW (634 kg; SEM = 5.2) were not affected ($P \geq 0.33$) by treatment. 3NOP linearly decreased ($P < 0.001$) methane emission by an average of 31% (303 vs. 441 g/cow/d for the control; SEM = 25.7). The 3 highest 3NOP inclusion rates had a similar

methane mitigation effect (average of 267 g/d, or a 40% reduction; $P \geq 0.93$). 3NOP linearly decreased ($P < 0.001$) methane yield (average of 12.6 vs. 17.0 g/kg DMI for the control; SEM = 1.15) and methane emission intensity (7.1 vs. 9.7 g/kg milk, respectively; SEM = 0.63). Emission of hydrogen linearly increased ($P < 0.001$) from 0.3 g/cow/d for the control to an average of 2.2 g/cow/d (SEM = 0.42) for the 3 highest 3NOP inclusion rates. Emission of carbon dioxide was not affected by treatment (average of 13.7 kg/cow/d; SEM = 6.16; $P = 0.97$). In this experiment, 3NOP linearly decreased enteric methane production in dairy cows. Maximum mitigation effect was achieved at 100 mg/kg feed DM with no further decrease in enteric methane emission with 150 and 200 mg/kg 3NOP.

Key Words: 3-nitrooxypropanol, enteric methane, dairy cattle

211 Effect of limit-feeding diets with different forage to concentrate ratios on the fecal bacterial and archaeal community composition in Holstein heifers. J. Zhang*, H. Shi, Y. Wang, Z. Cao, and S. Li, *State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Limit-fed high concentrate diet was proposed as an effective method to improve feed efficiency and decrease manure output in heifers' raising, while the effect on hindgut microbiota was still unclear. Therefore, this study aims to investigate the effects of wide range of dietary forage:concentrate ratios (F:C) on the compositions of fecal bacteria and archaea in heifers. Twenty-four Holstein dairy heifers with similar body condition (8–10 mo old) were assigned into 4 treatments and fed diets with different F:C (80:20, 60:40, 40:60, and 20:80, DM basis). The quantity of diets provided to high concentrate fed groups was restricted so that there was a similar intake of metabolizable energy to low concentrate fed groups. From d 20 to 23, fecal samples were collected from the rectum of heifers at 3-h intervals. At the end of sampling, equal amount of sample of each time point from the same heifer were homogenized, and the subsamples were used for nutrient and volatile fatty acids detection and DNA extraction. The fecal bacterial and archaeal communities were investigated by Illumina sequencing. With increasing dietary concentrate levels, the fecal neutral detergent fiber and acetate contents linearly decreased ($P < 0.01$), while fecal starch, propionate, and butyrate contents increased ($P \leq 0.05$). An increased concentrate level linearly increased ($P = 0.02$) *Proteobacteria* abundance, while linearly decreased ($P = 0.02$) *Bacteroidetes* abundance in feces. At the genus level, cellulolytic bacteria, namely unclassified *Ruminococcaceae* and *Paludibacter*, were linearly decreased ($q \leq 0.02$), while non-fibrous carbohydrate degraders, namely *Roseburia* and *Succinivibrio*, were linearly increased ($q \leq 0.05$). However, the abundance of most detected archaeal were similar across different F:C, and only *Methanosphaera* were linearly decreased ($P = 0.01$) with increasing concentrate levels. These results suggest that different F:C diets have significant effects on the fecal bacterial communities, while have slight effect on the archaeal communities. Our study might further be beneficial to enhance the feed digestibility and relieve environment problems in heifers' rearing.

Key Words: bacteria, archaea, heifer

212 Effect of essential oil extracted from tropical and/or sub-tropical plants on in vitro dry matter digestibility, ruminal fermentation, and methane production. D. H. Kim*[1,2], I. M. Ogunade[1], K. G. Arriola[1], D. Vyas[1], and A. T. Adesogan[1], [1]*Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL,* [2]*Division of Applied Life Science (BK*21*Plus, Institute of Agriculture and Life Science), Gyeongsang National University, Jinju, South Korea.*

The objective was to examine the effects of essential oils (EO) from *Syzygium aromaticum* (clove), *Cinnamomum zeylanicum nees* (cinnamon), *Rosmarinus officinalis* (rosemary), *Zingiber officinale* (ginger), and *Cymbopogon citratus* (lemon grass) on in vitro dry matter digestibility (IVDMD), ruminal fermentation and methane production using a TMR as substrate. A corn silage-based TMR was treated with each EO at doses of 0 (CON), 200 (Low), 400 (Med) and 600 (High) mg/L of buffered rumen fluid and with Monensin (MON) at 12 mg/kg substrate DM (positive control). A randomized complete block design with 3 replicates per treatment and 3 runs (blocks) was used to determine the effects of EO and MON. Data for each essential oil were separately analyzed with the GLIMMIX procedure of SAS and treatment effects were estimated using multiple comparisons. Each treated substrate was incubated in a 120-mL gas-tight culture bottle at 39°C for 24 h. The EO had no effect on IVDMD when compared with the CON. However, compared with MON, IVDMD was increased ($P < 0.05$) by the low dose of rosemary and ginger. Gas volume was reduced ($P < 0.05$) by the low dose of rosemary and low and high doses of cinnamon, compared with CON. All EOs except lemon grass reduced ($P < 0.05$) methane production at all doses when compared with the CON. The high dose of rosemary increased ($P < 0.05$) pH compared with CON and MON. Lactate content was significantly higher ($P < 0.05$) in med dose of cinnamon. Acetate concentration was reduced ($P < 0.05$) by medium and high doses of clove and cinnamon while isobutyrate concentration was increased with all doses of clove and ginger, compared with CON and MON. The high dose of lemon grass reduced ($P < 0.05$) propionate concentration compared with the MON. The high dose of cinnamon increased ($P < 0.05$) butyrate concentration compared with the CON and MON. In conclusion, compared with the CON, all doses of EOs reduced in vitro methane production without reducing digestibility whereas MON had no effect on digestibility, methane production or gas production.

Key Words: essential oil, methane, digestibility

214 Methane inhibition following fermentation and microbiota community response by different dose of chitosan in vitro. J. Tong*[1], H. Zhang[1], L. Jiang[1], and B. Xiong[2], [1]*Beijing Key Laboratory for Dairy Cow Nutrition, Beijing University of Agriculture, Beijing, China,* [2]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Chitosan, a non-toxic and biodegradable carbohydrate polymer, had diverse potential applications in the food processing, pharmaceutical, cosmetics, veterinary, environmental protection, agricultural industries and medicine. However, there is limited knowledge with regard to the different dose of chitosan in ruminant nutrition and whether chitosan is related to the altered bacterial community is still unclear. A completely randomized design was applied in vitro incubation. A basal diet with no chitosan was the control, and the treatment diets were supplemented with chitosan at the dose of 0.4, 0.8, 1.2, 1.6, and 2.0% DM. Six fermentation units were used per treatments. The concentrations of methane and volatile fatty acids were determined using a gas chromatograph. To explore methane inhibition following fermentation and the response of ruminal microbiota to different dose of chitosan, the bacterial 16S rRNA genes were sequenced by Miseq high-throughput sequencing and were confirmed by realtime-PCR. The results revealed that the dose of 2.0% chitosan had greatest reduction in methane emission and shifted the fermentation pattern toward propionate production. Real-time PCR

analysis showed that chitosan increased the population of *Fibrobacter succinogenes* and *Megasphaera* spp. relative to total bacterial 16S rDNA, while the dose of 2.0% CHI significantly increased the population of *Ruminococcus albus* compared with control ($P < 0.01$). Furthermore, 0.4% CHI could significantly increase the population of *Succinivibro* spp. ($P < 0.01$), but 2.0% CHI had no effect. ANOSIM showed no significant differences in bacterial community composition among all the treatments ($R = -0.03$, $P = 0.71$). Chitosan addition had a tendency of increase the feed digestibility and significantly increased the VFA production ($P < 0.10$). In particular, correlation analyses between relative abundances of bacteria genera showed that the propionate concentration was positively related with *Prevotellaceae* and *Bacteroidales* ($P < 0.05$), but negatively correlated with *Ruminococcaceae* ($P < 0.05$). Therefore, the effects of chitosan on microbial populations and bacterial communities, particularly on propionate production, are probably responsible for the discrepancy in its effects on rumen fermentation and methane emission, and thus it may be used as a potential alternative to fed to ruminants.

Key Words: chitosan, methanogenic, rumen fermentation

215 Relationship between residual feed intake and CH_4 production in dairy heifers. H. Flay*[1,2], B. Kuhn-Sherlock[1], K. Macdonald[1], M. Camara[1], D. Donaghy[2], N. Lopez-Villalobos[2], and J. R. Roche[1,3], [1]*DairyNZ, Hamilton, New Zealand,* [2]*Massey University, Palmerston North, New Zealand,* [3]*University of Auckland, Symonds St, Auckland, New Zealand.*

There is growing interest in improving feed conversion efficiency, through reducing residual feed intake (RFI), and in reducing agricultural methane (CH_4) emissions. As CH_4 is a major source of digestible energy loss in ruminants, it is plausible that selection for low RFI (i.e., high feed efficient) would also reduce CH_4 emissions. CH_4 production (g/d) and yield (g/kg DMI) for 56 heifers (20–22 mo old) were measured in a 2 × 2 factorial arrangement, including 2 breeds of dairy cattle (Jersey: Jer and Holstein-Friesian: HF; n = 28/breed) previously designated as either high (+2.0 kg DM) or low RFI (−2.1 kg DM; n = 28/RFI category). Breed × RFI category interactions were not significant; main effects are presented (see Table 1). HF heifers were significantly heavier and consumed more than Jer, but breed did not significantly affect DMI/kg BW or daily BW gain (BWg). Because of the lower DMI, Jer heifers produced less CH_4/d, but not per kg DMI or per kg BWg. RFI category had no significant effect on BW or BWg, but DMI and DMI/kg BW were 9.2% and 10.6% less in low RFI heifers. RFI category had no significant effect on CH_4/d or CH_4/kg BWg; but CH_4/kg DMI was greater in low RFI heifers because of their lower DMI. Results probably reflect more complete digestion of ingested feed in low RFI heifers, consistent with previously reported greater physical breakdown of feed and a higher apparent digestibility of organic matter in low RFI animals. In conclusion, selecting dairy heifers for low RFI is unlikely to affect daily CH_4 production (g/d), but could increase CH_4 yield (g/kg DMI). *This research was funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.*

Key Words: feed conversion, environmental sustainability, greenhouse gas

216 Variation in animal performance explained by the rumen microbiome or by diet composition. C. B. Gleason* and R. R. White, *Virginia Tech, Blacksburg, VA.*

Although the rumen microbiome is thought to impact feed digestion, the connection between microbiome and production in beef and dairy cattle remains unclear. The aim of this meta-analysis was to determine if the microbiome can serve as an accurate predictor of animal performance compared with predictions based on diet composition. To support this comparison, a set of models was derived and compared. Models predicted milk yield (MY), ADG, DMI, and feed efficiency (FE) using different sets of independent variables: diet (D), microbiome (M), and experimental methods (EM). Diet independent variables included dietary percentages of dry and organic matter, neutral and acid detergent fiber, crude protein, ether extract, non-fiber carbohydrate, starch, and forage. Microbiome variables included relative abundance of 3 major bacterial phyla, species richness, and species diversity. Experimental variables were publication year, breed type (dairy, beef, or *Bos indicus*), and rumen sampling fraction (fluid or solid). A second set of models used D and EM variables as predictors of the microbiome. Predictor variable sets were used individually and in combination. Linear mixed-effects regression, weighted by standard error, was used to derive models using data from 51 journal articles. Models were compared on the basis of CCC, root estimated variance associated with study and error, and AICc, where appropriate. The MY model using D+M+EM predictors outperformed all other MY models (CCC = 0.71). Average daily gain was most accurately predicted by D alone (CCC = 0.92). Interestingly, M+EM was more successful at predicting DMI than any model using D. Similarly, dairy FE was more accurately predicted by M+EM than D, albeit slightly (CCC = 0.69 vs. 0.65), while beef FE could only be modeled using D variables. Breed type proved a better predictor of phyla than D. Conversely, species richness and diversity indicators were unaffected by breed type, but could be predicted by D. This analysis concludes that, in some cases, the microbiome may serve as an accurate indicator of animal performance independent of diet.

Key Words: rumen microbiome, diet, production

Table 1 (Abstr. 215). LSM (± SEM) for BW, DMI, BWg, and CH4 in Jer and HF heifers from low or high RFI categories

	BW, kg	DMI, kg/d	BWg, kg/d	CH_4, g/d	CH_4, g/kg DMI	CH_4, g/kg BWg
Jer	408 ± 7.0	11.3 ± 0.29	1.2 ± 0.06	242 ± 4.3	21.6 ± 0.51	219 ± 9.6
HF	479 ± 7.0	12.4 ± 0.29	1.3 ± 0.06	267 ± 4.3	21.9 ± 0.51	211 ± 9.6
P	<0.001	0.01	0.09	<0.001	0.65	0.57
Low RFI	448 ± 9.8	11.3 ± 0.29	1.2 ± 0.06	253 ± 4.9	22.7 ± 0.47	222 ± 9.5
High RFI	439 ± 9.8	12.4 ± 0.29	1.3 ± 0.06	256 ± 4.9	20.7 ± 0.47	208 ± 9.5
P	0.50	<0.01	0.31	0.63	<0.01	0.30

217 Relationships between mean rumen pH and time spent under pH 5.8. D. M. Liebe*[1], J. L. Firkins[2], and R. R. White[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*The Ohio State University, Columbus, OH.*

Efforts focused on developing recommendations for how to prevent subacute ruminal acidosis (SARA) have identified nonlinearities between time spent below a threshold pH and mean ruminal pH in terms of their interpretation of likelihood of SARA. The goals of this study were to 1) define a relationship between mean rumen pH and time spent below pH 5.8 (TU5.8) and 2) to investigate how diet and cow characterizations influence TU5.8 when formulating a ration for a mean rumen pH between 6.0 and 6.1. Exponential models were fit with mean rumen pH as an independent variable and TU5.8 as the dependent variable. Metadata on rumen conditions were collected from 16 previously published studies (60 treatment means). Nonlinear least-squares regression was used to derive parameters for an exponential curve fitted through the center of the treatment means. Parameters of this curve were used as informative priors for a Bayesian hierarchical model. A Markov Chain Monte Carlo (MCMC) method was then used to sample randomly from each prior distribution and develop posterior estimates for the 2 parameters of our exponential equation: Φ_1 and Φ_2 within each study. Using these study-specific parameters derived by the MCMC approach as independent variables, linear models were fit to predict Φ_1 and Φ_2 using accompanying physical and chemical dietary data from each study. The rate of increasing TU5.8 was 30% greater at pH 6.0 than pH 6.1. The influence of dietary characteristics was determined by changing each parameter ± 1 SD and measuring area under the curve between 6.0 and 6.1 (AUC) compared with the mean diet. The most influential diet characteristics were DIM, CP, and butyrate concentration, changing AUC for TU5.8 by −6.1%, +3.5%, and +2.1% at +1 SD, respectively. Whereas increasing DIM appears to reduce TU5.8 between mean pH 6.0 and 6.1, increasing dietary CP and ruminal butyrate concentrations appear to increase this TU5.8 the most of the variables studied, although other variables were significant. Irrespective of diet, the targeting of mean rumen pH of 6.0 to 6.1, through dietary changes such as forage chop-size, is recommended to avoid SARA.

Key Words: subacute ruminal acidosis, rumen pH, fiber

218 Preparing and characterizing magnetic nanoparticles coated with cellulose for effective enrichment of cellulolytic microorganisms from rumen. L. Xing[1,2], S. G. Zhao*[1,2], N. Zheng[1,2], and J. Q. Wang[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Cellulolytic bacteria are considered to be one of the most important parts in rumen. However, it is still lack of effective approaches to enrich or isolate those uncultured cellulolytic bacteria. The magnetic nanoparticle-mediated isolation is a new method to isolate or enrich the uncultured bacteria. This research aimed to isolate and enrich ruminal cellulolytic bacteria. The magnetic nanocomposites (MNP) were synthesized and coated with cellulose. Transmission electron microscopy, X-ray diffraction, and value stream mapping were applied to characterize the characterization of MNP. MNP coated with cellulose (MNPC) (17.4 mg/mL) was mixed with *S. bovis*(1.0×10^8 cfu/mL) for 20 min to evaluate its capture efficiency, and for 60 h to evaluate the bacteria stability on MNPC. MNPC was mixed with *Cellulomonas flavigena* (*C. flavigena*, cellulolytic bacterium) and *Streptococcus bovis* (*S. bovis*, non-cellulolytic bacterium) for 60 h incubation to evaluate the isolation specificity of this approach. The bacterial in liquid were sucked out every 12 h and were determined by qPCR. To apply the method to enrich rumen cellulolytic bacteria, MNPC was mixed with rumen bacteria and incubated for 90 h. The bacteria in liquid were sucked out every 10 h and were determined by qPCR. Results showed MNP were globular and had average size of 20 nm and magnetization of 3.3–24.9 emu/g. The capture efficiency of MNPC was 99%. The stability of *S. bovis* on the MNPC was 90.24% after 60 h. The enriched *C. flavigena* raised from 6.49×10^2 cfu/mL to 2.20×10^6 cfu/mL while *S. bovis* could not metabolize cellulose and captured by the MNPC with 1.05×10^3 cfu/mL in the liquid. Relative abundance of the typed rumen cellulolytic bacteria, e.g., unclassified *Butyrivibrio*, unclassified *Fibrobacter*, and *R. flavefaciens* increased from 1.06%, 0.01%, 0.10% to 7.02%, 0.09%, 0.26%, respectively after 90 h incubation. The growth trend of a species from uncultured *Lachnospiraceae* (relative abundance of 28.33%) is related to that of the typed cellulolytic bacteria, so it was indicated that this species was a potential cellulolytic bacterium. This study provided a new approach to cultivate the uncultivable cellulolytic microorganisms.

Key Words: enrichment, magnetic nanoparticles

219 Effects of prepartum dietary cation-anion difference intake on dairy cows: A meta-analysis. I. J. Lean*[1,2], J. E. P. Santos[3], E. Block[4], and H. M. Golder[1,2], [1]*Scibus, Camden, NSW, Australia,* [2]*Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [4]*Arm & Hammer Animal Nutrition, Princeton, NJ.*

Effects of prepartum intake of DCAD equivalents (Eq)/d on concentrations of blood minerals and metabolites, production and health, were evaluated using classical meta-analytical methods. We hypothesized that reducing DCAD intake would reduce the risk of milk fever, hypocalcemia and increase production. There was a maximum of 58 comparisons from 31 experiments and 1,476 cows. Intakes of DCAD were 2.28 Eq/d and −0.64 Eq/d for control and treated groups. Diets with lower DCAD reduced urine pH (SMD = 1.90: −1.23 pH; $P < 0.001$), decreased prepartum DMI (SMD = 0.23; 0.29 kg/d; $P < 0$ 0.001), increased postpartum DMI (SMD = 0.40; 0.63 kg/d; $P < 0.001$), and increased milk production (SMD = 0.172; $P < 0.001$). However, treatment significantly increased milk in parous cows (SMD = 0.29; 1.1 kg/d; $P = 0.002$) but treated nulliparous (NP) cows produced 1.28 kg/d (SMD = −0.2; $P > 0.1$) less than controls. The FCM yield increased with treatment (SMD = 0.12; 0.56 kg/d; $P = 0.03$); but treatment tended to increase ($P = 0.06$) FCM in parous cows and decrease in NP cows compared with controls. Milk fat percentage, fat yield, and protein percentages were not affected by treatment, but protein yield tended to increase (SMD = 0.21; 0.02 kg/d; $P = 0.07$). Treatment increased blood Ca and P on the day of calving, (SMD = 0.53; 0.13 m*M*; $P < 0.001$) and (SMD = 0.40; 0.13 m*M*; $P < 0.001$), respectively, and Ca postpartum (SMD = 0.36; 0.06 m*M*; $P = 0.003$). Blood BHB was decreased in treated cows before calving (SMD = −0.39; −0.04 m*M*; $P = 0.007$). No other blood metabolite changes occurred. Cows fed lower DCAD diets (Eq/d) had less risk of milk fever by 68%, retained placenta (69%), metritis (219%), and morbidity (64%). There was no effect on risk of abomasal displacement or mastitis. There was no effect of differences in Ca intake g/d on outcomes evaluated. Increased Mg intake g/d between groups increased milk fat production and reduced risk of retained placenta. The DMI increased in cows of both parity and milk increased in parous cows. Health was improved for both parity groups, hence this study supports a positive effect of acidogenic diets on dairy cows. More studies are needed on dietary strategies for NP transition cows.

Key Words: DCAD, hypocalcemia, calcium

220 Blood metabolites as indicators of susceptibility to subacute ruminal acidosis in mid-lactation Holstein cows. S. M. Nasrollahi*[1], A. Zali[1], G. R. Ghorbani[2], and W. Z. Yang[3], [1]*University of Tehran, Tehran, Iran,* [2]*Isfahan University of Technology, Isfahan, Iran,* [3]*Lethbridge Research and Develpopment Centre, Lethbridge, AB, Canada.*

The objective of the study was to evaluate blood metabolites as indicators of susceptibility to subacute ruminal acidosis (SARA) in mid-lactation Holstein cows. Seventy-eight lactating dairy cows (DIM = 103 ± 27; BW = 630 ± 77 kg) were fed a high-concentrate diet (65% of DM) during a 24-d study. Cows were classified according to rumenocentesis pH, measured 4 h after the morning feeding on the last day of the study, as tolerant (pH ≥6.0; n = 26), marginal (5.8 ≤ pH <6; n = 21), and susceptible (pH <5.8; n = 31). Cows were also classified according to reticuloruminal pH, measured during the final 5 consecutive days for 14 cows via indwelling sensors, as susceptible if duration of an acidotic condition (pH <5.8) exceeded 330 min/d (n = 9) and tolerant if the acidotic condition persisted <330 min/d (n = 5). Blood was sampled 4 h after morning feeding on d 18 of the study and immediately serum were harvested. Data were analyzed using Mixed Procedures of SAS with a model included the fixed effects of SARA susceptibility (rumenocentesis-based or reticuloruminal-based) and the random effect of parity. The classification based on rumenocentesis pH showed serum activity of aspartate aminotransferase (AST) was greater ($P < 0.03$) in susceptible cows (70.7 U/L) than tolerant (56.6 U/L) and marginal (59.9 U/L) cows. Also, the blood urea nitrogen (BUN) concentration was greater ($P = 0.01$) with SARA susceptible cows (13.6 mg/dL) than marginal (12.2 mg/dL) and tolerant (12.5 mg/dL) cows. Similarly, blood albumin concentration was greater for SARA susceptible cows than for marginal and tolerant cows ($P < 0.02$). The classification based on reticuloruminal pH also resulted in a trend of greater AST ($P = 0.08$) activity and greater BUN concertation ($P < 0.05$) in the blood of susceptible cows. Glucose, triglyceride, cholesterol, globulin, alkaline phosphates and serum amyloid A did not differ among the different susceptibility classes. Among blood metabolites measured, serum concentration of AST was in the strongest correlation with rumenocentesis pH (n = 78; r = −0.32; $P = 0.01$) and milk fat percentage (n = 78; r = −0.23; $P = 0.04$) and therefor it may be a good indicator of acidosis susceptibility in mid-lactation dairy cows.

Key Words: acidosis, blood metabolites

221 Timing of initiation and duration of feeding ruminally protected choline (RPC) affects performance of lactating Holstein cows. J. M. Bollatti*[1], M. G. Zenobi[1], N. A. Artusso[1], G. F. Alfaro[1], A. M. Lopez[1], B. A. Barton[2], J. E. P. Santos[1], and C. R. Staples[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Balchem Corp., New Hampton, NY.*

The objective was to evaluate the timing of initiation and duration of feeding ruminally protected choline (RPC; ReaShure, Balchem Corp., New Hampton, NY) on dairy cow performance. The experiment was divided into the following 2 periods of time: −21 to 21 DIM and 22 to 105 DIM with data from periods analyzed separately using repeated measures within the MIXED procedure of SAS. From −21 to 21 DIM, multiparous Holstein cows (n = 99) were top-dressed with 0 or 60 g/d of RPC (12.9 g/d choline ion). Starting at 22 DIM, cows were switched to the alternate diet (control to RPC or RPC to control) or maintained on the same diet (control to control or RPC to RPC) resulting in a 2 by 2 factorial arrangement of treatments. Methionine (Perdue Ag Solutions LLC, Salisbury, MD) was formulated to 2.9% of metabolizable protein (MP) in the prepartum diets and to 2.4% of MP in the postpartum (PP) diets. Feeding RPC did not affect prepartum DMI, BW, or BCS (means of 11.7 kg/d, 774 kg, and 3.46, respectively). During the first 21 d PP, RPC did not affect DMI (16.2 vs. 16.4 kg/d; $P = 0.72$) or milk yield (35.1 vs. 36.8 kg/d; $P = 0.13$) of control and RPC-fed cows, respectively. Milk fat % was greater for cows fed RPC (4.41 vs. 4.60%; $P = 0.05$). As a result, cows fed RPC produced more energy-corrected milk (ECM; 39.0 vs. 42.0 kg/d; $P = 0.04$) and feed efficiency (FE; ECM/DMI) was greater (2.44 vs. 2.64, $P = 0.01$). Extent of BW loss was greater by cows fed RPC (24.4 vs. 37.7 kg, $P = 0.01$). Between 22 and 105 DIM, yield of milk tended to be greater (44.6 vs. 46.9 kg/d; $P = 0.06$) and yield of ECM was greater (43.2 vs. 45.6 kg/d; $P = 0.05$) for cows fed RPC in transition ((control-control + control-RPC) vs. (RPC-control +

RPC-RPC), respectively). Feed intake did not differ between treatment groups (23.5 vs. 23.3 kg/d), therefore FE was improved by feeding RPC during transition (1.81 vs. 1.92; $P = 0.01$). Concentration of milk fat (mean of 3.46%) and milk protein (mean of 2.85%) were unchanged by treatments. Initiation of feeding RPC at 22 DIM or extending RPC supplementation beyond 21 DIM did not improve milk yield; however, supplementation of RPC between 21 d prepartum and 21 d PP increased yield of ECM over 15 wk PP.

Key Words: choline, dairy cow, transition

222 Uptake of a fluorescent analogue of glucose (2-NBDG) by mixed rumen bacteria and identification of glucose utilizing species. J. Tao*, C. McCourt, H. Sultana, J. P. Driver, C. D. Nelson, and T. J. Hackmann, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Most rumen bacteria are uncultured, making it hard to identify their niche and what feed carbohydrates they consume. Our objective was to identify uncultured rumen bacteria that use one feed carbohydrate (glucose) by (1) incubating bacteria in a fluorescent glucose compound (2-NBDG), (2) using cell sorting to separate out cells that fluoresce, and (3) using high-throughput DNA sequencing to identify sorted cells. Mixed bacteria readily took up 2-NBDG, and flow cytometry showed that 10.0 (0.5 SEM) % cells became fluorescent (positive) (n = 18). Positive cells were separated with fluorescence-activated cell sorting, and post-sort analysis revealed sorted cells were highly [94.9 (0.3 SEM) %] pure (n = 18). The 16S ribosomal RNA gene of sorted cells was sequenced using the Illumina MiSeq platform. This sequencing revealed several bacteria related to cultured groups known to use glucose, including *Prevotella, Butyrivibrio,* and *Succinivibrio.* However, many bacteria belonged to uncultured groups, including those of family BS11, class koll 11, and order RF 39. Control experiments confirmed that RF 39 and other uncultured groups indeed cannot be easily cultured, as they were not detected among cells grown on media including glucose as the sole carbon source. Importantly, RF 39 has been previously associated with efficiency of milk production. Our work begins to reveal the niche of this group by showing some members consume glucose. Work is ongoing to synthesize and test other fluorescent glucose compounds. Our work supports that 2-NBDG, in combination with other compounds, could be used to identify which uncultured bacteria consume which feed carbohydrates, helping define what role uncultured bacteria play in the host.

Key Words: rumen bacteria, 2-NBDG, glucose utilization

223 Effects of lipopolysaccharide dosing on ruminal fermentation in a dual-flow continuous culture system. X. Dai*, E. M. Paula, A. L. J. Lelis, L. G. Silva, V. L. N. Brandao, H. F. Monteiro, and A. P. Faciola, [1]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective of this study was to evaluate the effects of lipopolysaccharide (LPS) dosing on ruminal digestibility and fermentation. Diets were randomly assigned to 6 fermenters in a duplicated 3 × 3 Latin square with three 11-d experimental periods that consisted of 7 d for diet adaptation and 4 d for sample collection. Treatments were control diet (CON); wheat and barley diet (WBD), to induce subacute ruminal acidosis (SARA); and control diet + LPS (LPSD). Fermenters were fed 72 g of DM/d. The forage:concentrate ratio of CON was 65:35. The WBD was achieved by replacing 40% of the DMI of the CON diet with equal amount of ground wheat and barley; the LPS concentration in LPSD was 200,000 EU which was similar to that observed in cows with SARA. The SARA inducing and LPS dosing started at d 8. Ruminal digestibility, VFA, and NH_3-N samples were analyzed in this study. Ruminal pH was measured every hour before SARA induced and every 30 min once ruminal pH went below 5.6. The ruminal pH of WBD was lower than LPSD and CON ($P = 0.05$), and WBD was able to keep ruminal pH below 5.6 for 240 min every sampling day, successfully inducing SARA. Ruminal pH of LPSD was not different from CON, and both were above 6 for the entire experimental period. Ruminal NDF ($P = 0.10$), ADF ($P = 0.09$) and CP ($P = 0.06$) digestibility of LPSD tended to be lower than that of CON, but not different from WBD. There were no differences on total VFA concentration, molar proportion of propionate, isobutyrate, valerate, and isovalerate among treatments. WBD had the lowest acetate and the highest butyrate and lactate concentrations; however, there were no differences between LPSD and CON. The dynamic patterns of VFA of LPSD stated to be similar to WBD at 6 h after LPS dosing. The WBD had the lowest concentration of NH_3-N while the highest dietary N; however, no differences were observed between LPSD and CON. In conclusion, grain induced SARA model was successfully set up in dual-flow continuous culture system; ruminal LPS dosing tended to decrease ruminal fiber and CP digestion but have no effects on ruminal pH and fermentation in a dual-flow continuous culture system.

Key Words: in vitro, SARA, pH

224 Effect of *Saccharomyces cerevisiae* fermentation product and clay sequestering agents on rumen fermentation and bacterial community of lactating dairy cows challenged with dietary aflatoxin B_1. Y. Jiang*[1], I. M. Ogunade[2], A. A. Pech-Cervantes[1], P. Fan[1], X. Li[3], D. H. Kim[1], K. G. Arriola[1], M. B. Poindexter[1], M. C. M. Gonçalves[4], K. C. Jeong[1], D. Vyas[1], and A. T. Adesogan[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Division of Food and Animal Science, Kentucky State University, Frankfort, KY,* [3]*Department of Animal Sciences, China Agricultural University, Beijing, China,* [4]*Institute of Agriculture and Environmental Sciences, Federal University of Mato Grosso, Sinop, MT, Brazil.*

This study was conducted to examine the effects of clay (CL) and *Saccharomyces cerevisiae* fermentation product (SCFP) sequestering agents on the ruminal bacterial community of Holstein dairy cows challenged with aflatoxin B_1 (AFB_1). Eight lactating cows stratified by milk yield and parity were randomly assigned to 1 of 4 treatments in a 4 × 4 Latin square with 2 replicate squares, four 33-d periods and 5-d washout between periods. Treatments were (1) Control (basal diet); (2) T (Control + 63.4 μg/kg AFB_1); (3) CL (T + 200 g/head/d sodium bentonite clay, top-dressed); (4) CL+SCFP (CL +19 g Diamond V NutriTek + 16 g MetaShield, top-dressed). During each period, cows were adapted to the basal diet (36.1% corn silage, 8.3% alfalfa hay and 55.6% concentrate; DM basis) from d 1 to 25 (pre-dosing period), orally dosed with AFB_1 from d 26 to 30 (dosing period), followed by toxin withdrawal from d 31 to 33 (withdrawal period). Data were analyzed using GLIMMIX procedure of SAS. During the pre-dosing period, feeding CL+SCFP increased the relative abundance of *Prevotella* compared with the Control (43.6 vs. 40.0%; $P = 0.02$). During the dosing period, feeding T had no effects on the ruminal bacterial community ($P > 0.10$) but CL+SCFP increased the abundance of *Fibrobacter* compared with T and Control (1.45 vs. 0.97 and 1.05%; $P = 0.04$ and 0.10, respectively). Ruminal fermentation parameters did not differ across treatments. Feed efficiency was positively correlated ($r > 0.30$, $P < 0.05$) with relative abundance of *Treponema*, and *Coprococcus*. Yields of milk and milk components correlated positively ($r > 0.42$; $P < 0.01$) with relative abundance of unclassified *Succinivibrionaceae,* or *Coprococcus*, suggesting they play an important role in increasing milk production. The ruminal microbial

community was not affected by T or CL but CL+SCFP increased the abundances of *Prevotela* and *Fibrobacter* in the predosing and toxin-dosing periods, perhaps resulting in the increased milk yield observed in our companion study.

Key Words: aflatoxin, microbiome, sequestering agents

225 Effect of *Saccharomyces cerevisiae* fermentation products on performance, diarrhea outbreaks, and plasma glucose and NEFA concentration in bottle-fed calves. L. Pisoni*, K. V. Whinnery, and A. E. Relling, *The Ohio State University, Department of Animal Sciences, Wooster, OH.*

The use of microbial additives in rations has become a common practice in ruminant's nutrition. The main purpose on their use is to increase cattle performance and production from early stages. One of these additives are yeast cultures like *Saccharomyces cerevisiae* fermentation products (SCFP). The objectives of this study were to evaluate the effect of SCFP on BW, DMI, diarrhea outbreaks, and plasma glucose and NEFA concentration in bottle fed calves. Eighty newborn calves (n = 40 per treatment) were involved in this experiment. They were randomly assigned to a control (C) treatment or SCFP supplemented treatment (SC). Calves received a total of 6 L of colostrum at birth and 3 L of milk twice daily. They were offered ad-libitum access to a solid concentrate since d 3. Calves on SC also received 1 g of SmartCare daily on the colostrum or milk in the morning feeding, and 0.7% of NutriTek on the solid feed. Body weight was recorded at birth and on d 14, 30, 45, and 60. Dry matter offered was measured daily and refusals twice a week to obtain an average DMI. Blood was sampled on d 7 and 14 to evaluate plasma glucose and NEFA concentration. Fecal scores were recorded daily. On d 30, fecal samples were collected for measurements of *Salmonella* and *E. coli* concentration on feces. Data were analyzed as a randomized complete block design with repeated measurements (SAS 9.4). The model included the random effect of birth day (block), and the fixed effect of treatment, time and the interaction of treatment by time. There was no effect ($P > 0.2$) on BW, DMI, diarrhea outbreaks, nor plasma glucose concentration. Bacteria count showed absence of *Salmonella* and *E. coli* on feces. There was a time by treatment tendency ($P < 0.11$) on plasma NEFA concentration in which C had a plasma NEFA concentration of 269.4 and 209.2 µM for d 7 and 14 and SC had a 242.5 and 241.2 µM for d 7 and 14, respectively. The results showed that, under the current conditions, SCFP do not improve performance. The changes on plasma NEFA concentrations cannot be explained based on the performance results.

Key Words: prebiotic, health, dairy

226 Effect of subacute ruminal acidosis (SARA) and *Saccharomyces cerevisiae* fermentation products on endotoxins and interleukin-6 in blood plasma. L. Xu[1], J. Guo[1], H. Khalouei[1], I. Yoon[2], E. Khafipour[1], and J. C. Plaizier*[1], [1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*Diamond V, Cedar Rapids, IA.*

Grain-induced SARA has been associated with an acute phase response and translocation of endotoxins from the digestive tract. It is assumed that the acute phase response is mediated by cytokines and that *Saccharomyces cerevisiae* fermentation products (SCFP) attenuate the effects of SARA. This study tested if grain-induced SARA increases the translocation of lipoteichoic acid (LTA), an endotoxin of gram-positive bacteria, and raises interleukin (IL)-6 cytokine. A total of 32 lactating Holstein dairy cows were assigned to 4 treatments in a complete randomized block design. Cows received a base TMR ration, containing 34% DM NDF and 18.6% DM starch. Treatments included control (base TMR and 140 g/d of ground corn), and 3 SCFP treatments: XPC (base TMR with 14 g/d of Diamond V Original XPC mixed in 126 g of ground corn), NTL (base TMR with 19 g/d of Diamond V NutriTek mixed in 121 g of ground corn), and NTH (base TMR with 38 g/d of Diamond V NutriTek mixed in 102 g/d of ground corn). Diets were fed from 4 wk before to 12 wk after calving. During wk 5 and 7 of lactation, SARA was induced by replacing 20% of DM of the base TMR with pellets containing 50% wheat and 50% barley. The SARA diet contained 28.2% DM NDF and 27.9% DM starch. Blood and rumen fluid samples were collected twice weekly during wk 4 (baseline), 5 (SARA 1), and 7 (SARA 2). The interaction between the effects of SARA and treatment were tested for significance. LTA was not detected in rumen fluid, but that may have been due to compounds that interfered with the assay. The interactions between the effects of SARA and SCFP on LTA and IL-6 in blood plasma were significant ($P < 0.05$). SARA increased this concentration from 0.66 to 0.71 ng/mL ($P < 0.05$) only in the cows on the control, XPC, and NTL treatments. SARA only increased ($P < 0.05$) the concentration of IL-6 in cows on the NTH treatment (380 vs. 569 ng/mL. Results show that grain-induced SARA increases LTA moderately, without increasing IL-6 in blood, and that NTH can reduce this increase in LTA. This increase in LTA is likely due to increased translocation of this endotoxin, and can be attenuated by LTH. The acute phase response resulting from SARA does not appear to be mediated by IL-6.

227 Evaluation of the effects of silage inoculants on shedding of *Escherichia coli* O157:H7 in dairy cows. D. H. Kim*, F. X. Amaro, M. C. N. Agarussi, V. P. Silva, T. Fernandes, A. A. Pech-Cervantes, Y. Jiang, I. M. Ogunade, D. Vyas, and A. T. Adesogan, *Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.*

The objective was to investigate if *Lactobacillus buchneri* (LB) could reduce *Escherichia coli* O157:H7 (EC) shedding by lactating dairy cows (Experiment 1) and inhibit the growth of the pathogen on and increase the aerobic stability of silage and a TMR (Experiment 2). In Experiment 1, 487 dairy cows were screened for EC counts using swab samples from the recto-anal junction. Twenty-eight infected animals were selected and blocked into low (EC count $<10^3$ cfu/swab) and high shedders (EC count $>10^3$ cfu/swab). Corn forage was harvested and ensiled with (Inoculated; INO) or without (Control; CON) LB inoculant and the resulting silages were mixed into separate TMR. Each TMR was offered to low or high shedder cows for 21 d. For Experiment 2, to measure aerobic stability and EC inhibition, CON and INO silages and TMRs were sampled at silo opening and aerated for 7 d after treatment with either distilled water alone or EC (5×10^5 cfu/g). Treatments were arranged in a 2 × 2 factorial to evaluate main effects of fecal shedding and LB inoculation (Experiment 1) or LB and EC inoculation (Experiment 2) and the respective interactions. Silage inoculation and level of EC shedding did not affect milk production or DM intake ($P > 0.10$). The prevalence of EC was greater for high compared with low shedders (2.67 vs. 2.12 log cfu/swab, $P < 0.01$). Inoculated silage tended to reduce EC shedding from low shedders on d 7 and 21 ($P = 0.13$). Inoculation with LB increased ($P < 0.05$) silage and TMR aerobic stability (144 vs. 30.2 h and 99.2 vs. 25 h, respectively) and inhibited the growth of EC in silage (0 vs. 6.62 log cfu/swab, $P < 0.01$). Inoculation with LB improved silage and TMR aerobic stability, inhibited the growth of EC in aerated silage and tended to reduce EC shedding by low shedder but not high shedder cows.

Key Words: *Escherichia coli* O157:H7 shedding, *Lactobacillus buchneri*, silage

228 The importance of the ruminal epithelial barrier for a healthy and productive cow. J. R. Aschenbach*[1], G. Greco[1], G. B. Penner[2], Q. Zebeli[3], and S. Amasheh[1], [1]*Institute of Veterinary Physiology, Freie Universität Berlin, Berlin, Germany,* [2]*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada,* [3]*Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, Vienna, Austria.*

The squamous ruminal epithelium (RE) is crucial for the performance of dairy cows because it is the main site for absorption of key nutrients (e.g., short-chain fatty acids, SCFA) and electrolytes (e.g., Na^+ and Mg^{2+}). The absorptive function has to be highly selective to prevent simultaneous entry of microbes and toxins from the ruminal content into the blood. As such, absorption is primarily via the transcellular route; whereas, the paracellular pathway is tightly sealed. The latter is accomplished by a network of tight junction (TJ) proteins (claudin-1, claudin-4, and occludin) and TJ-associated proteins (ZO-1); the network being most intense in the *stratum granulosum* where the decisive permeation barrier is located. When microbial fermentation activity is high, the integrity of absorbing cells and their connecting TJ is often challenged by acidity, high osmolarity, toxins, and by immune mediators released during inflammation of the RE. The epithelial damage induced by acidity has been studied most frequently. In contrast to the transient opening of the paracellular space induced by high luminal osmolarity, acidity-induced opening of the RE barrier is not immediately reversible and it even aggravates upon return to physiological pH values. A recent key finding has been that the RE can withstand low luminal pH values rather well in the absence of elevated concentrations of SCFA. It is essentially the combination of luminal acidity and high luminal SCFA concentration that determines the degree of RE damage during an acidotic incidence, with SCFA dose being more relevant than SCFA species. The initial insults set by luminal acidity and the increasing concentrations of microbe-derived toxins such as lipopolysaccharide during acidosis are among the key factors that trigger ruminal and systemic inflammation. Recent studies suggest that thiamine can ameliorate the local inflammation. Butyrate has also been claimed to reduce inflammation in recent studies; however, other studies support promotion of parakeratosis and epithelial injury by butyrate. Further research is needed to enhance the understanding of the various factors that make the barrier tight during epithelial adaptation to changing diets.

Key Words: rumen epithelial barrier, ruminal acidosis, tight junctions

229 Plasma response of histidine and histidine metabolites to incremental amounts of abomasal infusion of histidine in lactating dairy cows. B. L. Basiel*[1], Y. Zhang[1], A. F. Brito[1], N. L. Whitehouse[1], and M. Miura[2], [1]*University of New Hampshire, Durham, NH,* [2]*Ajinomoto Co. Inc., Kawasaki-shi, Japan.*

Supplementation of rumen-protected AA is often used to compensate blood deficiency of Met, Lys and His for optimal milk protein synthesis in dairy cows. The bioavailability of RP-Met and -Lys has been measured using the plasma free AA dose-response technique. However, the characteristic endogenous His pool may increase uncertainty to apply the technique on RP-His. Therefore, our objective was to determine the linearity in plasma His by infusing His abomasally. Five multiparous lactating Holstein cows (149 ± 92 DIM) fitted with ruminal cannulas were used in a 5 × 5 Latin squares with 7-d experimental periods. All cows were fed a basal diet to provide adequate energy and metabolizable protein (NRC, 2001). Treatments were 0, 6, 12, 18, and 24 g/d of abomasally infused His. Blood samples were collected at 2, 4, 6, and 8 h after the morning feeding on the last 3 d of each period and composited by day. Milk samples were also collected on the last 3 d of each period. Milk production and components data, and plasma metabolites were analyzed with the PROC MIXED procedure of SAS. Relationships between plasma His and His infused were described by the PROC REG procedure of SAS. Intake of DM was not affected by His infused. Milk lactose concentration increased quadratically to the His infusion. Milk yield and other milk components (fat and true protein) were not affected by treatment. Plasma His concentration increased linearly with increasing amounts of His infused from 54.7 μ*M* at 0 g/d to 70.2 μ*M* at 24 g/d. Treatments had no effect on all other plasma AA, carnosine, and hemoglobin. Both plasma His concentration (μ*M*) and the proportion of His in total AA were highly related to the amounts of His infused (r^2 = 0.93 and 0.97, respectively). We conclude that there is linear response of plasma His to His supplementation in this short-term study.

Key Words: abomasal infusion, dairy cow, histidine

230 Total-tract fatty acid digestibility responses to altering the dietary ratio of palmitic and oleic acids in dairy cows. A. N. Negreiro*, J. de Souza, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of altering the ratio of palmitic (C16:0; PA) and oleic (*cis*-9 C18:1; OA) acids in supplemental fat on total-tract digestibility responses of dairy cows. Cows were blocked by milk yield and assigned to 3 groups (12 cows per group) in a main plot. Production groups were: a) low (45.2 ± 1.7 kg/d); b) medium (53.0 ± 1.6 kg/d); and c) high (60.0 ± 1.9 kg/d). Within each production group, a truncated Latin square arrangement of FA treatments was used in 2 consecutive 35-d periods with the final 5-d used for sample and data collection. The fatty acid (FA) treatments supplemented at 1.5% diet DM were: 1) 80:10 (80% PA + 10% OA); 2) 73:17 (73% PA + 17% OA 3) 66:24 (66% PA + 24% OA); and 4) 60:30 (60% PA + 30% OA). The statistical model included the random effect of cow within production group, and the fixed effect of treatment, production group, period, and their interactions. Linear, quadratic, and cubic contrasts were used to determine effects of increasing dietary OA. Results are presented in the following sequence: 80:10, 73:17, 66:24, and 60:30. We observed no treatment by production group interactions for any variable of interest. FA treatments did not affect intakes of DM (29.1, 29.0, 28.9 and 29.3 kg/d; $P = 0.68$), NDF (8.43, 8.42, 8.40, and 8.35; $P = 0.66$), or total FA (1164, 1148, 1132, and 1142 g/d; $P = 0.70$). Increasing OA in FA treatments decreased the intake of 16-carbon FA (465, 425, 379, and 370 g/d; $P < 0.01$, linear) and increased the intake of 18-carbon FA (644, 661, 693, and 721 g/d; $P < 0.01$, linear). Increasing OA in FA treatments increased total FA digestibility (76.5, 80.1, 79.9 and 80.6%; $P = 0.02$, linear), 16-carbon FA digestibility (72.9, 76.8, 75.2 and 78.1%; $P < 0.01$, linear), and 18-carbon FA digestibility (79.8, 83.5, 82.8 and 82.7%; $P = 0.05$, quadratic). Increasing OA in FA treatments decreased absorbed 16-carbon FA (337, 326, 285 and 287 g/d; $P = 0.03$, quadratic), increased absorbed 18-carbon FA (518, 551, 573 and 595 g/d; $P < 0.01$, linear), and tended to increase absorbed total FA (896, 921, 900, 924 g/d; $P = 0.1$, quadratic). Our results demonstrate that increasing OA up to 30% in a FA supplement increased FA digestibility and did not affect nutrient intake.

Key Words: digestibility, oleic acid, palmitic acid

231 Effect of plane of nutrition in pre- and post-weaning phases on feed sorting behavior of dairy calves. L. E. Engelking*[1], J. P. Rosadiuk[1], T. C. Bruinje[1], T. J. DeVries[2], and M. A. Steele[1], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*University of Guelph, Guelph, ON, Canada.*

Nutrition has an impact on the behavior of dairy calves in the pre- and post-weaning phases; however, an interaction between these 2 phases is poorly understood. Thus, the objective of this study was to investigate how plane of nutrition in the pre- and post-weaning phases affects feed sorting behavior post-weaning. Twenty Holstein heifer calves were randomly assigned to treatments according to a 2 × 2 factorial design. Treatments were (1) low pre- and low post-weaning, (2) low pre- and high post-weaning, (3) high pre- and low post-weaning, and (4) high pre- and high post-weaning. Calves were fed 5 L/d (2.5 L, 2 × /d; low) and 10 L (2.5 L, 4 × /d; high) of milk on an automated calf feeder from 1 to 8 wks. Water, concentrate, and chopped straw were provided free choice during the pre-weaning phase. During the post-weaning phase calves were fed a dry TMR containing (as-fed) 30% chopped straw and 70% concentrate (low) or 15% chopped straw and 85% concentrate (high) from wk 9–16. Feed refusal samples were collected once weekly from wk 10 - 16 to assess feed sorting using the Penn State Particle Separator (3 screens: 19, 8, and 1.18 mm) separating into long, medium, short, and fine components. Sorting was calculated as the percentage of actual intake relative to predicted intake. Values > 100% indicated sorting for, and values < 100% indicated sorting against. Statistical analyses were performed using the MIXED procedure of SAS 9.4. Plane of nutrition fed pre-weaning did not have affect feed sorting behavior post-weaning. However, calves fed a low plane of nutrition post-weaning tended to sort for more short particles than calves fed high plane of nutrition post-weaning (102.8 vs. 100.8%; $P = 0.10$). Post-weaned low plane calves sorted against medium particles, whereas post-weaned high plane calves sorted for medium particles (95.6 vs. 101.3%; $P < 0.001$). Our results suggest that plane of nutrition fed before weaning does not influence post-weaning sorting behavior, whereas providing a higher plane of nutrition post-weaning through an increased concentrate proportion decreases the incidence of feed sorting.

Key Words: feed sorting, plane of nutrition, pre- and post-weaning

232 Use of tail movement to predict calving time in dairy cattle: Validation of a calving detection technology in dairy cattle. S. E. Mac*, C. M. Truman, and J. H. C. Costa, *University of Kentucky, Lexington, KY.*

Early detection of calving allows the farmer to manage the parturient cow, to be present during calving if necessary and to monitor cases of dystocia in dairy cattle. Dystocia, when not assisted, has the potential to increase calf mortality, decrease milk yield, lower conception rate, and increase uterine disorders. The objective of this study was to evaluate the ability of a precision technology, Moocall (Moocall, Dublin, Ireland), to detect the onset of calving in dairy cattle. Data from 73 cows were collected from September 2016 to January 2017 at the University of Kentucky Coldstream Dairy. The calving detection device was attached to the tail 4 ± 3 d (mean ± SD) before expected due date, and video was recorded for behavior analysis. The tail-mounted technology sends 2 SMS alerts per calving, one at 2 h and the second at 1 h before calving. Accuracy of the calving device was evaluated by comparing the alerts times to the actual time of calving. Tail behavior was monitored and analyzed for frequency and duration of tail lifts 2 h before the first alert (baseline period), the h before the first alert, and the h before the second alert. All analysis was through SAS 9.3. PROC TTEST was used to analyze the alert data. A lower one-sided analysis for significance of the difference in means was performed, with the average difference between alert 1 and alert 2 as 150 and 90 min, respectively. The average time interval between the first alert and calving was 107 ± 10 min (mean ± SEM, $P < 0.01$) and the average time interval between second alert and calving was 71 ± 10 min ($P < 0.01$). Video was evaluated for the frequency and duration of tail lifts during the control period, h before the first alert, and the h before the second alert. Mean frequencies were 3.37, 7.95, and 8.47 (lifts/h), respectively. Mean durations of tail lifts were 55, 124, and 134 s, respectively. The calving detection device has the potential to alert farmers approximately 2 h before calving. The farmer being present during birth can reduce dystocia problems and increasing timely delivery of colostrum, improving cow and calf health.

Key Words: calving, tail behavior, precision technology

233 Balancing diets for intestinal protein digestibility in lactating dairy cattle. C. K. Hoff*, P. A. LaPierre, D. A. Ross, and M. E. Van Amburgh, *Cornell University, Ithaca, NY.*

The excessive use of nitrogen (N) has a negative impact on the environment and increases feed costs for dairy farmers. Work to identify the absolute metabolizable protein (MP) requirements for high producing lactating dairy cattle indicate that those requirements can be met at much lower dietary crude protein levels than are currently in use in the field. However, the intestinal digestibility (ID) of N should be known to formulate closer to requirements. The objective of this study was to evaluate the ID of animal proteins using the assay of Ross et al. (2013) when applied in the Cornell Net Carbohydrate and Protein System (CNCPS) v6.5. A blend of animal proteins were developed from blood meal with an ID of 74.6% N and a feather meal with an ID of 54.6% N. Both diets were isocaloric and supplied metabolizable energy allowable milk of 51 kg at 27 kg DMI and 15.8% CP. The high intestinal digestibility (HID) diet was formulated with 1.18 kg blood meal, whereas the low intestinal digestibility (LID) diet was formulated with 1.3 kg of a blend of 82.8% feather meal and 17.2% of the blood meal, and when the ID was used for formulation, the MP allowable milk was 45.8 kg/d and 43 kg/d, respectively. Multiparous and primiparous cows (n = 96) between 80 and 150 d in milk (DIM) were balanced between the 2 treatments in a replicated pen design of 16 cows per pen, 3 pens per treatment. Milk yield and dry matter intakes were measured daily, while components, body weight (BW), and body condition score (BCS) were measured weekly and the study was conducted for 63 d. Data were analyzed using JMP and the Mixed Model procedure. These results indicate that cattle responded to the difference in predicted ID digestibility and the ID assay can be useful in diet formulation to improve predictive capacity (Table 1).

Table 1 (Abstr. 233).

Item	HID	LID	SE	*P*-value
N	48	48		
BW, kg	743	745	0.4	0.5
DMI, kg/d	26.2	25.7	0.4	0.5
Milk, kg/d	44.5	43.1	0.5	0.1
ECM, kg/d	49.5	46.2	1.7	0.04
Fat, %	4.08	3.81	0.10	0.04
Protein, %	3.03	2.93	0.04	0.06
Fat, kg/d	1.88	1.70	0.05	0.01
Protein, kg/d	1.39	1.31	0.01	0.008

Key Words: intestinal digestibility, nitrogen efficiency, modeling

234 Rumen gene expression in dairy calves fed one of two diets differing in form. N. R. Hardy*, T. T. Yohe, and K. M. Daniels, *Virginia Tech, Blacksburg, VA.*

In the US, there are 2 main feed management strategies for rearing dairy calves: (1) offer high amounts of liquid feed to capitalize on efficiency of non-ruminant metabolism, or (2) offer moderate amounts of liquid feed and expect high starter intake to capitalize on ruminant metabolism. Effects of these programs on ruminal volatile fatty acid (VFA) metabolism remain understudied. The objective was to measure relative gene expression of transporters important for VFA absorption in rumens of calves fed 1 of 2 diets formulated to differ in the primary site of digestion after calves were challenged with a supraphysiological concentration of VFA. Neonatal Holstein bull calves (n = 12) were individually housed and randomly assigned one of 2 dietary treatments, balanced to be isoenergetic and isonitrogenous. Diets were milk replacer only (MRO; n = 6), or milk replacer with starter (MRS; n = 6). Milk replacer was 22% CP, 21.5% fat (DM basis) while the textured calf starter was 21.5% CP (DM basis). Milk replacer and starter were adjusted weekly to provide 4.0 MCal of ME/d and 0.17 kg/d of apparent digestible protein. Water was available ad libitum. Milk replacer and starter intake were recorded daily. Calves were euthanized on d 42–44 after a 6 h exposure to supraphysiological concentration of VFA. Rumen tissue was obtained from the ventral sac region; the epithelial portion was separated and preserved for RNA extraction and cDNA synthesis. cDNA was used in quantitative reverse transcription PCR assays. Genes of interest were *MCT1*, *MCT2*, *MCT4*, *NHE1*, *NHE2*, and *NHE3*. Reference genes were *RPS9*, *RPS15*, and *RPS26*. Data were analyzed with a linear model that included the fixed effect of treatment and random effect of calf within treatment. Rumen epithelial gene expression was not affected by diet. It could be surmised that MRS calves had more total gene abundance than MRO calves based on empty reticulorumen weight (0.67 ± 0.04 kg and 0.39 ± 0.04 kg, respectively; $P < 0.01$), but this data remains to be analyzed. Considering the lack of differences in gene expression it

appears that the rumen is able to undertake VFA absorption irrespective of calfhood diet.

Key Words: dairy calf, rumen, gene expression

235 Effects of housing on Holstein calf well-being during hot weather. H. A. Young*, A. L. Adams Progar, and A. Lopez Ayala, *Washington State University, Pullman, WA.*

Dairy calves are susceptible to heat stress when environmental temperatures exceed 20°C. Heat stress conditions cause decreases in feed intake and compromises calf health. The objective of this study was to compare the behavior and well-being of Holstein heifer calves housed in 2 different housing systems during hot weather over the course of 2 summers (Trial 1 and Trial 2). At 24 – 48 h of age, calves were assigned to 1 of 2 treatments: (1) housed in stalls in a 3-sided barn (S; n = 14) or (2) housed in hutches placed outside (H; n = 8). Each calf was observed until weaning at 42 d of age. Calf BW were measured every 7 d and ADG was calculated. Calf body temperatures were measured hourly using temperature recording devices (iButton) attached to the underside of each calf's tail. Blood samples were collected at 7, 24, and 42 d of age via jugular venipuncture and analyzed for cortisol and thyroxine concentrations using enzyme-linked immunosorbent assays (ELISA). Temperature and relative humidity within the housing systems were recorded at 1 h intervals using data loggers (HOBO) and used to calculate the temperature-humidity index (THI). Calf lying and standing behavior was recorded at 5-min intervals using time-lapse video cameras and analyzed using the scan sampling method. Data were analyzed using Pearson correlations and mixed model ANOVAs with repeated measures. The overall THI was significantly lower in hutches than in stalls ($P < 0.0001$). Calves housed in hutches had higher body temperatures ($P < 0.0001$), spent more observed time lying ($P = 0.001$), and had higher plasma thyroxine concentrations ($P = 0.02$) than calves housed in stalls. Feed efficiency improved ($P = 0.004$) and plasma cortisol concentrations decreased as calves aged ($P = 0.04$), but neither was affected by housing. Future studies should further analyze the environmental conditions each housing system offers calves during hot weather. For example, air flow and black globe temperatures should be measured to provide a better explanation of why hutches remained cooler during hot weather but calves housed in hutches had higher body temperatures.

Key Words: dairy calf, well-being, hot weather

236 Development of a scoring system to estimate fly counts on organic cows. C. Auman*[1], L. Hardie[1], I. Haagen[1], L. Han[1], B. Heins[2], and C. Dechow[1], [1]*Pennsylvania State University, University Park, PA,* [2]*University of Minnesota, Minneapolis, MN.*

Flies can be very irritable to cows, especially during the summer months when populations tend to be high and cows are grazing. These flies can have a negative impact on the cow's health and production. The main objective of this study was to develop an efficient and effective way to score fly populations on cows. Cows for this study were housed on 4 organic dairy farms in the Midwest and Eastern United States. Cows were given a subjective score based on visible flies located on the side of their body that was closest to the scorer. The subjective score scale was from 0 to 4, with 0 being few to no flies and 4 being a high population of flies. Pictures were then taken of those cows to objectively count the flies. Flies were counted if they were in the region between the withers and point of shoulder back to the hip bones and down to the bottom of the barrel. Flies within this region were counted and defined as the total count for that cow. A second count of flies only on the chine was made to determine infestation levels in a smaller but more fly concentrated region. Flies were counted using the software ImageJ, which totaled and marked the flies in an image as the user clicked on them. Statistics were calculated using PROC MEANS and PROC CORR in SAS 9.4. In total, there were 144 pairs of observations of subjective scores and objective counts with 15, 51, 24, 8, and 2 percent of scores assigned as 0, 1, 2, 3, and 4, respectively. In the chine region, the mean (±SD) count of flies was 2.6 (4.7), 13.8 (16.0), 39.8 (44.7), 69.0 (57.9), and 178.0 (81.9) for scores assigned as 0, 1, 2, 3, and 4, respectively. Mean total counts for scores of 0 to 4 were 17.4 (35.0), 37.5 (36.8), 106.4 (61.9), 217.5 (94.3), and 419.3 (164.0), respectively. In order of ascending subjective score, median fly counts in the chine region were 2, 10, 23, 70, 198, and in total were 9, 26, 83, 216, and 378. On average, 35.7% of the total flies were located in the chine region and the rank correlation between chine and total side counts was 0.66 ($P < 0.01$). In conclusion, the subjective scores recorded on farm accurately reflected differences in fly counts, suggesting that subjective scoring is an efficient and effective method for determining fly populations on dairy cows.

Key Words: fly count, organic, dairy

237 Effect of nightly mixing versus separation of dams and calves on behavior, production, and calf growth. A. D. Campeaux*[1], A. R. Lee[1], M. C. Cantor[2], J. H. C. Costa[2], L. G. Schneider[1], and P. D. Krawczel[1], [1]*Department of Animal Science, University of Tennessee Knoxville, Knoxville, TN,* [2]*Dairy Science Program, Department of Animal and Food Sciences, University of Kentucky, Lexington, KY.*

Early separation of dairy calves and dams is viewed negatively by the public. The objective was to observe the effects of nightly mixing of dams and calves versus early separation on behavior, milk production, and average daily gain (ADG) over a 14-d period. Primiparous cows and their calves (n = 20) were separated within 6 h of birth. Cows were housed in a freestall barn in the same pen. From d 0 to d 4, calves were housed in individual straw-bedded pens. Starting at d 5 postpartum until d 19, calves with successful passive transfer (n = 20) were housed individually in hutches from 6:00 to 20:00 h. Nightly, (20:00 to 6:00 h) cow-calf pairs randomly were housed either as control (C); dams in freestalls 24 h/d and calves housed in groups on pasture), or mixed housing (M; dams on pasture with calves). Calves were fed 4 L 26% crude protein and 20% fat milk replacer (Ag Central Co Op, Madisonville, TN) from d 0 to 4 and 6 L from d 5 to d 19. Calves were provided ad libitum starter grain (Calf Primer 1, AG Central Co Op, Madisonville, TN) and water. Cows were milked twice daily at 7:30 and 19:30 h. Loggers were attached to dams and calves' rear legs before d 5 to measure step count, standing, and lying behavior. Milk yield was measured on a daily basis and sampled twice weekly for components. Calf weights were recorded twice weekly. A linear mixed model was used to evaluate the effect of treatment and sex on milk production, milk components, and calf and dam step count, standing behavior, and lying bouts. *t*-tests were conducted to evaluate the effect of treatment on ADG. Control dams took less steps per d than M dams (1795.9 ± 86.1 vs 2114.5 ± 76.0, $P = 0.01$), and produced more milk (28.9 ± 1.9 vs 23.8 ± 1.7 kg, $P = 0.047$. Differences in milk yield should be interpreted with caution as cows were not balanced by projected milk yield. Nightly mixing of calves and dams did not influence calf or dam lying behavior, SCS, milk components, or ADG ($P \geq 0.13$). Calf final body weights were 44.32 ± 5.13kg for (C) and 45.59 ± 4.58kg for (M). Partial exposure of cows and calves may be a viable opportunity to address public concerns.

Key Words: dam-calf, group housing, early separation

238 Supplementing early lactation dairy cows with branched-chain amino acids. C. J. McCabe*[1], F. Leal-Yepes[1], and D. Van Nydam[2], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Cornell University College of Veterinary Medicine, Ithaca, NY.*

The transition period for dairy cows has been highlighted as the period of time where cows are most susceptible to metabolic disorders due to the strong nutrient demands from lactation (Drackley, 1999). This leads to negative energy balance and cows must mobilize their tissue reserves to compensate for the difference, which can cause immune dysfunction and lead to disorders such as hyperketonemia and mastitis (Overton and Waldron, 2004). Hyperketonemia is defined as a concentration of β-hydroxybutyrate ≥1.2 mmol/L in blood and a case is estimated to cost a farmer up to $289 (McArt et al., 2015). The branched-chain amino acids (BCAA) leucine, isoleucine, and valine are all essential amino acids (AA) cows must obtain through their diet or microbial protein synthesis to meet daily requirements. BCAA are unique compared with other AA because they are not directly metabolized by the liver. Branched-chain aminotransferase (BCAT2), the first enzymatic step in the oxidation of BCAA shows higher activity in muscle tissue than in liver (Herman et al., 2010). Leucine plays a particularly important role in activating the signaling factor of the mammalian target of rapamycin (mTOR) pathway to promote protein synthesis in skeletal muscle. Previous studies have shown the mTOR pathway results in decreased food intake by stimulating leptin release and improving glucose metabolism (Herman et al., 2010; Laplante and Sabatini, 2012). Studies in lactating dairy cows have shown no significant difference in milk protein yield, milk volume yield, or changes in the milk profile with BCAA supplementation (Appuhamy et al., 2011; Mackle et al., 1999; Huhtanen et al., 2002). Thus, indicating that BCAAs play a role in muscle protein synthesis with a particular emphasis on leucine (Mackle et al., 1999). By supplementing BCAAs in the early postpartum diet, there is an opportunity to reduce the negative energy balance by stimulating skeletal muscle tissue synthesis through the BCAA mTOR pathway.

Key Words: branched-chain amino acids, transition cows, health

239 Judicious use of antibiotics in pre-weaned dairy calves: A sustainable future for producer, calf, and consumer. W. Jenkins*, K. Alward, and J. Bohlen, *University of Georgia, Athens, GA.*

Antibiotic use in today's animal agricultural industries is heavily scrutinized by the public due to increased risk of antibiotic resistance of pathogens. Development of these antibiotic resistant pathogens causes great concern for both the welfare of animals and the potential of today's antibiotics becoming ineffective for human health. Modifications of the Veterinary Feed Directive has limited the concerns with human health but productivity of dairy calves remains a topic necessitating discussion with regards to prophylactic use of antibiotics. Pre-weaned dairy calves are often fed a milk replacer containing antibiotics (medicated) and antibiotics are additionally used to treat health conditions such as scours and respiratory disease. While many feed medical milk replacers as part of a preventative program, recent studies have shown that the overuse of antibiotics in healthy pre-weaned dairy calves can potentially have a negative effect on their growth and can even lead to increased morbidity cases. These studies also suggest that restricting the use of medicated milk replacers and only therapeutically treating calves with antibiotics, increases the daily feed intake and average daily gain of calves. Further, in healthy calf groups, morbidity and mortality rates did not differ whether calves were fed a non-medicated versus medicated replacer. The additional incentive to a producer is a reduced price point for milk replacer. Coupled with higher rates of gain, this represents significant economic value. Producers must work to improve management programs to ensure healthy calf groups and reduce reliance of medicated milk replacers as preservation of today's antibiotics and the reduction of antibiotic resistance needs to be a top priority for dairy producers. Judicious use of antibiotics correlates to improved calf health, growth, and financial incentive while simultaneously reducing the use of antibiotics in otherwise healthy calves. Restricting antibiotics to only a therapeutic use in dairy calves represents a sustainable future for dairy producers.

Key Words: antibiotics, pre-weaned calves, sustainable

240 Automated temperature reading systems to detect fever in dairy cattle. M. M. Woodrum*, G. Mazon, and J. H. C. Costa, *University of Kentucky, Lexington, KY.*

Fever is a biological response in animals that comes from their co-evolution with pathogens. When the immune system recognizes pathogens, a chain of events is activated that stimulates the hypothalamus to raise the body temperature above thermal homeostatic levels. Cattle's normal body temperature range is from 38.0 to 39.3°C. Fever is defined as a cow's body temperature reaching a temperature of 39.5°C. The rise in body temperature makes it difficult for bacteria and some viruses to replicate within cattle, giving a better chance for the animal to overcome the illness. The rapid immuno-response that produces fever often makes it the first detectable sign of illness, coming before changes in physical appearance. Rectal temperature has been the gold standard for disease detection for decades. However, reliance on rectal temperature is being questioned for its accuracy and convenience. To detect the onset of illness via initial change in body temperature, rectal temperature would need to be taken continuously. Individuals' body temperatures can vary between animals, throughout the day, and in response to disease or stress. Because of this variance, a single temperature measurement has a high probability of being a false positive or false negative when identifying a fever. All individuals' temperature patterns should be considered for accurate fever detection. The act of taking the rectal temperature of an animal may increase temperature, reducing the accuracy of the reading further. Automated temperature reading systems may provide a solution for inaccurate fever detection. Systems including infrared cameras, rumen boluses, and implanted or vaginally inserted temperature data loggers automatically measure temperature. Automated temperature measurements can provide more accurate readings and early fever detection. Studies show improvement with non-invasive and automatic systems that could allow for management practices and early detection of diseases. This would allow producers to limit the spread of disease and more effectively treat their livestock, reducing disease cost, and improving overall cattle health and welfare in the dairy field.

Key Words: disease, health, precision dairy technology

241 Using technologies to enhance respiratory disease detection in calves. C. L. Henderson*, C. S. Petersson-Wolfe, D. R. Winston, and K. M. Daniels, *Virginia Tech, Blacksburg, VA.*

Diseases in dairy calves affect dairy herd profitability. The ability to identify respiratory diseases can be difficult, but is a critical part of calf care. With different technologies, such as accelerometers, pedometers,

and automatic calf feeders, farmers are able to detect when a calf is becoming ill sooner. Bock et al. (2013) used accelerometers alone to monitor changes in calf activity to indicate onset of disease. Placement of the technology on the right hind leg was most effective. Swartz et al. (2016) used pedometers in addition to accelerometers to monitor lying bouts and calf activity in efforts to detect respiratory diseases in calves. Both steps from pedometers and lying bouts from accelerometers provided critical information to detect respiratory disease. Automatic calf feeders can provide information such as drinking speed, number of visits, and intake. Swartz et al. (2017) used a combination of an automatic calf feeder, accelerometers and pedometers to validate the ability of these technologies to detect respiratory disease. Sick calves ate less, drank slower, had an increase in lying bouts and took less steps than healthy calves. With the introduction of accelerometers, pedometers, and the automatic calf feeders, workers are able to indicate diseases in calves at an earlier stage to ensure that calves are treated promptly, thereby reducing recovery times and mortality rates.

Key Words: accelerometer, pedometer, automatic calf feeder

242 Sustaining the dairy industry. G. E. Rodriguez* and C. D. Nelson, *University of Florida, Gainesville, FL.*

As the global population continues to grow, food production must rise to meet increasing demands. The dairy industry is a highly dynamic sector of the agricultural market, from both a national and international perspective. The United States is encountering a unique issue in the industry when compared with overseas operations: the greatest American challenge is countering the drop in fluid milk consumption due to negative consumer perspective. In comparison, densely populated nations, such as China and other countries of Southeast Asia, are still struggling to balance consolidation and growth with resource usage and pollution. While economies, markets, and social attitudes toward the dairy industry vary between individual countries, a clear theme of increased quality and quantity of product with ever-decreasing input resonates globally. Sustainability is a practice ideology that is becoming more prevalent as it relates to the challenges of the dairy industry across the world. The traditional concerns such as water and land consumption and air pollution remain as relevant as always. However, developed dairy economies will find that sustainability challenges exist beyond the physical aspects of resources into social perspectives. An example is the falling fluid milk consumption rates in the United States due to consumer misconceptions and the increasing popularity of milk-alternatives. Well-established industries are moving toward transparency in their operations, or risk their survival in the fast-paced market shifts. Growth demands constant change and challenges. The evolution of today's dairy farm is rooted in the difficulties that our predecessors faced. Like the industry always has, it will continue to meet obstacles head on, and create efficient, sustainable solutions out of struggle. Somewhere along the line may be an answer to the ever-present question: what is the future of the dairy industry?

Key Words: dairy, sustainability, production

243 Strategies for reducing methane emission by dairy cattle. N. P. Uzee* and C. C. Williams, *Louisiana State University, Baton Rouge, LA.*

Methane is the second most prevalent greenhouse gas, and plays an important role in global warming. Methane is generated from many sources, including natural production in the environment, by livestock, and as a result of human activity. Approximately one-fourth of anthropogenic methane emission in the United States is from enteric fermentation primarily from livestock, particularly ruminants. While the effects of methane are generally focused on its global warming potential, methane also results in a net loss of feed energy to the animal. There are numerous strategies that have been utilized to potentially reduce methane production in ruminants. Increasing feed efficiency by feeding highly digestible forages is one approach that has been used. Feeding ionophores has also been shown to reduce methane production in the rumen. Grinding and pelleting of forages may also reduce methane emissions, but this practice is not cost effective for producers. Increasing dietary fat has the potential to decrease methane emissions, but this method could negatively affect milk components and producer income. Methane emission inhibitors are chemical compounds with inhibitory effects on rumen methanogenic microorganisms. These compounds have been studied for their efficacy in reducing methane emissions and effects on production in dairy cattle. Among the more efficient methane inhibitors is 3-nitrooxypropanol (3NOP), which blocks an enzyme necessary for microbial production of methane in the rumen. This inhibitor has been shown to decrease methane emission by 30% and increase body weight gain in dairy cattle, with no effects on dry matter intake, milk production, or feed efficiency. Research with dairy cattle, as well as other livestock species, has shown that methane inhibitors as feed supplements could lead to substantial reductions in greenhouse gas emissions. While there are several strategies that have been proven to reduce methane emission from ruminants, the FDA approval of 3NOP could prove to be a more efficacious method of reducing greenhouse gas emission while improving livestock performance.

Key Words: methane emission, feeding strategies, methane inhibitors

244 Environmental enrichment in dairy cows and calves. J. E. Uren* and M. L. Eastridge, *The Ohio State University, Columbus, OH.*

Environmental enrichment for dairy cows and calves has gained interest with increased focus on animal well-being. Much of the focus with cows has been with mechanical brushes, but broader research has been conducted with calves with less in-field use. Also, limited research has occurred to address enrichment with mechanical brushes for calves in comparison to adult cows. Wilson et al. (Applied Animal Behavior Sci; 76:259, 2002) highlighted the importance of grooming tools, such as mechanical brushes, and revealed that enrichment tools for scratching purposes are used most among the items studied and have sustained interest for feedlot cattle. DeVries et al. (J. Dairy Sci. 90:2241, 2007) illustrated that duration and frequency of grooming behaviors increased (by 508% and 226% respectively) after dairy cows were exposed to a mechanical brush. Furthermore, Mandel et al. (J. Dairy Sci. 96:6506, 2013) examined the possibility of using analysis of cow behavior around a mechanical brush as a gauge of well-being because mechanical brush use may be a luxury activity since use of the brush declined during periods of decreased energy (e.g., food placed far away or post artificial insemination). Research has provided evidence that calves utilize enrichment devices, such as lollies, artificial teats, stationary brushes, and rubber chain links (Pempek et al., J. Dairy Sci. 100:4807, 2017). Finally, Zobel et al. (Animal 7:84, 2017) focused on dairy calf enrichment via manila ropes and mechanical brushes, demonstrating that calves housed in pairs utilized both mechanical brushes and manila ropes for 27.1 min/d. The differences in brush to rope use between individual calves in this study demonstrate a need for additional research to determine preferred and effective enrichment devices. Review of past

studies suggests that mechanical brushes are beneficial enrichment tools for dairy cows and that they may be convenient tools to monitor dairy cow welfare and possibly health. As animal welfare research continues to expand, additional studies need to be conducted focusing on dairy calf enrichment and behavior in individual and group housing systems.

Key Words: enrichment, behavior, welfare

245 Factors that affect lying times of dairy cattle and the effect of increased lying time on milk production. J. R. Sexton* and P. Erickson, *University of New Hampshire, Durham, NH.*

Lying time is affected by factors such as proper stocking density, specific stall dimensions, feed time management and comfortable, compressible bedding that encourages increased lying times while still keeping the incidence of mastitis low. Lying times are indicators of stress because cows that spend longer than normal periods standing have more stressors in their environment, while cows that spend most of their day lying down and ruminating are more comfortable. Increased lying times in dairy cattle increases milk yield and profit.

Key Words: lying time, cow comfort, production

246 Controlling potassium from the field to the diet. D. M. Dietz* and D. R. Olver, *The Pennsylvania State University, University Park, PA.*

One of the most expensive metabolic diseases facing dairy producers is milk fever. In addition to the costs associated with this disease, cows with milk fever are susceptible to other health problems such as retained placentas, displaced abomasums, and ketosis. Although calcium is the key mineral in controlling milk fever, the role of potassium cannot be overlooked. Elevated potassium levels have a profound impact on dietary cation-anion difference (DCAD). Many dry cow forages contain high levels of potassium that can disrupt the calcium homeostasis of close-up dry cows. These high potassium levels are often associated with fields that have been heavily fertilized with manure. Because potassium is a cation, diets with high potassium levels cause an increase in blood pH. Alkaline blood pH levels decrease the mobilization of calcium from bone, thus increasing the cow's risk of developing milk fever. Anionic salts can be used as a method to reduce the effects of excess potassium in the diet. These acidic compounds lower incidences of milk fever by increasing calcium mobilization, but many of these products are unpalatable. The need for these anionic salts to decrease DCAD can be reduced by controlling potassium levels in forages. Forage potassium levels should be kept under 2%. Soil tests can be used to designate specific fields for production of dry cow grasses. Earlier cuttings often contain higher potassium levels than other cuttings, so later cuttings can be designated for use in dry cow rations. Finally, reducing manure application on fields can help lower forage potassium levels. Careful management of potassium levels in the field and in the dry cow diet can help reduce the risks of milk fever and other health issues.

Key Words: potassium, dietary cation-anion difference, milk fever

247 Udder morphology, milk production, and composition in pasture-based dairy ewes during lactation. V. Batinic[1], D. Salamon[2], S. Ivankovic[1], N. Antunac[2], and A. Dzidic*[2], [1]*Faculty of Agriculture and Food Technology, University of Mostar, Mostar, Bosnia and Herzegovina,* [2]*Faculty of Agriculture, University of Zagreb, Zagreb, Croatia.*

The aim of this article is to determine udder morphology and milk production changes during lactation in different pasture-based dairy ewes mainly used for the production of fine cheese varieties. Milk production, udder morphology and body weight were measured and milk composition samples (milk fat, protein, lactose and dry matter percentage) were collected during early (d 80–100), mid (d 140–160) and late (d 200–220) lactation in Kupres (n = 267), Privor (n = 204) and Stolac (n = 226) pramenka ewes. For the statistical analysis, a repeated measures model was used with ewe as a random effect and breed, lactation number and day of lactation, defined as fixed effects. Milk yield was positively correlated with udder width (r = 0.78), udder height (r = 0.66), teat width (r = 0.40), teat length (r = 0.35) and negatively with cistern height (r = −0.16) and teat angle (r = −0.36; $P < 0.05$). Teat length and teat width were positively correlated (r = 0.89; $P < 0.05$). Throughout lactation stages, there was an increase in milk fat, protein and nonfat dry matter content. In contrast, milk production and lactose content decreased throughout lactation. Largest differences between and within breeds were found in early compared with mid and late lactation measurements. More pronounced decrease during lactation was observed in udder width and height and slight in teat width and length. Cistern height ranged from 0.76 cm in early for Kupres to 1.25 cm in late lactation for Privor pramenka ewes. Teat angle ranged from 26° for Kupres in early to 46° for Privor pramenka ewes in late lactation. Interaction between breed and lactation was significant ($P < 0.05$) for all observed variables. Breed with highest milk production and udder height and width was Kupres compared with Privor and Stolac pramenka ewes. Udder and teat measurements in all breeds were medium-sized and acceptable for machine milking resulting in low teat angle and cisternal height below the teat orifice. Most of the observed udder morphology traits had positive correlations with milk yield and therefore could be applied in breeding programs.

Key Words: udder morphology, milk production, dairy ewes

248 Performance and metabolic effects of heat stress in Lacaune dairy ewes in late lactation. N. Mehaba*[1], W. N. Coloma[1], A. A. K. Salama[1,2], X. Such[1], and G. Caja[1], [1]*Universitat Autonoma de Barcelona, Bellaterra; Barcelona, Spain,* [2]*South Dakota State University, Brookings, SD.*

Aiming to assess the performance and metabolic effects of heat stress (HS) in dairy ewes, 8 Lacaune ewes (75.5 ± 3.2 kg BW; 165 ± 4 DIM; 2.31 ± 0.04 L/d) were submitted to thermoneutral (TN) and HS conditions in a crossover design (2 × 21 d periods, 6 d washout). Conditions were (day-night, 12–12 h; THI, thermohygrometric index by NRC, 1971): TN (indoors, 20–15°C and 50 ± 5%; THI = 65–59) and HS (chamber; 35–28°C, 45 ± 5%; THI = 83–75), respectively. Ewes were milked × 2-daily and fed ad libitum. Milk was recorded and sampled for composition (MilkoScan FTIR) on the last week of each period. On first period (d 17, 19 and 21), 2 ewes from each treatment, were used for glucose (GLU) tolerance test (GTT, 0.25 g/kg BW), and lipogenic and lipolytic challenges by insulin (INS, 4.6 μg/kg BW) and epinephrine (2 μg/kg BW), respectively. Blood samples were collected at 12- time points (min −30 to 120) in respect to infusions and plasma ovine-INS analyzed by ELISA (Mercodia). Data were analyzed by PROCMIXED of SAS v.9.4 (SAS Inst. Inc., Cary, USA). Results (Table 1) showed that HS reduced 10% DM and increased 29% water intakes, without effects on milk yield and ECM-6.5%. Milk fat and protein contents decreased by 16% each, and SCC increased by 5%. Basal and final INS values in GTT were similar for TN and HS ewes, peaking at min 10, although HS tended to show higher INS levels than TN (min 5 to 30; $P = 0.09$). No differences between TN and HS ewes were detected in GLU responses to INS challenge, GLU reaching a similar nadir at min 30 (33.9 ± 2.4 mg/dL; $P < 0.001$). On average, NEFA response to INS challenge was lower in HS vs. TN (0.24 ± 0.03 vs. 0.36 ± 0.03 mmol/L, respectively; $P = 0.032$), whereas their response to epinephrine was greater in TN vs. HS ewes, the difference being observed from min 5 to 45 ($P < 0.001$). In conclusion, lactational performances of dairy ewes in late-lactation were moderately impaired by the HS conditions used, although milk fat and protein contents and fat mobilization were severely worsened. Dramatic impacts on cheese yielding and energy balance are expected in dairy ewes exposed to hot weather conditions. *Study supported by MINECO Spain (Project AGL2013-44061-R).*

Table 1 (Abstr. 248).

Item	TN	HS	SEM	Trt	Period	Trt × Period
Feed, kg DM/d	2.49	2.23	0.08	0.040	0.51	0.62
Water, L/d	7.11	9.20	0.33	0.001	0.004	0.18
Milk, kg/d	1.71	1.87	0.13	0.37	0.14	0.77
Fat, %	6.81	5.74	0.18	0.21	0.70	0.62
Protein, %	6.37	5.51	0.16	0.003	0.69	0.07
SCC, $\log_{10}$	4.40	4.63	0.06	0.015	0.89	0.56

Key Words: dairy ewe, heat stress, metabolism

249 Genotype effects on energy and protein requirements for gain in goats. I. A. M. A. Teixeira*[1], A. K. Almeida[1], E. Kebreab[2], and K. T. Resende[1], [1]*Unesp, Jaboticabal, SP, Brazil,* [2]*University of California, Davis, CA.*

The objective of this study was to verify genotype effect on net energy (NE_g) and protein (NP_g) requirements for growth of dairy, meat, and indigenous goats, as well as to provide estimates of energy and protein requirements for growth in goats. For that, 11 comparative slaughter studies were gathered in a database with 240 individual records of meat (n = 79), dairy (n = 94), and indigenous (n = 67) goats. Allometric equations were fitted to estimate body protein and energy contents in the empty BW (EBW) as dependent variables and EBW as allometric predictor. Body protein (g) was also used as allometric predictor for estimating energy body composition. Parameter estimates were acquired by linearized (log-transformed) allometric equations using the MIXED procedure in SAS software (SAS Institute Inc., Cary, NC). The model included the random effect of the study and fixed effects of goat type (meat, dairy, and indigenous). Net requirements for growth were estimated as the first derivative of the generated allometric equations with respect to EBW. Additionally, fitting the allometric equation of body energy (kcal) on total body protein as independent variable (i.e., protein weight) was done. Monte Carlo method was used to estimate standard

deviations of the calculated NP_g and NE_g. Irrespective of goat type, NP_g did not change as goats grew from 5 to 45 kg BW (i.e., ranging from 185.1 ± 1.82 to 192.5 ± 4.33 g/kg of EBW gain). On the other hand, NE_g increased from 1,699 ± 65.8 to 3,450 ± 219 kcal/kg of EBW, from 1,732 ± 52.2 to 2,899 ± 133 kcal/kg of EBW, and from 1,785 ± 44.8 to 4,119 ± 198 kcal/kg of EBW for meat, dairy, and indigenous goats between 5 and 45 kg BW, respectively. In conclusion, net protein requirement does not differ across genotypes. On the other hand, net energy requirements are influenced by genotype. Energy and protein requirements for growth in goats reflect on body composition differences, as a result of maturity degree of the goat type at a given weight, which was indicated by the lack of difference in net energy required per gram of protein gain. Future research should focus on the effect of maturity degree on NE_g.

Key Words: body composition, comparative slaughter, goat type

250 Rumen fatty acid profile of dairy ewes fed contrasting sources of energy supplementation. F. E. Miccoli*[1,2], D. Colombatto[2,3], and R. A. Palladino[1,3], [1]*School of Agriculture Science, National University of Lomas de Zamora, Buenos Aires, Argentina,* [2]*Department of Animal Production, University of Buenos Aires, Buenos Aires, Argentina,* [3]*Consejo Nacional de Investigaciones Cientificas (CONICET), Buenos Aires, Argentina.*

Energy supplementation can alter ruminal biohydrogenation (BH) of polyunsaturated fatty acids, producing diverse intermediates which may lead to different milk fatty acid (FA) composition. The aim of the study was to evaluate rumen FA profile in dairy ewes supplemented with contrasting sources of carbohydrates on a fresh ryegrass-based diet. Six mature fistulated sheep (30 DIM; 78.2 ± 13.53 kg) were assigned to treatments: SH (soybean hulls) and CG (corn grain), 3 ewes in each treatment in a crossover design with 14 d of adaptation. Daily ration was offered at 4% of BW in a 50:50 forage to concentrate ratio and balanced with soybean meal to achieve 16% CP (NRC, 2001). Fifty-milliliter ruminal samples were collected at 08:00, 12:00, 16:00, 20:00, 24:00 and 04:00 h and pooled per animal in each experimental period. Then a 50 mL subsample of the pooled sample (n = 18) was taken for further fat extraction and FA methylation for gas chromatography. Data were analyzed as a crossover design (Infostat, 2009), and means were compared by Tukey test (a = 0.05). No differences were observed for C8:0 C12:0, C13:1, C14:0, C14:1,C16:0, C16:2, C16:3, C18:0,C18:1t9, C18:1c11, C20:5 and C22:6 ($P > 0.05$) although C15:0, C16:1c9 and C17:0 were higher in SH ($P < 0.05$). Vaccenic acid and C18:1c9 concentrations were higher in CG than in SH (4.83 vs. 2.93; 0.81 vs. 0.38g/100g FA; $P < 0.05$) in contrast with linolenic acid, C18:4 and C20:4 (1.26 vs.1.79, 0.02 vs. 0.07 and 0.43 vs. 0.57g/100g FA). Surprisingly, and in contrast to several reports in cows, linoleic and rumenic acid contents tended to be higher in CG (4.21 vs.2.66 and 0.15 vs. 0.04g/100g FA) and C18:2t10c12, higher in SH (0.14 vs. 0.09 g/100g FA) ($P < 0.1$). Despite this fact, we have previously informed no differences in either C18:2c9t11 or C18:2t10c12 in milk fat (Miccoli et al., 2017). As less information is available in small ruminants compared with cows, results should be taken with caution. These findings are consistent with previous reports showing that concentrates alter BH pathways in a different manner among species.

Key Words: rumen fatty acid, energy supplementation, dairy ewes

251 Changes in blood key metabolites and insulin in late-pregnant prolific Afec-Assaf ewes drenched with propylene glycol or glycerol. U. Moallem*[1], A. Tamir[1,2], A. Rosov[1], L. Lifshitz[1], H. Dvir[1], and G. Elisha[1], [1]*Department of Ruminant Science, ARO, Volcani Center, Rishon LeZion, Israel,* [2]*Department of Animal Science, University of Jerusalem, Rehovot, Israel.*

Propylene glycol (PG) and glycerol (GL) are common glucogenic supplements for treatment of hyperketonemia or hypoglycemia in sheep. The objective was to determine the diurnal changes (24 h) in blood key metabolites and insulin in response to PG or GL drenching in ewes. Eighteen late-pregnant ewes (~132 d pregnant), averaging 2.97 ± 0.17 BCS, bearing 2–4 fetuses and with blood β-hydroxybutyrate (BHB) concentration of 0.5–1.4 mmol/L were included. Ewes were divided into 3 groups according to BHB levels, expected litter size, BW and BCS, and were drenched with: 1) Control (CTL) – 55 mL of water; 2) PG - 106 mL of PG (448 calories), and 3) GL – 108 mL of Koforin 80 (80% GL; 448 calories). Blood samples were taken before, and post-drenching (PD) every hour for 24 h. Concentrations of glucose, BHB, NEFA, lactate, glycerol and insulin were determined. Data were analyzed using the PROC MIXED procedure of SAS. According to the response pattern, data were analyzed in 3 time-periods PD: (1) 1–6 h; (2) 7–14 h; and (3) 15–24 h. In period 1, glucose concentrations were highest in the GL ($P < 0.001$), BHB were lowest in the PG ($P < 0.001$), and NEFA levels were lower in the GL than in the CTL ($P < 0.02$). Lactate concentrations were highest in the PG ($P < 0.0001$); glycerol were 5-fold higher in the GL than in the CTL ($P < 0.01$) and insulin was lower in the CTL than in the GL ($P < 0.03$). In period 2, glucose concentrations were higher in the PG than in the GL ($P < 0.04$), BHB were higher in CTL than in GL ($P < 0.03$), NEFA and insulin were similar, lactate were highest in the PG ($P < 0.0004$), and glycerol were higher in both PG and GL than in the CTL ewes ($P < 0.004$). The differences between groups in period 3 were negligible. These results suggest that GL is more effective than PG in enhancing the glucose in the first few hours, but less effective than PG in reducing BHB. In addition, the increased concentrations in lactate in response to PG, and the faster increase in blood glucose in response to GL, suggests on different metabolic path in which these 2 glucogenic precursors enter the gluconeogenesis cycles: PG through lactate and GL through the shortest pathway, which influences the immediate response.

Key Words: sheep, propylene glycol, glycerol

252 Effects of early prenatal heat stress on the postnatal performance of dairy goats. W. N. Coloma*[1], N. Mehaba[1], A. A. K. Salama[1,2], X. Such[1], and G. Caja[1], [1]*Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*South Dakota State University, Brookings, SD.*

Environmental conditions affecting mothers during pregnancy may modify the fetal programming through physiological and epigenetic changes. This study aimed to evaluate the postnatal effects of heat stress (HS) during early pregnancy on dairy goats. Thirty lactating Murciano-Granadina dairy goats (41.8 ± 5.7 kg BW) in late lactation were housed in pens of 5 and synchronized with vaginal sponges to be mated naturally at 2-d intervals. Goats were allocated to 2 balanced groups to which thermoneutral (TN, n = 15; 15–20°C) or HS (n = 15; 12-h day at 37 ± 0.5°C, 12-h night at 30 ± 0.5°C) conditions were applied before (d −12 to 0) and after fertile mating (d 1 to 45). Does were fed a total mixed ration (70% alfalfa hay and 30% concentrate) ad libitum and water was freely available. Rectal temperature and respiration rate were recorded 3-d per week at 8, 12 and 17 h. Feed and water intake were recorded daily. From d 45 onwards of pregnancy all the goats were joined in a unique group and conducted under semi-intensive conditions. Two weeks before the expected parturition, does were weighed and moved to kidding pens for permanent supervision. At delivery, litter size, pregnancy length and litter weight were recorded. Kids were fed with a milk replacer ad libitum and weighed at d 35 of age. Data were analyzed by

PROC MIXED for repeated measurements of SAS (v.9.1.3). Table 1 summarizes the obtained results (RMSE, residual mean square error). Although litter size did not vary by treatment, pregnancy length was shorter (−3 d) in HS than TN does and, consequently, birth weight of the kids tended to be 7% lower in the HS group ($P < 0.10$). Neither growth until d 35 nor body weight of 35-d-old kids were affected by the treatments. In conclusion, a short period of HS (i.e., heat wave) during the mating period and early pregnancy will reduce the duration of pregnancy with possible epigenetic effects on the kids. Further research is needed to know the long-term effects produced by fetal programming on the kids. *Study supported by MINECO Spain (Project AGL2013–44061-R).*

Table 1 (Abstr. 252).

				P-value	
Item	TN	HS	RMSE	Trt	Litter size
Litter size, no. kids	2.31	2.23	0.79	0.806	—
Litter weight, kg	5.40	4.71	0.71	0.061	0.001
Pregnancy length, d	146	143	2	0.006	0.915
Kid birth weight, kg	2.34	2.18	0.38	0.122	—
Kid weaning weight d-35, kg	7.88	7.64	1.34	0.520	—

Key Words: fetal programming, heat stress, dairy goat

253 Effect of 2,4-thiazolidinedione treatment on milk fat synthesis in lactating dairy goats in optimal nutritional status. S. Jaff*[1], F. Rosa[1], M. Moridi[1,2], J. Osorio[1], J. Lohakare[1,3], E. Trevisi[4], S. Filley[1], C. Estill[1], G. Cherian[1], and M. Bionaz[1], [1]*Oregon State University, Corvallis, OR,* [2]*Guilan University, Rasht, Guilan, Iran,* [3]*Kangwon National University, Chuncheon, South Korea,* [4]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

In a prior in vivo experiment, daily injection of the putative Peroxisome Proliferator-activated Receptor gamma (PPARγ) agonist 2,4-thiazolidinedione (TZD) in dairy goats with less-then-adequate nutritional status did not affect milk fat. In the present experiment, we tested the hypothesis that PPARγ activation affects milk fat synthesis in goats with adequate nutritional status. Saanen multiparous goats in early-mid lactation received a diet that met NRC requirements and were injected daily with 8 mg/kg BW of TZD (n = 6) or saline (n = 6; CTR) for 3 wk. Blood and milk samples were collected at 9 time points during the experiment. Blood samples were used to measure several parameters related to metabolism and inflammation. Milk yield and milk components including fatty acid profile were assessed. Expression of fatty acid metabolism-related genes was measured via RTqPCR in adipose tissue and mammary epithelial cells (MEC) isolated from milk. Adipocytes size was assessed by histological analysis. Data were analyzed by GLIMMIX of SAS with TZD and time and their interaction as main effects and goats as random effect. Decreased ($P < 0.05$) plasmatic NEFA, NEFA/Albumin, BHB, and fatty acids and increased ($P < 0.05$) glucose were detected in TZD. Milk yield and milk fat were not affected by the treatment. TZD increased ($P < 0.05$) the size of adipocytes and increased ($P < 0.05$) the expression of genes related to glucose metabolism and insulin signaling such as *GAPDH* and *IRS1* and tended ($P \leq 0.09$) to increase *SLC2A4* and to decrease the expression of *CD36* and *YWHAZ*, a putative inhibitor of insulin signaling. The expression of *SCD1* was decreased ($P = 0.06$) in MEC by TZD treatment. In milk TZD increased ($P < 0.05$) the proportion of long and very long chain fatty acids of dietary origin, such as C18:0, several 18:1 isomers, all-trans C18:3, and C22:0. No other parameters were affected by TZD. Results failed to support the hypothesis that TZD activates PPARγ in MEC of animals in good nutritional condition. TZD had minimal effect on milk fat synthesis but milk fat production was maintained at the level of CTR despite a lower availability of NEFA and a possible larger de-novo fatty acid synthesis using glucose in adipose tissue.

Key Words: thiazolidinedione, dairy goat, milk fat synthesis

254 Influence of vitamin B_2, ascorbic acid, and melatonin on reproductive performance of ewes during the summer season. M. M. Waheed*[1,2], K. H. El-Shahat[2], A. A. Sallam[3], B. E. El-Saidy[3], and T. A. A. Khalifa[4], [1]*King Faisal University, Alhufof, Alahsa, Saudi Arabia,* [2]*Cairo University, Giza, Egypt,* [3]*Animal Production Research Institute, Sakha, Kafr Alskeikh, Egypt,* [4]*Veterinary Research Institute, Ionia, Thessaloniki, Greece.*

Trials were conducted to evaluate the influence of vitamin B_2, ascorbic acid and melatonin on the reproductive performance of 42 healthy pluriparous mature cross (3/8 Finnish × 5/8 Rahmani) ewes during summer. Ewes were allocated into 4 groups: the first group (G_1; n = 11, 18–27 mo, 36–41 kg, BCS = 3) injected with 2mL saline, served as control. The second (G_2; n = 10, 20–31 mo, 34–40 kg, BCS = 3) and third group (G_3; n = 11, 19–28 mo, 35–42 kg, BCS = 3) were injected intramuscular daily for 3 wk with 2 mL (1 5mg) of vitamin B_2 and ascorbic acid, respectively. Ewes in the fourth group G_4 (n = 11, 22–29 mo, 33–39 kg, BCS = 3) received double daily subcutaneous injections of 0.5 mg melatonin at 8AM and 4PM at the dorsal surface of the ear. All treated groups (G_2, G_3, and G_4) received 1g zinc chloride and 2.5mg chromium (Merck Millipore, USA, Cat #. 1088161000 and 1120970500, respectively) orally with ration once per week for 3 weeks. All animals were checked for the onset of estrus 2 times daily using an intact ram and ewes were bred via natural mating using 3 rams. Reproductive performance parameters (onset of estrus, lambing rate and number of lambs) were recorded. Laparoscopic examinations were carried out throughout the study for detection of ovulation rates. Data are presented as means ± SEM and compared by Student's *t-test* using SPSS program. Results revealed that, ascorbic acid had a significantly ($P < 0.05$) decreased mean interval (days) from treatment to estrus as compared with control group (15.73 ± 1.83 vs. 22.64 ± 2.50, respectively). A remarkable improvement in the lambing rate in G_2, G_3 and G_4 treated groups than in the G_1 (80.0, 90.9, 90.0 Vs. 72.7%, respectively). The highest proportion of ewes bearing single lamb observed in G_3 (100%). Incidence of lamb mortality found only in G_2 (triplet) in one ewe. The ovulation rate was significantly ($P < 0.05$) elevated in G_4 than that in G_2 and G_1 (1.60 ± 0.16 vs. 1.20 ± 0.13 and 1.14 ± 0.00, respectively). The follicular development did not vary among groups. In conclusion, vitamin B_2, ascorbic acid and melatonin have a beneficial effect on the reproductive performance of ewes during summer.

Key Words: ewes, ascorbic acid, melatonin

Teaching, Undergraduate and Graduate Education Symposium: Active Learning—From Theory to Practice

255 There is a difference between presenting and teaching. N. Fuhrman*, *Department of Agricultural Leadership, Education, and Communication, University of Georgia, Athens, GA.*

As the title suggests, there is a difference between simply "presenting" information and truly teaching it to others. Through this discussion and demonstration, participants will engage in a hands-on experience with active learning theories and will leave thinking differently about their teaching. Constructivism, experiential learning, and Bloom's taxonomy will be used to help teachers realize that some of the most profound, memorable learning experiences occur through "teachable moments"—even those that are intentionally planted in a lesson. The importance of reflection following active learning will be used to transition into methods of evaluating and documenting student learning following active learning experiences.

Key Words: teaching, active learning

256 Promoting active learning in teaching and assessment. W. J. Warner*, *North Carolina State University, Raleigh, NC.*

Learning is not a passive pursuit. To engage in meaningful learning, retain key information, and demonstrate important tasks and/or behaviors, individuals need the opportunity to actively participate. Educational researchers have suggested a holistic model of active learning consisting of information and ideas, experience, and reflective dialog. This presentation will provide pedagogical strategies, assignment ideas, and assessment approaches to support this holistic approach to active learning.

257 How active learning can develop intercultural competencies. M. Russell*, *Purdue University, West Lafayette, IN.*

The objectives of this presentation are to (1) apply experiential learning pedagogies to active learning practice, (2) define and integrate intercultural competencies as learning objectives, and (3) map course goals, objectives, activities to the assessments. A form of experiential learning, active learning has been defined as "any instructional method that engages students in the learning process and requires students to do meaningful learning activities and think about what they are doing" (Prince, 2004; Eison, 2010). Applications of active learning include engaged, team- or student-centered, inquire-based, and even service-learning and are independent of place. Regardless of learning strategy, instructors must strategically select desired learning outcomes and objectives. Most of us are trained to teach and assess agricultural science content, yet increasingly, employers demand affective competencies as well as discipline content learning outcomes (Crawford et al., 2011). Seemiller (2014) and others have identified the ability to work in multicultural and intercultural teams as a critical employability skill. Vande Berg, 2016 identified 4 phases of diversity and intercultural effectiveness; self-awareness, awareness of others, managing emotions and thoughts in the face of differences, and shifting frames and behaviors to other cultural contexts. As with any other learning objective, instructors must intentionally map the learning objectives to appropriate interventions (activities) and then to the assessment of outcomes (Moore, 2014; Kyndt et. al., 2016). Examples of course syllabi will emphasize the importance of congruence among learning objectives, specific interventions, and appropriate assessment methods and instruments. As agricultural educators develop courses for students to grow and learn, we must include social science competencies that engage students in deeper learning. By the end of this presentation, we will have increased the participants' comfort in curriculum design that addresses the intercultural affective domains.

Key Words: affective domain, course design, employability skills

258 Integrating active learning strategies in study abroad programming. E. L. Karcher*, *Purdue University, West Lafayette, IN.*

Undergraduate students in agriculture must have an appreciation for global issues. Imparting a global perspective on students aims to help those in agricultural disciplines to form a link between production and the consumer. Currently, 37% of undergraduate students in the Purdue College of Agriculture participate in at least one international experience during college. The selection of programs available to students varies substantially in design and academic rigor. International exposure does not necessarily equate to learning, and program leaders may struggle to overcome the "tourist vacation" factor while abroad. Therefore, the objective of this presentation is to discuss the selection and assessment of active learning strategies best used to facilitate learning in study abroad programming. Study abroad participation is considered a high-impact practice. However, program leaders must facilitate student development with carefully planned learning activities to maximize student benefits. Selected activities must align with specific program learning objectives that challenge students to become global learners through a structured framework created by the instructor. Examples of active learning strategies include blogging, group projects, reflection assignments, and journaling. Proper assessment is critical to gaining evidence of each assignment's effectiveness in developing a global learner. Predefined rubrics, such as those available from the Association of American Colleges & Universities, provide guidance for assessing competencies, such as student's global world-view development. Developing study abroad programming that facilitates growth in students' intercultural competence is critical as the world continues to globalize. The ability for undergraduates to participate in well-designed study abroad experiences is a necessary component for the development of future leaders in the global agricultural industries.

Key Words: active learning, high-impact practices, global learning

259 College classrooms as active learning environments. M. A. Wattiaux*, *University of Wisconsin-Madison, Madison, WI.*

Active learning has been defined as "anything that involves students in doing things and thinking about the things they are doing." Compared with classrooms in which students are primarily (passive) listeners and instructors are primarily (active) transmitters, active learning environments are characterized by teaching strategies that engage students in pre-planned and structured activities (in and out of class). Such activities have improved learning skills, reduced the achievement gap among students with contrasting levels of preparation (academic and socio-economic backgrounds), and lowered failure rates in large enrollment, introductory, or gateway courses. A recent meta-analysis (n = 158 studies) indicated that traditional lecturing increase failure rates by 55% and student performance on tests and concept inventories increased

by 0.45 standard deviation in active learning classes. These beneficial effects were proven across science, technology, engineering, and math (STEM) disciplines, course types and levels, and are usually stronger in smaller enrollment courses. Although class activities are sometimes viewed as temporary diversion strategies to maintain students' engagement in lecture, their impact are likely to increase when designed and implemented with clearly defined objectives and expected learning outcomes. Using classroom time to deliver course content becomes increasingly obsolete. With ubiquitous educational technology, courses can be designed so as to make students accountable for engaging thoughtfully with materials before class. In flipped classrooms (and to a lesser extent in blended classrooms), in-class time privileges individual, pair, small group or large group activities that engage students in higher order of analytical thinking and confront them with diverse and alternative modes of understanding. As such, active learning creates a community of learners who begin to think more like scientists (on a quest for new discoveries). Active learning nudge students toward an understanding that knowledge is neither given nor gotten, but constructed, a greater ability to assess their own beliefs, and the realization that learning is a worthy life-long goal.

Key Words: undergraduate education, teaching

260 Impacting student career path ideas and options through industry career centers. T. S. Heady*, *Elanco Animal Health, Greenfield, IL.*

Elanco Animal Health is a global research-based company that develops and delivers product and services to enhance animal health and production, based in Greenfield, Indiana. We value innovation, both in scientific research and daily operations, and strive to cultivate a collaborative work environment for more than 6,100 employees in more than 70 countries. Founded in 1954, Elanco is a division of Eli Lilly and Company. Our summer intern program offers undergraduate and graduate students an opportunity to learn more about our company, their chosen career path, themselves, and industry in general. Roles are found in areas including research and development, sales, marketing and manufacturing. The 12-wk paid roles include a week's long orientation and a fun-filled presentation week at our global headquarters, and running from mid-May to mid-August. As a courtesy, we find safe, affordable housing for the interns as well. We look for highly motivated and driven leaders, and those who want to enrich the lives of others through food and companionship. We also seek individuals with integrity, excellence and respect for people, as these are our company values. As a company, we offer a purposeful career, a balance of work and life, a diverse culture and a vibrant community. All eligible interns will be considered for full-time positions based on their performance over the course of their summer internship. Elanco also arranges various intern activities including sporting events, dinners, team lunches, etc. to provide opportunities for socializing, professional development, and learning more about the company.

Key Words: intern, career, students

T1 Sample size estimates for assessing lameness, leg injuries, and body condition. J. M. C. Van Os*[1], D. M. Weary[1], J. H. C. Costa[1,2], M. J. Hötzel[2], and M. A. G. von Keyserlingk[1], [1]*Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada,* [2]*Laboratório de Etologia Aplicada e Bem-Estar Animal (LETA), Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil.*

There is increased demand for herd-level animal welfare evaluations but little consensus on how best to sample for this purpose. Our objective was to evaluate the effect of different sample size approaches on farm classifications relative to thresholds of acceptability for animal-based measures. We predicted more accurate classifications when more cows were sampled and when selecting from all lactating cows compared with only the high-producing pen. On 38 freestall farms, we assessed all 12,375 lactating cows for lameness, injuries on the tarsal and carpal joints, and BCS and then compared these herd prevalence measures with 9 sampling strategies (using precision of 15, 10, or 5% applied to the high pen, all lactating cows, or the entire herd). For each sampling strategy, we selected cows randomly in 10,000 replicates, calculating the prevalence for each replicate and classifying farms as meeting (below) or failing to meet (above) 16 thresholds for cow-based measures. For each threshold, we determined how many farms were classified correctly in ≥95% of sample replicates. Across thresholds, the number of farms meeting the ≥95% target increased with the number of cows sampled (i.e., when using narrower precision values and when applying the formula to lactating cows rather than the total herd size). Contrary to predictions, sampling from the high pen resulted in similar accuracy to selecting from among all lactating cows. For example, with a threshold of < 10% severely lame cows, 33 farms met the ≥95% target when calculating sample size using 10% precision applied to lactating cows. This decreased to 32 or 26 farms when the formula was applied to the high pen or the whole herd, respectively. Using a precision of 15 or 5% changed the number of farms meeting the target by –4 to –7 and 4 to 8, respectively. Narrower precision greatly increases sample size requirements (e.g., by up to 183 cows when using 5 vs. 10% precision), and assessment programs will need to balance misclassification with feasibility constraints. Our findings suggest sampling high-producing cows can serve as a practical proxy for the larger population of lactating cows.

Key Words: welfare assessment, sampling, validation

T2 Farm-level housing and management factors associated with clinical lameness in freestall-housed dairy cows in the United States. K. M. Luchterhand*, L. Collings, C. Cook, K. Ito, J. Kelsey, M. Mouw, and K. Wood, *Novus International Inc., St. Charles, MO.*

The objective of this study was to examine housing and management factors associated with clinical lameness in Holstein freestall housed dairy cattle across USA. Data were collected from 535 commercial freestall dairy farms from November 2012 to July 2017. Herd size averaged 1,434 (range 105–13,000) lactating cows. The high production Holstein pen was scored for locomotion (LS; 1 = normal gait, 5 = severely lame; clinically lame, LS ≥ 3), hock injuries (hair loss and/or swelling; %) and knee injuries (swollen; %). Accelerometers were placed on 40 randomly selected cows for pen 3 d averaged daily lying time. Lying time difference was calculated from longest and shortest 3 d averaged lying times of the pen. Lameness prevalence averaged (mean ± SD, %) 28.3 ± 13.2, pen lying time and pen lying time difference averaged (mean ± SD, h/d) 10.89 ± 1.12 and 8.79 ± 1.92, respectively. Hock and knee injuries averaged (mean ± SD, %) 23.1 ± 21.5, 3.3 ± 2.9, respectively. Bedding type (manure solids, MS; none, NO; other organic, OR; or sand, SA) were associated with lameness ($P = 0.01$). Manure solids (lsmeans ± SE; 28.6 ± 1.5) were associated with lower lameness prevalence than SA (33.0 ± 1.4; $P < 0.01$) and tended to be lower than OR (32.0 ± 1.6; $P = 0.06$), but not different from NO. There we no differences among NO, OR and SA. The number of rows of stalls (2 row, 2R; 3 row, 3R; or 4 or greater rows, 4R) were associated with lameness prevalence ($P = 0.01$). There were no differences between 2R (28.5 ± 1.5) or 3R (30.0 ± 1.6), however 4R (38.6 ± 3.5) were associated with increased lameness than 2R ($P < 0.01$) and 3R ($P < 0.01$), respectively. Additional factors associated with lameness included hock and knee injury prevalence ($P < 0.01$, $P = 0.02$, respectively). Each additional unit increase in hock injury and knee injury prevalence was associated with an increased lameness by 0.2 ± 0.04 and 0.5 ± 0.2, respectively. Each additional hour of pen lying time difference were associated with an increase in lameness prevalence (1.0 ± 0.3; $P < 0.01$). The findings of this study suggest that by selecting fewer rows per pen and providing certain bedding types can reduce the prevalence of lameness.

Key Words: freestall, lameness

T3 The impact of episodic heat stress on lying behavior and lameness of lactating dairy cows on northern New York farms. A. R. Cate*, C. S. Ballard, M. D. Miller, M. G. Green, and R. J. Grant, *William H Miner Agricultural Research Institute, Chazy, NY.*

The aim of this study was to evaluate the impact of heat stress on lying behavior and lameness of lactating dairy cows in northern New York, where episodic heat stress is more typical than prolonged heat stress. Four commercial dairy farms with varying housing conditions and heat abatement systems were selected and monitored for lying behavior and locomotion score from June to October, 2017: Farm A = sand-bedded freestall with natural ventilation; Farm B = sand-bedded freestall with fans over stall beds; Farm C = tiestall with sawdust covered rubber mats enlisting naturally assisted ventilation; Farm D = sawdust covered mattress freestall with fans over stall beds. Environmental conditions of animal housing were monitored in 10-min intervals using a data logger mounted in pen/barn at cow-level. Thirty early to mid-lactation focal animals were selected on each farm (not balanced for DIM) with a locomotion score <3 (not lame, 1 to 5 scale). Lying behavior was monitored continuously using data loggers. Locomotion score was measured at end of study period and classified as Lame or Not Lame. Four days of cool weather (mean THI <65, COOL) and 4 d of hot weather (mean THI >70, HOT) were selected and lying behavior summarized and analyzed by farm using Proc Mixed procedure in SAS to evaluate differences for COOL and HOT days. Lameness status from beginning to end of study period within farms were analyzed by Chi-squared analysis using Proc Freq. Each of the 4 farms, regardless of heat abatement system employed, experienced a decrease in lying time from cool to hot days of 20, 10, 13, and 16% (Farm A, B, C, and D respectively). Lying bouts (n/d) were greater on hot days for cows with only natural ventilation (Farm A; 9.5 vs 8.9 ± 0.53; $P = 0.02$), whereas cows with heat abatement systems showed no differences. While episodic heat events resulted in reduced lying time across all farms, increased incidence of lameness were significant for only Farm A and D (17 and 14% respectively, $P < 0.05$). Results are shown in Table 1.

Table 1 (Abstr. T3).

Item	COOL	HOT	SE	*P*-value
THI (mean ± SD)	60.9 ± 3.7	72.3 ± 1.1	—	—
Minutes THI ≥68 (mean ± SD)	74.0 ± 127	1,279.0 ± 155	—	—
Lying time (h/d)				
Farm A	12.8	10.3	0.3	<0.01
Farm B	11.5	10.3	0.3	<0.01
Farm C	12.8	11.1	0.3	<0.01
Farm D	10.3	8.6	0.3	<0.01

Key Words: heat stress, lameness, lying behavior

T4 Effect of managment system on the lying behavior of organic dairy cows. V. L. Couture*[1], P. D. Krawczel[1], S. R. Smith[2], L. G. Schneider[1], A. G. RÍus[1], and G. M. Pighetti[1], [1]*University of Tennessee, Knoxville, TN,* [2]*University of Kentucky, Lexington, KY.*

Lying behavior is often used to measure dairy cow welfare in confinement systems. However, grazing dairy cows spend more time foraging, which alters the time budget. The objective was to identify variations in the lying behavior of organic, grazing dairy cows. Lactating cows (n = 230) from certified organic dairy farms (n = 5) were enrolled. Farms were categorized by housing and feeding management. Low input (LI) farms (n = 3 farms; 171 cows; 188.6 ± 92.8 DIM) used loose housing and relied on pasture for > 50% DMI. High input (HI) farms (n = 2 farms; 59 cows; 197.9 ± 90.5 DIM) used tie-stalls and relied on pasture for 30–50% of DMI. Accelerometers were affixed to the cows' rear leg for 28-d periods during the spring (P1), summer (P2), and fall (P3) on LI farms and during P1 and P3 on HI farms. Data were analyzed using the MEANS and MIXED procedures in SAS (v9.4). A linear mixed model was developed using backward manual elimination to test the effects of milk yield (MY), parity, and DIM on lying time in LI and HI farms. HI cows laid longer than LI cows (11.16 ± 0.06 vs 8.49 ± 0.03 h/d). Lying time of the LI cows increased from P1 to P3, but HI cows did not differ (P1: LI = 7.41 ± 0.07 h/d; P3: LI = 9.21 ± 0.05 h/d; P1: HI = 11.13 ± 0.08 h/d; P3: HI = 11.20 ± 0.09 h/d). On LI farms, increased MY was associated with decreased lying time and as DIM increased, so did lying time ($P \leq 0.01$). LI primiparous cows laid less (7.62 h/d) than second (8.85 h/d; $P = 0.001$) or third (9.3 h/d; $P = 0.0004$) parity cows, but were did not differ from cows in their fourth or greater parity (8.21 h/d; $P = 0.44$). Lying time was also decreased for cows in the 4 or greater lactation compared with those in their third ($P = 0.02$). On HI farms, as DIM increased, lying time increased ($P < 0.01$), but MY and parity did not have an effect ($P > 0.05$). LI cows were more active overall and lying behavior was more sensitive to MY and parity in comparison to HI cows. Closer examination of the environmental factors effecting lying behavior on LI and HI systems will aid in formulating welfare and production recommendations for organic, grazing dairy cows.

Key Words: dairy, grazing, lying behavior

T5 Effect of outdoor space allowance on the behavior and preference of free-stall housed cows provided access to an outdoor bedded pack. A.-M. Smid*, D. Weary, and M. von Keyserlingk, *University of British Columbia, Vancouver, BC, Canada.*

Providing dairy cows access to pasture is desirable, but farmers can face a range of practical constraints including the lack of available pasture. A possible alternative to pasture is a bedded outdoor area that requires less space than pasture. However, little is known about space requirements for outdoor packs. This study investigated how space allowance on an outdoor open pack affected the number of agonistic interactions on the pack and the time cows spent outdoors. Seventy-two pregnant, lactating, healthy Holstein cows were assigned to 3 groups (24 animals/group). At the start of each group, animals were given 3 d to allow for the social dynamics of the group to stabilize. Thereafter, a habituation phase of 5 d followed in which animals were given access to the outdoor pack with a space allowance of 16 m^2 per cow. After this habituation period, 13 different space allowances were tested differing in 1-m^2 increments, ranging from 4 to 16 m^2 per cow. Each group was tested for 1 d on each treatment, with treatments allocated randomly without replacement. The location of animals (i.e., in the freestall barn or on the outdoor pack) and displacements from a lying position on the outdoor pack were scored using continuous video observations. Data of the effect of space on time spent outside were analyzed using a mixed model that specified cow within group as a random effect; the same model with group as random effect was used to analyze the effect of outdoor space on the amount of agonistic interactions on the pack. Cows spent on average (±S.D.) 4.7 (±0.8) h/d outside, and spent more time outside with increasing outdoor space allowances (3.3 ± 1.2 min/m^2 increase). On the outdoor pack, cows engaged in a total average of 0.11 (±0.05) interactions/h outside, and this was not influenced by space. These results suggest that a bedded pack can be used to provide freestall-housed cows free access to the outdoors, with cows spending an increasing amount of time outside with increasing outdoor space allowance.

Key Words: animal welfare, free range, exercise yard

T6 Impact of freestall neck-rail position on stall and cow hygiene. I. Robles[1], D. F. Kelton[2], H. W. Barkema[3], G. P. Keefe[4], J.-P. Roy[5], M. A. G. von Keyserlingk[6], and T. J. DeVries*[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [3]*Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada,* [4]*Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada,* [5]*Faculté de Médecine Vétérinaire, Université de Montréal, Montréal, QC, Canada,* [6]*Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada.*

The objective of this study was to determine how freestall design affects stall cleanliness and dairy cow hygiene. Four pens, including 30 lactating cows/pen (182.7 ± 92.8 DIM; parity = 2.5 ± 1.2), were exposed in a crossover design, with 2, 28-d treatment periods (February–March; March–April 2017), to each of 2 treatments: 1) neck rail 175 cm from the vertical plane above the rear curb, and 2) neck rail 20 cm closer to the rear of the stall; 155 cm. Stalls were 125cm wide and 259 cm long. Cow hygiene scoring (4-point scale; 1 = clean to 4 = dirty), was done 2x/wk. Cows were categorized as clean (≤2) or dirty (≥3), and proportion of cows with dirty lower legs, udder (DU), upper-legs and flank per pen were calculated. Freestalls (n = 30/pen) were raked and bedding leveled 2×/d. Base of the stalls were mattresses covered with chopped straw (~2cm deep), with additional bedding added 1×/wk. Stall cleanliness was assessed 2×/wk using a 1-m^2 metal grid, containing 88 squares (sq), centered between stall partitions of every fifth stall, and then counting the squares containing visible urine or fecal matter. Data were summarized by pen and analyzed using multivariable mixed-effect linear regression models. Treatment of stall length did not affect cow cleanliness ($P = 0.46$), and no associations were found between stall treatment and stall cleanliness ($P = 0.62$). Mean proportion of soiled sq/stall per pen was 37.8 ± 7.7 (range = 21–56%). Number of days since bedding was added (2 ± 2 d) was associated with proportion of dirty sq/stall (intercept = 33.3; β = 2.4; SE = 0.6; $P < 0.001$); for every 2 d since

bedding was added, there was a 5 percentage point increase in mean proportion of soiled sq/stall. There was a tendency for an interaction between period and treatment ($P = 0.07$) for proportion of cows with DU; in the first period there were a lower proportion ($P < 0.001$) of cows with DU when the neck rail was closer to the rear of the stall (9.8 ± 1.9%) as compared with having the neck rail position further away (13.3 ± 2.1%). A greater proportion ($P < 0.001$) of cows with DU were reported in the second period (20.0 ± 1.1%) as compared with the first period (12.0 ± 1.4%), possibly due to seasonal differences. These results confirm that frequent bedding addition, as well as stall size, is important to maintain clean free-stalls and good cow hygiene.

Key Words: hygiene, freestall

T7 Improving welfare as a strategy to increase productivity and profitability in tiestall farms. M. V. Robichaud*[1,2], J. Rushen[2], A. Marie de Passillé[2], E. Vasseur[3], D. Haley[4], and D. Pellerin[1], [1]*Université Laval, Québec, QC, Canada,* [2]*University of British Columbia, Vancouver, BC, Canada,* [3]*McGill University, Ste-Anne-de-Bellevue, QC, Canada,* [4]*University of Guelph, Guelph, ON, Canada.*

To stimulate dairy producers to invest in their animals' comfort, it is essential to determine any associations between cow welfare and farm productivity and profitability. We evaluated those associations at the farm level. Animal welfare data were collected on 100 Canadian tiestall dairy farms, including animal-, environmental-, and management-based measures. The productivity indicators included average milk production, SCC, culling rate and proportion of cows in third lactation or greater. The profitability indicator "margin over replacement costs" (MORC) per cow was calculated for each farm using milk and culling revenues minus replacement and dead animal disposal costs. Univariable and multivariable linear regression models were used to analyze associations between farm productivity or profitability and welfare indicators. A 1-h increase in average daily lying time was associated with an increase of 273 kg in average yearly corrected milk production per cow ($P = 0.003$). The percentage of obviously lame cows was modified by the milk genetic index in its association with average yearly milk production, leading to greater improvement in milk production with increasing genetic index in farms with lower lameness prevalence. Farms with a greater proportion of cows with low body condition score and stalls mostly soiled with manure had higher average somatic cell count per year (5,000 and 3,680 per %; $P \leq 0.001$). Farms with greater variability in their average herd lying time had lower proportion of cows in their third lactation or greater (6% per h; $P = 0.007$). Farms with higher average MORC per cow (higher benefits) had significantly lower prevalence of stalls soiled with manure ($7 per %; $P = 0.001$) and with very wet bedding ($16 per %; $P = 0.018$), and longer average daily lying time ($147 per h; $P = 0.017$). The farm's MORC per cow was also associated with the interaction between lameness prevalence and milk genetic index. These results show a complex relationship between farm profitability and animal welfare and that improving some aspects of animal welfare on tie-stall farms is beneficial for farm's productivity and profitability.

Key Words: cow welfare, productivity, profitability

T8 Lameness, injuries, and lying behavior on New York tiestall dairies. L. K. M. Ferlito* and B. J. Hicks, *Cornell University, Ithaca, NY.*

The objectives of this study were to measure lameness, injuries, and lying behavior of lactating cows on tiestall dairies, and to measure cow size and stall dimensions to determine if facilities are adequately sized. This was an observational study in which New York State dairies were evaluated during 2 farm visits in the spring and summer of 2017. A total of 10 facilities, 400 lactating cows (40 per herd), and 85 stalls were evaluated (8 to 9 stalls per herd). Cows were evaluated for lameness while standing in the stall and while moving, hock, knee, and neck injuries, and lying behavior. Facility evaluations included stall dimensions, bedding amount, and bedding cleanliness. On average, 20% of the stalls had no bedding, 39% had a little, 24% had 1–2 inches, and 17% had >2 inches of bedding covering the stall. The majority (62%) of stalls were clean, 21% slightly soiled, and 17% very soiled. Cows had an average rump height of 58.8 inches (range: 55.7 to 62.4), and an average hook width of 22.9 inches (range: 19.2 to 25.5). Stall length, width, and tie rail height averaged 67.9, 52.4, and 39.1 inches respectively. Based on cow size, only 4 farms met the size recommendations for stall length, 9 farms for stall width, and only 2 had adequate tie rail height. Locomotion score lameness prevalence averaged 24% (20% mild and 4% severe), with a range of 5% to 45%, and the in-stall lameness prevalence average was 14%, with a range of 3% to 25%, indicating the in-stall system underestimates lameness. Farms averaged 58% for hock injuries (49% mild and 9% severe), ranging from 20 to 88%; 4% for knee injuries, ranging from 0 to 10%; and 18% for neck injuries (11% mild and 7% severe), ranging from 3 to 50%. Lying time averaged 10.7 h/d (range: 7.7 to 12.7), with 10.3 bouts/day (range: 6.6 to 13.0), and a bout length of 68.1 min/bout (range: 54.4 to 81.4). While lameness and injuries are a challenge on individual tiestall dairies, some dairies were performing very well compared with industry benchmarks and guidelines. Additional research on more herds is needed to understand the relationship between housing and management factors with lameness and lying time to improve cow comfort on tiestall dairies.

Key Words: dairy, tiestall, lameness

T9 Assessing comfort of lactating dairy cows housed in tiestalls with recycled manure solids bedding. S. Oueslati*[1], M. Villettaz Robichaud[1], S. Godbout[2], S. Fournel[1], P. Ruel[3], E. Vasseur[4], and D. Pellerin[1], [1]*Université Laval, Québec, QC, Canada,* [2]*Institut de Recherche et de Développement en Agroenvironnement, Québec, QC, Canada,* [3]*Centre de Recherche en Sciences Animales de Deschambault, Deschambault, QC, Canada,* [4]*McGill University, Ste-Anne-de-Bellevue, QC, Canada.*

The use of recycled manure solids (RMS) as bedding in tie-stall housing has gained interest but its effect on the comfort of dairy cows has not yet been documented. The objective of this research was to assess the comfort of lactating cows housed in tie-stall with different RMS bedding depth. An experiment was conducted to compare cow cleanliness, lying time, and stall surface moisture when the bedding used was either straw (6 cm deep), RMS (6 cm deep) or RMS (15 cm deep). The 18 lactating Holstein cows enrolled in this experiment were blocked in groups of 3, according to parity and days in milk and housed for 3 weeks period on each treatment, using a crossover design. Flank, leg, udder, and teat cleanliness were assessed twice a week using a scoring chart based on a 4-point scale (1 = clean to 4 = very dirty). Stall surface moisture was also evaluated twice per week using the knee test and a 6-point scale (0 = dry to 6 = very wet). Finally, lying time was recorded during the last 14 d of each 3-week period using accelerometers. Mixed effects logistic regression models that included a random intercept for animal were used to analyze the effects of bedding treatment on the parameters monitored. Results showed that the use of RMS bedding led to increased odds of wet lying surface (knee test score ≥3) when compared with straw, for both the 6 cm depth (Odds ratio (OR) = 14; 95% Confidence interval (CI) = 1.8 – 115; $P = 0.012$) and 15 cm depth (OR = 10; 95%

CI = 1.3 – 84; $P = 0.029$). Cows' flank, leg, udder cleanliness and daily lying time were not influenced by the bedding treatments. However, the use of RMS lead to lower teat cleanliness (score ≥3) for both the 6 cm depth (OR = 5; 95% CI = 2 – 15; $P = 0.003$) and 15 cm depth (OR = 9; 95% CI = 3 – 26; $P \leq 0.001$). Our results show that RMS could be a good bedding alternative for dairy cows kept in tie-stall, based on the comfort indicators measured, but its use may require greater attention to teat cleaning for milking.

Key Words: recycled manure solids, tiestall housing, cow comfort

T10 Would cows benefit from "king-size" beds? V. Boyer*[1], E. Edwards[2,1], M. F. Guiso[1,3], S. Adam[4], P. Krawczel[2], A.-M. de Passillé[5], and E. Vasseur[1], [1]*McGill University, Animal Science, Montréal, QC, Canada,* [2]*University of Tennessee, Animal Science, Knoxville, TN,* [3]*Università degli Studi di Sassari, Sassari, Sardinia, Italy,* [4]*Valacta, Ste-Anne-de-Bellevue, QC, Canada,* [5]*University of British Columbia, Dairy Research and Education Centre, Agassiz, BC, Canada.*

Tiestall dairy cows spend their whole days in the same space, which, therefore, must be designed to accommodate all the activities they conduct. Lying is a very important behavior for dairy cows and a critical response variable for assessing stall designs, to ensure that their needs for resting space are met. The objective of this study was to determine if increasing tie-stall width alters the lying behavior of lactating dairy cows. Two treatments were compared: the current recommendation (139 cm) and a double stall (284 cm). Stall length was of 188 cm. Sixteen cows were blocked by parity and lactation stage, then randomly allocated to a treatment and a stall within 1 of 2 rows in the barn, for a period of 6 wk. Leg-mounted accelerometers were used to record lying behaviors. Cows were recorded on video one full 24h/wk, using surveillance cameras positioned above the stalls. Video data from wk 1, 3 and 6 were recorded at a rate of 1 frame per minute, and analyzed by a trained observer to assess the position and the location of the cows' body, head, and limbs during the lying hours. Lying behaviors, and frequency of each position and location were analyzed in SAS using a mixed model in which treatment, block and week were included as fixed factors, and cow and row, as random factors. Multiple comparisons were adjusted for using the Scheffé method. Results indicate that cows in the double stalls fully extended their hindlimbs more often than the control cows (21.7% vs 7.64%, $P = 0.015$). They also intruded in the neighboring stalls with their hindlimbs less often than the control cows [2.39 vs 16.3% for the left side ($P = 0.0004$), 0.14 vs 13.1% for the right side ($P = 0.0045$)], instead favoring the second half of the double stall, in 38.3 ± 6.37% ($P = 0.0039$) and 29.2 ± 6.57% ($P = 0.012$) for the left and right hind legs, respectively. Total lying time did not differ ($P = 0.24$) between the double stall group (716.1 ± 25.04 min/d) and the control group (670.8 ± 24.98 min/d). These results suggest that dairy cows utilize the additional space they are granted with, and that they would likely benefit from stall widths larger than what is currently recommended for tiestall and freestall systems.

Key Words: dairy cow, stall width, lying behaviour

T11 Effect of prepartum lying time on stillbirth in transition dairy heifers and cows. B. T. Menichetti*[1], J. M. Piñeiro[1], A. A. Barragan[1], A. Relling[2], A. Garcia-Guerra[2], and G. M. Schuenemann[1], [1]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [2]*Department of Animal Sciences, The Ohio State University, Wooster, OH.*

The objective was to assess the effect of prepartum lying time (LT) on stillbirth in transition dairy heifers and cows. A total of 1,051 Holstein dairy cows (401 primiparous and 650 multiparous) from 3 commercial dairy herds were enrolled at 14 d before calving until 14 d post-calving. Weekly, a cohort of 10 to 15 cows was enrolled at each farm and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual cows to assess their lying time. All heifers and cows were housed in similar prepartum freestall barns and moved into a contiguous individual maternity pen for parturition. Stillbirth was defined as a calf born dead or died within 24 h after birth, and with normal gestation length. Blood samples were collected at 7 d before (dpp) and at calving to assess NEFA and calcium (Hypocalcemia ≤ 0.8 mg/dL), respectively. Data were analyzed using MIXED procedure of SAS. Multiparous cows had greater (736 ± 20 min; $P < 0.05$) LT before parturition compared with primiparous cows (597 ± 30 min). Multiparous cows with a stillborn calf had reduced pre-partum LT (716 ± 28 min; $P < 0.05$), increased NEFA 7 dpp (416 ± 44 µEq/L) and increased hypocalcemia (56.7 ± 11%) at calving compared with cows with a calf born alive (762 ± 12 min, 313 ± 8 µEq/L, and 33.6 ± 6%, respectively). Primiparous cows with stillborn calf had reduced LT (570 ± 35 min) and increased hypocalcemia (45.4 ± 15%) at calving ($P < 0.05$) but NEFA did not differ compared with primiparous cows with a calf born alive (625 ± 25 min and 18.7 ± 9%, respectively). These results suggest that LT, prepartum energy status, and calcium at calving of dams are critical for calf survival.

Key Words: stillbirth, lying time, dairy cattle

T12 Effect of parity, season, and group size on dairy cows and heifers' preferences for calving location. E. M. Edwards*[1], K. L. Proudfoot[2], H. M. Dann[3], L. G. Schneider[1], and P. D. Krawczel[1], [1]*The University of Tennessee, Knoxville, TN,* [2]*The Ohio State University, Columbus, OH,* [3]*The William H. Miner Agricultural Research Institute, Chazy, NY.*

Cows unable to cope with the challenges of calving often succumb to disease, negatively affecting their welfare. This may be improved by accommodating the cows' preferences at calving. The objective was to determine the effects of parity, season, and group size on dairy cows and heifers' preference for calving location when group housed and provided free access to pasture. This study was implemented using an observational study design. Multiparous Holstein cows (n = 33) and nulliparous Holstein heifers (n = 32) were dynamically enrolled −21 d before their expected calving date and removed on the day of calving. Cows had continuous access to a bedded-pack barn (area 1; 167.4 m^2), open pasture (area 2; 1.82 ha), and an area of natural forage cover (i.e., trees and tall grasses; area 3; 0.24 ha). Video data were used to determine calving location. Cows calved from August to December 2016. Season was categorized as summer (August 6 to September 21), fall (September 22 to December 20) and winter (December 21 to December 28). For each cow, mean daily group size was calculated over the days she was enrolled, including at calving. Multinomial logistic regression (SAS 9.4) was used to test if factors were associated with the probability of calving in the 3 designated areas of the environment, and model selection was performed by backward manual elimination. Twenty-five calvings (38%) occurred in the barn, 17 calvings (26%) occurred on open pasture, and 23 calvings (35%) occurred in the area of natural forage cover. Parity was associated with the location of calving ($P = 0.02$); heifers were more likely to calve in the natural forage area compared with the barn (OR = 5.88; 95% CI = 1.69, 20.42). However, heifers and cows were equally as likely to calve in open pasture or the barn (OR = 2.89; 95% CI = 0.8, 10.53). Season, group size at calving, and mean daily group

size were not associated with calving location preference ($P > 0.25$). These results suggest parity plays a role in preference for calving location when group housed. Calving areas that accommodate both heifers and cows' environmental preferences for calving when grouped may improve calving outcomes.

Key Words: dairy cow, calving location, parity

T13 Lying behavior as an indicator of diarrhea and navel inflammation in veal calves. M. J. Studds[1], L. L. Deikun*[1,2], D. E. Sorter[1], and K. L. Proudfoot[1], [1]*The Ohio State University, Columbus, OH,* [2]*Provimi, Brookville, OH.*

Many veal calves arrive at growing facilities with inadequate immunity, leaving them susceptible to diseases including diarrhea and navel inflammation. Observing calf behavior, such as lying behavior, can be used as a tool to understand calf health. The aim was to investigate the effect of navel inflammation and diarrhea on lying behavior in veal calves. A total of 125 calves from 3 cohorts were included in the study. Calves were housed individually on slatted flooring (Tenderfoot) in 3 rooms of a mechanically ventilated barn. On d 4 after arrival, calves were fitted with 3D accelerometers (HOBO Pendant Data Loggers) on their hind legs to continuously measure lying time, number of lying bouts, and lying bout duration. Health exams were conducted twice weekly for 2 wk starting on the day after arrival. Exams included a rectal temperature, a navel score (0 = normal, 1 = mildly inflamed, 2 = moderately inflamed, 3 = severely inflamed), and a fecal score (0 = normal, 1 = semi-formed, 2 = loose, 3 = watery). Calves were considered to have "navel inflammation" (n = 22) or "diarrhea" (n = 15) if they scored ≥2 during at least 3 of the 4 health exams. "Normal" calves (n = 18) had no signs of illness and rectal temperatures <39.4°C during 3 of the 4 health exams. Week 1 included d 4 to 9, and wk 2 included d 9 to 14. Data were analyzed using a repeated measures ANOVA; the model included cohort, health status (normal, navel inflammation or diarrhea), week (1 or 2) and a health × week interaction. There was an effect of health status on lying time ($P = 0.03$), whereby normal calves spent more time lying (18.9 ± 0.2 h/d) compared with calves with inflamed navels (18.0 ± 0.2; $P = 0.01$) and diarrhea (18.1 ± 0.2; $P = 0.05$). There was no effect of health status on the number of lying bouts per day ($P = 0.62$), nor on the duration of lying bouts ($P = 0.36$). There were no health × week interactions. Results indicate that veal calves with navel inflammation and diarrhea may be less comfortable than calves without these conditions. Veal producers should consider changing their facilities to create more comfortable lying environments to help calves better cope with illness.

Key Words: calf comfort, sickness behavior, housing

T14 Impacts of wild birds on pathogen dissemination and behavioral interactions in dairy cattle. T. P. Caskin*[1], J. M. Gay[2], K. M. M. Steensma[3], J. H. Harrison[4], B. Garries[3], A. Sarchet[3], and A. L. Adams Progar[1], [1]*Department of Animal Sciences, Washington State University, Pullman, WA,* [2]*Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA,* [3]*Departments of Biology and Environmental Studies, Trinity Western University, Langely, BC, Canada,* [4]*Departments of Animal Science and Extension, Washington State University, Puyallup, WA.*

Feed loss and pathogen dissemination from wild birds may cost Washington dairy farmers $200,000 per year per farm. Wild birds may also alter cattle feeding, aggression, and aversion behaviors. The objectives of this study were to (1) record the number of birds present on Washington dairies; (2) observe behavioral changes in cattle feeding; and (3) determine the pathogens present in bird fecal matter on dairies. Based on bird migration patterns, fall and winter seasons were selected for data collection. Bird counts were recorded every 7 d over the course of 4 wk using direct observations on 11 dairies. Pathogen and behavior studies were conducted in 15 lactating pens on 5 farms, averaging 4 pens per farm. Bird fecal samples were collected for pathogen analysis. On-farm cameras recorded cattle and wild bird behaviors that included the number of cows eating, occurrences of cow intra-species aggressive and aversive behaviors, and incidences of aggressive and aversive behaviors between cows and birds at the feed bunk. Pathogen data were analyzed using PROC LOGISTIC and behavioral data were analyzed using PROC GENMOD in SAS. The number of birds observed at any specific time was 0–1,000 birds per farm. *Escherichia coli* was isolated from 34/88 samples, and *Campylobacter jejuni* was isolated from only one sample. No significant differences in the number of positive *E. coli* samples were found between locations (2.3 ± 0.4 samples; $P = 0.17$). Preliminary results suggest that the percentage of head gates used by cows (0.03 ± 0.005% vs. 0.8 ± 0.01%; $P < 0.001$), and the number of cows eating (1 ± 0.09 vs. 19 ± 0.3 cows; $P < 0.001$) differed among locations. Additionally, the number of birds present (1 ± 0.07 vs 41 ± 6.7 birds; $P < 0.001$) and the number of birds eating at the feed bunk (1 ± 0.03 vs. 28 ± 5.01 birds; $P < 0.001$) differed among locations. However, no significant relationship was detected between the number of birds and the number of cows eating at any given time (13 ± 0.4 cows; $P = 0.91$). Determining bird preferences for feeding location will aid in developing effective deterrence methods and minimize economic losses on farms.

Key Words: fecal pathogen, feeding behavior, wild birds

T15 Assessing human-conditioned sorting behavior in dairy cows in farm research trials. D. Manriquez*[1], L. Chen[2], G. Albornoz[2], J. Velez[2], and P. J. Pinedo[1], [1]*Department of Animal Science, Colorado State University, Fort Collins, CO,* [2]*Aurora Organic Dairy, Platteville, CO.*

Cow-human interactions can influence and modulate group and individual behaviors of dairy cows. Our objective was to test the effectiveness of human sorting to separate subgroups of lactating dairy cows in on-farm studies and to assess the level of conditioning to this activity. As part of a previous nutritional trial cows were sorted daily for 150 d into 2 contiguous sub-pens. Subsequently, we compared 3 sorting methods applied to the same cows: (1) human active sorting (AS) at the pen gate; (2) human presence as passive sorting (PS); and (3) non-human gate sorting (GS). We hypothesized that after a training period cows become conditioned to sorting. Holstein cows (n = 176; parity = 2.5 ± 1.3), were randomly assigned into 2 subgroups (A = 91 cows; B = 85 cows) to be sequentially separated by 3 sequentially applied sorting methods (AS; PS; and GS). Each sorting method was evaluated once per day after the morning milking during 5 d. The counts of correctly allocated cows and the error rate (misplaced-cows/d) of each subgroup were compared by sorting method. Additionally, an individual error index rate was calculated and analyzed considering treatment group, parity, and general activity ratio (daily rumination / daily activity). When AS was applied, the total proportion of animals correctly sorted was of 99.8%, whereas PS had 94.8% of sorting accuracy ($P < 0.001$). Non-human GS could not be accurately assessed because the cows lost the self-sorting behavior overcrowding one side of the pen making impossible the data collection. The average of animals correctly placed was greater in AS when compared with PS (175 ± 1.7 cows vs. 166.6 ± 3.5 cows; $P = 0.005$). Cows that had longer walking distance to their research sub pen had greater individual error rate, especially when PS was applied. No association was found between parity and general activ-

ity ratio with the individual error index. Researchers observed a clear self-sorting behavior in response to human sorting, for both AS and PS, with average values for correct placement above 90%. Therefore, after a period of training, lactating dairy cows became operant conditioned to human sorting, which represent an opportunity for animal separation without intense human labor or stressing practices for the animals.

Key Words: sorting, operant conditioning

T17 Effects of 2,4-thiazolidinedione on milk fatty acid profile and vitamins in dairy goats with subclinical mastitis. C.-Y. Tsai*[1], F. Rosa[2], M. Bionaz[2], and P. Rezamand[1], [1]*University of Idaho, Moscow, ID,* [2]*Oregon State University, Corvallis, OR.*

Mastitis is a major disease that affects the profitability of dairy farms. Peroxisome proliferator-activated receptor gamma (PPARγ) may improve the response to mastitis and regulate differentiation of adipocyte. A putative PPAR agonist, 2,4-thiazolidinedione (TZD), modulates metabolism and inflammation. The objective of this study was to determine the effects of TZD during induced subclinical mastitis on the milk fatty acid profile and lipid-soluble vitamins in dairy goats. The experiment included 24 Saanen lactating dairy goats receiving low-energy diet without vitamin supplementation. Six goats in each group received a daily I.V. injection of either 8 mg/kg BW of TZD or saline. A week later, goats were challenged with intramammary infusion (IMI) of *Streptococcus uberis* (MTZD or MCTR) or saline (CTZD or CTRL). Milk samples were obtained on d −8, 1, 4, 7, and 12 relative to IMI and were analyzed for retinol, α-tocopherol and fatty acid profile. Data were analyzed using the Proc Mixed of SAS with significance declared at $P \leq 0.05$. CTZD had greater retinol concentration compared with CTRL on d 1 (658.7 vs 376.0 ± 147.5 μg/mL), and MTZD had lower retinol concentration over time. On d 4 after IMI, MCTR had greater α-tocopherol concentration than CTRL (1.44 vs 0.69 ± 0.39 μg/mL) and tended to be greater than MTZD. The MTZD had lower α-tocopherol over time. The TZD effect on fatty acid profile was detected on d 12; CTZD group was greater than CTRL in C18:0 (7.63 vs 5.68 ± 0.79%), C18:1 *cis* (25.9 vs 21.4 ± 1.8%), C18:3n-3 (1.5 vs 1.2 ± 0.1%), and C20:5 (0.07 vs 0.04 ± 0.01%). In addition, during subclinical mastitis, MTZD had greater α-linolenic acid on d 12 (1.4 vs 1.2 ± 0.1%) and C22:6 on d 4 (0.05 vs 0.01 ± 0.01%) compared with that for CTRL group. Overall, during sub-clinical mastitis with low energy diet, and no vitamin supplementation, TZD increased the presence of several dietary long-chain fatty acids with anti-inflammatory features and retinol in milk. This suggests that TZD affects milk fat and may possibly improve anti-inflammatory responses of the mammary gland.

Key Words: thiazolidinedione, mastitis, dairy goat

T18 Precalving body condition score affects leukocytes count following pegbovigrastim treatment in Simmental cows around calving. V. Lopreiato*, D. Britti, and V. M. Morittu, *Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro, Italy.*

Treatment with granulocyte colony stimulating factor (G-CSF) has been shown to increase neutrophil count and function in transition cows. In this study we tested the hypothesis that cows with high or low body condition score (BCS) may have different response in peripheral blood leukocyte populations count after the treatment with pegbovigrastim (recombinant bovine G-CSF). Twenty-six Simmental cows were randomly allocated to one of 2 treatment groups, homogeneous for parity: pegbovigrastim (PEG; 14 cows) or saline control (CTR; 12 cows) injections. Within each treatment group, cows were divided in 2 subgroups according to a 5-point scale precalving BCS: BCS ≤3.5 (LowBCS; 7 PEG and 6 CTR cows) and BCS ≥3.75 (HighBCS; 7 PEG and 6 CTR cows). Cows received s.c. injections approximately 7 d before (1st) and within 24 h from calving (2nd). Blood samples collected at −7, 0, 1, and 3 d relative to calving (time) were used to determine the complete blood count using an Advia 2120 Hematology Analyzer (Siemens Healthcare Diagnostics inc., Deerfield, IL). The data were analyzed using a MIXED model procedure of SAS (Ver. 9.3) with fixed effects of treatment, BCS, time, and their interactions, and cow was the random effect. Overall, PEG-treated cows resulted in significantly higher neutrophil, basophil, and monocyte counts. These differences were also observed during time from calving onwards. LowBCS cows resulted in a tendency for higher neutrophil, basophil, and lymphocytes count compared with HighBCS cows. Furthermore, a BCS x treatment interaction was observed for neutrophil due to significantly higher count in PEG-LowBCS compared with PEG-HighBCS, while no differences were detected in CTR cows. These results shed light on the pegbovigrastim response in cows with different BCS. Simmental cows with BCS 3.5 or lower before calving experienced a greater responsiveness to pegbovigrastim treatment. However, further studies are required for the understanding mechanism of G-CSF response associated with the degree of adiposity around parturition.

Key Words: BCS, pegbovigrastim, leukocytes

T20 Effect of central administration of an acute phase protein, α-1-acid-glycoprotein, on feed intake in sheep. M. K. Waller*[1], B. A. Gregg[1], P. A. Parker[1], M. Garcia[2], B. J. Bradford[2], J. A. Daniel[3], and B. K. Whitlock[1], [1]*College of Veterinary Medicine, University of Tennessee, Knoxville, TN,* [2]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS,* [3]*Department of Animal Science, Berry College, Mount Berry, GA.*

Systemic inflammation and sub-optimal feed intake are common in dairy cows in the transition to lactation, and both conditions are associated with greater risk for removal from the herd and less productivity. No compelling mechanistic link has tied postpartum inflammation to suppression of feed intake. Recent findings in rodents demonstrated that an acute phase protein, α-1-acid glycoprotein (AGP), could provide this link by acting as a leptin receptor agonist. To determine the feed intake dose response of sheep to central AGP administration, nonlactating, non-pregnant, adult (≥1 yr of age) mixed breed black face ewes (n = 4) weighing 79.0 ± 5.0 (SD) kg were ovariectomized and surgically implanted with a cannula into a lateral ventricle of the brain. Ewes were kept indoors in individual pens with an environment consisting of a 12-h light/dark photoperiod and approximately 22–24°C. Ewes were fed a diet calculated to meet 100% of daily maintenance requirements and had ad libitum water. Ewes received 1 of 4 treatments [0 (control), 12 (low), 60 (medium), or 300 (high) μg/kg BW AGP (AGP from bovine plasma; Sigma Aldrich Co. Saint Louis, MO)] administered in 500 μL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride into the lateral ventricle. Sheep were allowed ad libitum access to feed and intake was determined at −72, −48, −24, 0, 2, 4, 6, 8, 12, 24, 36, and 48 h relative to treatment. The study was repeated until all sheep received all treatments with a 10-d washout period between treatments. Data were analyzed using procedures for repeated measures with JMP software (version 10.0.0; SAS Inst. Inc., Cary, NC) and tested for effects of replication, treatment, time, and treatment × time interaction. There was an effect of time ($P < 0.0001$) and no effect of treatment ($P = 0.31$), replication ($P = 0.45$) or treatment × time interaction ($P = 0.84$) on feed intake. These results indicate central AGP administration may not affect feed intake in

sheep or concentrations of AGP reached during systemic inflammation, that may affect feed intake, were not achieved in our model.

Key Words: appetite, inflammation, sheep

T21 The effects of central administration of a leptin receptor antagonist on endotoxin-induced hypophagia and fever in sheep. B. K. Whitlock*[1], B. A. Gregg[1], P. A. Parker[1], M. K. Waller[1], M. Garcia[2], B. J. Bradford[2], and J. A. Daniel[3], [1]*College of Veterinary Medicine, University of Tennessee, Knoxville, TN,* [2]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS,* [3]*Department of Animal Science, Berry College, Mount Berry, GA.*

Inflammation is often modeled with endotoxin (LPS from *E. coli*). Intracerebroventricular (ICV) infusion of appetite-stimulatory neuropeptides, many of which act "downstream" of leptin, and fasting inhibit LPS-induced reduction in feed intake and changes in body temperature. The objective of this study was to test the effects of a leptin receptor antagonist on LPS-induced hypophagia and fever in sheep. Adult black face ewes [n = 16; 81.2 ± 23.1 kg] were ovariectomized and fitted with a cannula for ICV infusion. Ewes were kept in individual pens with a 12-h light/dark photoperiod and ~22°C. Ewes had ad libitum access to a diet calculated to meet their requirements. Ewes received 1 of 2 treatments [0 (saline) or 500 (OLA; ovine super-active leptin antagonist) µg of OLA] administered via ICV infusion 30 min before receiving 1 of 2 peripheral treatments [0 (saline) or 0.4 (LPS; from *E. coli* O55:B5) µg/kg BW LPS] administered via the jugular vein. Feed intake and rectal temperature was determined at −72, −48, −24, 0, 2, 4, 6, 8, 12, 24, 36, and 48 h relative to peripheral treatments. Data were analyzed using repeated measures procedures with JMP software and tested for effects of LPS, OLA, and time, and all their interactions. As expected, LPS decreased feed intake at 2 and 4 h, but increased at 8 h compared with saline (LPS × time, $P < 0.05$). For ewes receiving LPS, OLA decreased feed intake at 2 h but increased at 12 h compared with saline (LPS × OLA × time, $P = 0.07$). Rectal temperatures were affected by LPS and OLA in a time-dependent fashion. LPS increased rectal temperatures at 2, 4, and 6 h (LPS × time, $P < 0.0001$) and OLA increased it at 6, 8, and 12 h (OLA × time, $P < 0.0001$), both compared with saline. We found no evidence that OLA moderated the hypophagic and febrile effects of LPS (OLA × LPS, $P > 0.10$). In contrast, OLA administration can increase rectal temperature in sheep.

Key Words: inflammation, appetite, fever

T22 Impact of fumonisin B_1 on rumen environment: An in vitro study. C. Stoiber[2], U. Hofstetter*[1], J. Faas[2], B. Doupovec[2], and D. Schatzmayr[2], [1]*Biomin Holding, Getzersdorf, Austria,* [2]*Biomin Research Center, Tulln, Austria.*

Ruminants are less susceptible to mycotoxins than monogastrics. However, a long-term intake of a diet contaminated with fumonisins (FUM) can lead to reduced feed intake and a loss in milk production. In this study we investigated the effect of fumonisin B_1 and B_2 (FB_1, FB_2) on rumen fermentation parameters using batch fermentations. Pyrex bottles (n = 15) were inoculated with 100 mL of a mixture containing 50% rumen fluid from bulls, 30% water and 20% synthetic saliva. Culture material of *Fusarium verticilloides* was added to 7 reactor bottles to achieve an end concentration of 7.2 mg/L FB_1 and 2.8 mg/L FB_2 (FUM treatment). Eight reactor bottles remained untreated (negative control). All reactors were incubated for 24 h at 39°C. Samples of the fermentation broth were taken at 0 h, after 1 h and 24 h of fermentation to determine the concentrations of volatile fatty acids (VFA) and the total bacterial counts by flow cytometry. The pH value was measured directly in the reactors. Statistical analysis was performed using SPSS 19.0. Lactate concentrations were significantly higher in reactors with FUM than in reactors without FUM treatment after 24 h of incubation. In the FUM treated reactors, propionate concentrations were significantly lower after 1 h of incubation and concomitantly, the acetate/propionate ratio was significantly higher. Total bacterial counts were significantly lower in the FUM treated reactors compared with the negative control after 1 h of incubation. The pH value was not significantly different between the treatments. While FB_1 concentrations did not change during the incubation (average concentration = 5.3 mg/L), FB_2 was significantly decreased after 24 h of fermentation (0 h: 1.7 mg/L; 24 h: 1.1 mg/L). In conclusion, the addition of FUM-containing culture material partially affected parameters of rumen fermentation in vitro. The toxicologically most relevant fumonisin FB_1 was not degraded by the rumen microbiota.

Key Words: fumonisin, rumen fluid, in vitro

T23 In vitro effect of cowpea polyphenols on bovine rumen microbiome. S. Adjei-Fremah*, K. Ekwemalor, E. Asiamah, B. Osei, E. Eluka-Okoludoh, K. Schimmel, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Bovine rumen is inhabited by diverse microbiota that plays important roles in feed digestion, nutrient uptake and energy metabolism. Rumen microbiota is essential to animal production and is affected by animal feeds and dietary additives. Dietary polyphenols can modulate the diversity and function of rumen microbes and improve animal health and productivity. Cowpea based feeds have health and production benefits for ruminants but the effect of cowpea polyphenols on bovine rumen microbiome is yet to be elucidated. The objective of this study was to evaluate the effect of cowpea polyphenols on bovine rumen microbial composition and population. Rumen fluid collected from Holstein-Friesian heifers (n = 3) was treated with cowpea phenolic extract (CPE) for 48 h. Genomic DNA was isolated from the rumen of CPE-treated and control groups and high-throughput sequencing of 16s rRNA gene on an Illumina HiSeq platform was performed. Sequence data were aligned, assembled, and analyzed using MSR: Metagenomics Version: 1.0.0, MSR Version: 2.4.60.8. CPE treatment resulted in variation in rumen microbial communities and composition ($P < 0.05$) at all taxonomic levels.The phyla *Firmicutes* (40.9%), *Proteobacteria* (30.5%) and *Bacteroidetes* (15.7%) were the dominant bacterial communities in the rumen. CPE treatment enhanced the abundance of many taxa belonging to *Bacteroidetes*, *Firmicutes* and *Tenericutes* phyla, while methanogenic archaea were reduced. Cellulolytic and fibrolytic bacterial communities were altered by CPE. Overall, treatment with CPE affected the rumen microbial population and diversity. In addition to the impact of cowpea on animal health and production, these results show that cowpea polyphenols modulate the rumen microbiome in cow.

Key Words: bovine rumen, cowpea polyphenols, microbiome

T24 Relationship between in vitro ceftiofur minimum inhibitory concentration and quarter somatic cell count response after the occurrence of clinical mastitis caused by *Klebsiella spp*. M. J. Fuenzalida*[1] and P. L. Ruegg[2], [1]*University of Wisconsin, Madison, Wisconsin,* [2]*Michigan State University, East Lansing, MI.*

The objective was to describe in vitro ceftiofur minimum inhibitory concentration (MIC) to inhibit growth (INH) of *Klebsiella* spp. (KLE) and its association with quarter somatic cell count (QSCC) during a

28-d follow-up period (FUP). Cases of clinical mastitis (CM) confirmed as gram-negative were randomly assigned to receive 2-d (short), 8-d (extended) intramammary ceftiofur or to a non-treated (control) group. Only cases confirmed as KLE were included in this analysis (n = 38). The primary outcome was QSCC collected 7, 14, 21, and 28 d after enrollment. The Wisconsin Veterinary Diagnostic Laboratory (WVDL) performed in vitro antimicrobial susceptibility tests according to the Clinical and Laboratory Standards Institute (CLSI). The WVDL categorized isolates based on the MIC at which ceftiofur (test ranged from 0.5 to 4.0 μg/mL) completely inhibited KLE (≤0.5, 1.0 and > 4.0 μg/mL). Survival analysis was performed using PROC LIFETEST to measure the probability of QSCC reduction (<200,000 cells/mL) during the FUP by MIC categories of INH. Repeated measures analysis was performed to analyze QSCC changes using PROC MIXED. Proportion of MIC categories of INH were not different among treatment groups ($P = 0.78$). The probability of QSCC reduction over the FUP was not different among MIC categories of INH of KLE ($P = 0.49$). The $\log_{10}$SCC for quarters that received 8-d (5.8, $P < 0.01$) therapy was less than QSCC of quarters that received 2-d (6.4) or no therapy (6.6). Time ($P = 0.24$) and ceftiofur MIC ($P = 0.48$) were not associated with QSCC. However, an interaction between MIC categories, and treatment group with QSCC was observed ($P = 0.04$). Quarter SCC did not change with time and was associated with ceftiofur therapy. The exposure to ceftiofur intramammary therapy seemed to modify the QSCC response when compared with the control group depending on the MIC category of INH.

Table 1 (Abstr. T24).

	LSM of $\log_{10}$ SCC at MIC (μg/mL)		
Treatment	≤0.5 (n = 17)	1.0 (n = 11)	>4.0 (n = 10)
Control	6.3	6.7	6.8
Short	6.2[a]	6.2[a]	6.7[b]
Extended	6.2[a]	5.6[b]	5.6[b]

[a,b]Within rows, means with different superscripts differ ($P < 0.05$).

Key Words: *Klebsiella*, ceftiofur, MIC

T25 Effects of postpartum oral calcium supplementation on productive and reproductive outcomes in Jersey cows. A. Valldecabres* and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.*

The effects of postpartum prophylactic oral Ca supplementation on the first Dairy Herd Improvement Association (DHIA) test milk yield, first-service conception and days open within 150 d in milk (DIM) were evaluated on 1,095 multiparous Jersey and Jersey × Holstein cows from 2 commercial herds. After calving, cows were systematically assigned to control (no oral Ca supplementation; n = 553) or oral Ca supplementation (CaOS; 50 to 60 g of Ca as boluses; QuadricalMINI, Bio-Vet Inc., Barneveld, WI; n = 542) at 0 and 1 DIM. Blood samples for serum Ca determination were collected before 1st treatment administration for a subset of cows (n = 768). Productive outcomes and days open within 150 DIM were analyzed by ANOVA with the MIXED procedure, with herd as a random effect and first service conception was analyzed by logistic regression with the GLIMMIX procedure of SAS. Additional variables considered for inclusion in the final model were previous lactation milk yield and DIM, gestation length, dry and close-up period lengths, body condition and locomotion scores at calving and calving easiness. There was a dry period length × treatment ($P = 0.006$) and a tendency for a DIM at 1st test × treatment ($P = 0.08$) interaction for 1st test milk yield. For cows with a dry period length larger than the herd mean (60 and 73 d for Herd 1 and 2, respectively), CaOS produced 1.6 kg of milk more than control cows ($P < 0.05$) whereas for cows with a dry period length shorter than the herd mean, CaOS produced 0.9 kg of milk less than control cows ($P = 0.05$). When the 1st DHIA test occurred > 15 DIM, CaOS tended to produce 1.1 kg more of milk than control cows. No significant effect of treatment was observed on reproductive outcomes nor interactions with serum Ca were observed in the preliminary analysis. Our results suggest that the response to postpartum oral Ca supplementation may vary according to different peripartum factors and time relative to treatment administration, but further analysis is required to define these interactions.

Key Words: calcium, hypocalcemia, dairy cow

T26 Establishment of an in vitro rumen model with primary rumen epithelial cells. N. Reisinger*, D. Baranski, D. Wendner, V. Nagl, and E. Mayer, *Biomin Research Center, Tulln, Austria.*

The rumen and its function gain more and more importance in cattle industry. Only a limited number of studies are available, using primary rumen epithelial cells isolated from cows (Sun et al., 2017; Stumpf et al., 2009). Establishing a cell based rumen in vitro model provides an important tool to investigate not only the rumen metabolism, but will also help to evaluate the effects of different toxins (e.g., bacterial toxins, mycotoxins) on rumen cells. The aim of the study was to isolate, cultivate, and characterize rumen epithelial cells (REC) isolated from the rumen tissue of dairy cows. Furthermore, it was assessed, if deoxynivalenol (DON) and fumonisin B_1 (FB_1) affect cell viability of REC. Rumen tissue was obtained from a local abattoir and transported on ice to the laboratory. Epithelial cells were isolated from the tissue by enzymatic dissociation with trypsin. Cell growth was visually checked with a light microscope. In addition, cells were characterized via immunostaining for cytokeratin and tight junction proteins. The water-soluble tetrazolium salt (WST-1) assay was used to assess the effects of 2 mycotoxins on cell viability: DON (0–25 μ*M*) and FB_1 (0–25 μ*M*). For statistical evaluation of data, GraphPad Prism software (Version 7) was used. As data were normally distributed, ANOVA was performed with Dunnett's as post-hoc test. *P*-values of <0.05 were considered as significant. REC were successfully isolated from the rumen tissue and could be cultivated for up to 8 passages without changes in cell morphology. Cells were positively stained for cytokeratin (epithelial cell marker) and all 3 tested tight junction proteins (claudin-1, occludin and zonula occludens-1). DON significantly decreased cell viability at a concentration of 1 μ*M* ($P < 0.05$), whereas FB_1 significantly decreased cell viability at 12.5 μ*M* ($P < 0.05$).The establishment of an in vitro model with REC was successful as cells could be cultivated and showed cell type specific characteristics. Furthermore, DON seems to exhibit higher cytotoxicity than FB_1 in REC.

Key Words: rumen, epithelial cells, in vitro

T27 Effects of pegbovigrastim administration on periparturient diseases, milk production, and reproductive performance of Holstein cows. M. Zinicola*, R. Bicalho, H. Korzec, A. G. Teixeira, E. K. Ganda, and L. Bringhenti, *Cornell University, Ithaca, NY.*

Treatment with granulocyte colony-stimualting factor (G-CSF) increases neutrophil counts in periparturient cows.The aim of this experiment was to evaluate the effects of treating Holstein cows with pegbovigrastim on periparturient diseases, milk production, and reproductive performance. Cows were randomly allocated into 1 of 2 treatment groups: untreated control (CTR, n = 423) and pegbovigrastim (PEG, n = 417). At 7 d

from anticipated calving date (d −7), cows allocated to PEG received a subcutaneous injection containing 15 mg of pegylated recombinant bovine G-CSF (pegbovigrastim injection, Imrestor, Elanco Animal Health). A second injection was administered within 24 h after calving (d 0). Blood samples were obtained from a subset of cows at −7 d, and at 0, 3, 7, and 14 d relative to parturition. Samples were used for hemogram and quantification of haptoglobin, nonesterified fatty acids, and β-hydroxybutyrate concentrations. Postpartum disease occurrence was recorded from calving until 30 DIM. Milk yield was recorded for the first 12 wk after calving. Multivariable mixed models were performed using repeated measures ANOVA, logistic regression, or proportional hazard models. PEG-treated animals exhibited 3- to 4-fold increase in circulating granulocyte, lymphocyte, and monocyte numbers 3 d after treatment, and this increase decreased over time but always remained significantly higher compared with CTR. The administration of PEG was not associated with clinical and subclinical mastitis, retained placenta, metritis, and endometritis. Primiparous cows treated with PEG tended to have lower odds of developing hyperketonemia than CTR. PEG cows tended to have higher odds of being diagnosed with puerperal metritis compared with CTR. Cows treated with PEG had higher odds of being diagnosed lame within 30 DIM compared with CTR. Primiparous cows treated with PEG produced more milk than CTR primiparous cows during the first 12 wk postpartum. Treatment did not alter reproductive performance. We conclude that treating dairy cows with PEG did not influence periparturient disease incidence, increased milk yield in primiparous cows, and did not influence reproductive performance.

Key Words: periparturient diseases, dairy cow, pegbovigrastim

T28 Impacts of various milk replacer supplements on the health and performance of high-risk calves. E. M. Davis*, Y. Liang, T. A. Batchelder, and M. A. Ballou, *Texas Tech University, Lubbock, TX.*

Objectives were to determine the effects of supplementing a blend of probiotics, β-glucan, mannanoligosaccharides, and small-molecular-weight molecules from colostrum on the performance and health of high-risk Holstein calves. One hundred bull calves were acquired from a local calf ranch within 24 h of birth and randomly assigned to 1 of 5 treatments added to the milk replacer: Control (CON), no additive; Immu-Prime (ImmPr), 1.5 g/d ImmPr first 3 d only; (3) Beta glucan (BG), 1 g/d BG; (4) Mannanoligosaccharide + *Bacillus subtilis* (MOS+Bs), 3 g/d MOS + 4×10^9 cfu/d *Bacillus subtilis*; and PROVIDA (PRO), blend of 2×10^9 *Lactobacillus casei* and *Enterococcus faecium* + 2×10^9 *Saccharomyces cerevisae*. Calves were weaned at d 56, comingled, and treatment carry-over effects were evaluated through d 84. Starter intake was measured daily and BW weekly. Peripheral blood samples were collected on d 1, 3, 7, 14, 21, 42, 56, and 84 and analyzed for hematology and serum analyzed for haptoglobin concentrations. Neutrophil function was assessed through surface L-selectin and phagocytic and oxidative burst activities. Data were analyzed using Proc Mixed. The BG consumed the most starter from d 1 to 28 and both the MOS+Bs and ImmPr consumed more than the CON ($P = 0.016$). Preweaned ADG was greater for PRO and BG when compared with the CON ($P = 0.038$), both the MOS+Bs and ImmPr were not different than the other treatments. There was no carry-over effect on ADG ($P = 0.879$). There was a tendency for BG, MOS+Bs, and PRO to have reduced serum haptoglobin throughout the study ($P = 0.075$). Total leukocyte, neutrophil, and lymphocyte counts were reduced among MOS+Bs calves ($P \leq 0.003$), whereas BG calves tended to have the greatest neutrophil:lymphocyte ratio (0.051). Neutrophil L-selectin was reduced among BG ($P = 0.030$), whereas PRO was not different than BG or the other treatments. Neutrophil oxidative burst was reduced among BG and PRO when compared with CON and ImmPr ($P = 0.011$), but MOS+Bs was not different than any treatment. Supplementing BG, MOS+Bs, and PRO all influenced performance and health of high risk calves, but the mechanisms appear to be different.

Key Words: calf, health, immune

T29 Carvacrol residues in milk after prophylactic intrauterine infusion of a natural oregano essential oils product in postpartum dairy cows. D. Manriquez*[1], E. Freitas[3], A. Velasquez[1], J. Velez[2], and P. J. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Aurora Organic Dairy, Platteville, CO,* [3]*Van Beek Natural Science, Orange City, IA.*

Withholding information for multiple therapeutic natural products used in organic production is not available. The objective was to determine whether the administration of a prophylactic intrauterine infusion of an oregano extract and oregano essential oils product (Optimum UterFlush (UF), Van Beek Natural Science, Orange City, IA), in postpartum dairy cows results in detectable levels of the active ingredient carvacrol (4-isopropyl-2-methylphenol) in milk. Five primiparous Holstein cows (DIM = 3.8 ± 0.4 d) were randomly selected from the fresh pen of an organic certified dairy farm. After a clinical inspection to ensure the enrolment of healthy individuals, a baseline composite milk sample was collected from each of the 5 cows. All the study cows were treated with an intrauterine infusion consisting in 3.75 mL of UF diluted in 117 mL of distilled water, applied every other day for 3 applications, as indicated by the manufacturer. Two sampling schemes were used: (1) composite milk samples were collected from all cows at 6, 12, 24 and 48 h after the first treatment administration; (2) composite milk samples were collected from a subgroup of 2 cows at 6, 12, 24 and 48 h after the last treatment. After collection, milk samples were frozen at −20°C and stored until lab analysis. The presence of carvacrol was analyzed using gas chromatograph mass spectrometry (ABC testing, Tustin, CA). Carvacrol was not detected in the baseline samples. In contrast, carvacrol was detected in all the samples collected after treatment application. Means and standard deviations were calculated using PROC MEANS from SAS. The average concentration of carvacrol reached its highest point at 6 h after the first UF infusion (0.217 ± 0.159 ppm) and then decreased to 0.084 ± 0.098 ppm at 12 h, to 0.005 ± 0.006 ppm at 24, and to 0.0006 ± 0.001 ppm at 48 h after the first treatment. Samples following scheme 2 resulted in average carvacrol concentrations of 0.097 ± 0.057 ppm at 6 h, 0.089 ± 0.068 at 12 h, 0.042 ± 0.038 ppm at 24 h, and 0.002 ± 0.002 ppm at 48 h after the last treatment. These results indicate that under the recommended dose of UF, carvacrol can be detected in milk after prophylactic intrauterine infusion.

Key Words: metritis, organic, oregano

T30 A mathematical modelling approach to dynamic networks: Potassium homeostasis and glucose-insulin in dairy cows. J. Ploentzke*, M. Berg, and S. Roeblitz, *Zuse Institute Berlin, Berlin, Germany.*

Lactating dairy cows require a particular composition of nutritional ingredients depending on their production status. The optimal supply of energy and minerals in diet, one of them potassium, is indispensable for the prevention of disbalances, such as hypokalemia or hypoglycaemia. Potassium balance is the result of potassium intake, distribution in the organism, and excretion, and closely interacts with glucose and electrolyte metabolism, in which postpartum veterinary treatments frequently intervene. We present a mechanistic, dynamic model for

potassium balance together with a glucose-insulin model in nonlactating and lactating dairy cows based on ordinary differential equations. Parameter values were obtained from fitting to data of a clinical trial as well as from literature. To verify the mechanistic functioning of the model, we validate the model by comparing simulation outcomes with clinical study findings. Furthermore, we perform numerical experiments and compare them with expected behavior according to mechanistic knowledge. The results give insight into the dynamic behavior of the network and open the way for further open questions and hypotheses to be tested, as hypokalemia development and dynamics of different potassium substitution therapeutic strategies.

Key Words: ODE model, potassium, glucose-insulin

T31 Effect of metritis on endometrium tissue transcriptome during puerperium in Holstein lactating cows. S. Genís*[1,2], A. Arís[1], M. Kaur[2], and R. L. A. Cerri[2], [1]*Department of Ruminant Production, IRTA, Caldes de Montbui, Spain,* [2]*Applied Animal Biology, UBC, Vancouver, BC, Canada.*

The objective of this prospective cohort study was to evaluate the gene expression profile of endometrium in dairy cows regarding metritis incidence and parity. Thirty Holstein cows from a group of 90 cows initially enrolled were randomly selected (22 multiparous (MP) and 8 primiparous (PP)) and endometrium biopsies were collected on d 1, 3, and 6 after calving and clinically monitored for metritis. Rectal temperature was measured twice and fever was defined as a temperature ≥39.5°C. A case of metritis was defined as a cow with a red-brown watery foul-smelling uterine discharge or purulent discharge with more than 50% pus and fever on d 1 to 6 postpartum. Twenty-four cows from 30 were selected to analyze the expression of 66 genes measured on the NanoString nCounter Analysis System. The genes selected were related with adhesion, immune system, steroid and prostaglandin biosynthesis regulation, insulin metabolism and transcription factors, and nutrient transporters. The results indicated a different pattern on genes related to immune function by parity. *PTX3*, involved in antigen presentation, was increased in healthy MP compared with healthy PP whereas inflammatory cytokine *TNF*α and complement-related protein *SERPING1* were upregulated in MP compared with PP ($P \leq 0.05$). As expected, metritis incidence affected gene expression pattern related to immune function with a clear increase in expression of antiviral factor *MX2* and myosin *MYH10* gene, involved in macrophages recruitment, in metritic cows compared with healthy cows ($P \leq 0.05$). Differences in uterus involution with metritis were reflected by downregulation of *IGF1* ($P < 0.10$), involved in endometrium remodeling, and a compensatory upregulation of its receptor *IGFR1* in metritic cows compared with healthy cows ($P \leq 0.05$). A greater expression of receptors of prostaglandins and oxytocin (*PGR* and *OXTR*), involved in involution processes, were observed in metritic PP compared with healthy PP ($P \leq 0.05$). Overall, these results reflect the effect of metritis in involution and immune response along with the parity influence in post-calving status of the animal.

Key Words: endometrium, metritis, NanoString

T32 Stabilized rice bran addition in milk of non-weaned organic Holstein calves. A. Velasquez*[1], D. Manriquez[1], S. Paudyal[1], H. Hyungchul[1], R. Callan[2], E. Ryan[3], and P. Pinedo[1], [1]*Department of Animal Sciences, College of Agricultural Sciences, Colorado State University, Fort Collins, CO,* [2]*Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO,* [3]*Department of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.*

The objective was to evaluate the effect of stabilized rice bran (SRB) addition into milk on health and performance of non-weaned dairy calves. Holstein heifer calves (n = 90) were enrolled at 6 ± 1 d old and monitored for 28 d, from July to August 2017. A paired comparison design with 2 treatment groups was considered. Calves were randomly assigned into a control (n = 45) or a treatment group (n = 45) receiving SRB. The treatment group received a daily dose of SRB corresponding to 10% of the daily calories (120 g). Calves were offered milk 3 times a day, in 2.8 L bottles. The SRB dose was divided in 2 feedings (0500 and 1900 h) to allow for adequate suspension in milk. Calf starter was offered in increasing amounts from d 4 of life. A health evaluation was conducted at enrollment and the initial health status was subsequently included as a covariate in the statistical models. Body weight was measured in the first and last day of the study. Additionally, a daily health evaluation was performed to assess health status and disease severity through diarrhea, dehydration, attitude, and milk intake scores. Milk intake was individually recorded after AM and PM feeding. Health status was categorized as not sick, slightly affected, moderately sick, and severely sick. Data were analyzed using repeated measures for binary responses, time to event analyses, and repeated measures ANOVA. Overall, average daily weight gain was not affected by treatment. The total number of calf-days classified as healthy or sick were not different between treatment groups. Similarly, the number of calf-days categorized as slightly affected, moderately sick, or very sick did not differ between treatment groups. The survival analyses indicated no differences in time to first moderate case of disease by treatment group and by health status at enrollment. Time to recovery from a moderate disease status showed a tendency for a shorter recovery time ($P = 0.052$) for healthy control calves. Our results indicated that the addition of SRB in milk did not have an effect in the performance and health of non-weaned dairy calves.

Key Words: neonatal diarrhea, stabilized rice bran, dairy calves

T33 Evaluation of the incidence of health events compatible with recumbency at dry-off in Denmark. H. L. Hyttel[1], K. Krogh*[2], and A. de Prado-Taranilla[2], [1]*Danish Cattle Association, SEGES, Skejby, Denmark,* [2]*Ceva Sante Animale, Libourne, France.*

The transition from gestation to lactation is marked by significant physiological changes for the individual cow in such a way that disease incidence is the highest in early lactation. Similarly, the transition from lactating to dry state is a critical period for the cow. Cows need to adapt to changes in diet, pen, pen mates and the milk synthesis must be stopped during this period. Most of the research at this period has focused on udder health issues and not on other diseases that cows may experience. The objective of this retrospective study was to evaluate the incidence of health events (HE) compatible with recumbency (REC) at dry-off (DO) in Danish dairy cows using descriptive statistics. Data provided by the Danish Cattle Association (DCA) from lactating cows with registered DO dates for 2 years were used for analysis of incidence of REC at DO. DCA maintains a central database for dairy farmers which contains data on HE. To evaluate the incidence of REC in the period ± 7 d to registered DO dates, 2 different proxies were included in the analysis: A) records of the following clinical disease codes compatible with recumbency: atypical milk fever, abomasal dilatation/displacement/ulcer/disorder, ketosis, fatty liver or milk fever (REC-1); and (B) records of the use of products containing calcium intended for Intravenous use to treat diseases compatible with recumbency (REC-2). The average number of events reported compatible with recumbency were 1.56‰ and the average of number of treatments compatible with recumbency

were 0.93‰. Disease events at dry off are rarely reported but occur with varying incidence. We conclude that health events compatible with recumbency (REC) happen between 7 d before and 7 d after DO in Danish dairy herds in the period of 2015 and 2016. There was no variation between the years 2015 and 2016. More research is needed to evaluate the risks cows may encounter in the transition from lactation to dry period that can lead to health events.

Table 1 (Abstr. T33).

	No. of events per 1000 cows dried off		No. of DO dates/
Dry-off year	REC-1	REC-2	lactations
2015	1.55	0.94	269,162
2016	1.57	0.92	282,424

Key Words: dry off, recumbency, paresis

T34 Udder health, milk production, and longevity parameters across three OmniGen-AF feeding trials. S. C. Nickerson[1], F. M. Kautz[1], L. O. Ely[1], A. D. Rowson*[2], D. J. McLean[2], and J. D. Chapman[2], [1]*University of Georgia, Athens, GA,* [2]*Phibro Animal Health Corporation, Teaneck, NJ.*

Effects of feeding OmniGen-AF (OG) to bred heifers and cows in 3 separate trials in a university dairy herd was summarized. In trial 1, 15 heifers received OG beginning at 6 months of age through day of calving; 18 animals served as controls. In trial 2, 35 heifers received OG beginning 60 d prior calving through day of calving; 38 animals served as controls. In trial 3, 11 late lactation cows received OG beginning 60 d prior to dry-off through 30 d post calving; 11 controls received OG at dry-off through 30 d post calving. OG was fed at 9g/100 kg BW/d. Prior to and/or during each trial, quarter milk samples were collected for microbiological analyses and SCC to monitor mastitis status. Mastitis treatments were recorded, and DHIA records monitored for milk production and cow longevity. Data were analyzed as Control vs. Treatment using ANOVA and means separation with Least Squared Means (PROC GLM, SAS 9.4, 2017). Results demonstrated that for heifers in trials 1 and 2, controls exhibited a greater number of clinical mastitis quarters than OG heifers during the precalving period (12 vs. 9). Likewise, after calving through dry-off for all trials, controls exhibited a greater number of clinical mastitis quarters than OG animals (7 vs. 0). Across all trials, 11 quarters from controls were treated for mastitis, whereas no OG animals were treated. Eleven control quarters became chronically infected, while only 2 OG quarters became chronic. At dry-off, the number of mastitis cases present in control and OG animals were 7 and 2. Numbers of quarters from control and OG animals diagnosed with new IMI between calving and dry-off were 4 and 0. DHIA overall lactation SCS were 3.04 and 2.48 for control and OG animals (P<0.10), and lactation SCC were 209,000/ml and 121,000/ml for control and OG animals (P<0.14). The 305-d ME for milk yield were 11,020 and 11,286 kg for control and OG animals. The 305-d ME for fat was numerically higher for control than OG animals (415 vs. 409 kg), and protein was numerically lower (317 vs. 324 kg). Cull rates were 10.52% for controls and 5.88% for OG animals. OG is effective in maintaining udder health and lowering SCC in dairy cattle when fed at least 2 months prior to calving.

Key Words: mastitis, SCC, OmniGen-AF

T35 Induction of leaky gut through feed restriction or abomasal infusion of resistant starch in healthy post-peak lactating cows. P. Piantoni*[1], M. A. Abeyta[2], G. F. Schroeder[1], H. A. Ramírez-Ramírez[2], H. A. Tucker[3], and L. H. Baumgard[2], [1]*Cargill Animal Nutrition Innovation Campus, Elk River, MN,* [2]*Iowa State University, Ames, IA,* [3]*Novus International, St. Charles, MO.*

Leaky gut (decreased intestinal barrier function) decreases milk yield and DMI, and might be related to metabolic disease in dairy cows. Six cannulated Holstein cows (36.3 ± 1 kg/d milk; 212 ± 97 DIM; 691 ± 70 kg BW) were used in a replicated 3 × 3 Latin square design experiment with 21-d periods (d 1–16 wash-out and d 17–21 challenge) to develop a model to induce leaky gut via feed restriction or altered hind gut fermentation. Treatments during the challenge were ad libitum feeding (CTR), 50% feed restriction (FR), and 500 g/d DM resistant starch (RS; abomasally infused once a day before feeding). Blood samples were collected 4 h after feeding on d 13 and 14 to create a baseline for responses measured, and on d 17, 19, and 21 of each period. Fecal samples were collected 4 and 8 h post-feeding on d 14 and 21. Data were analyzed using the GLIMMIX procedure of SAS with fixed effects of treatment, challenge day (repeated measures) and their interaction, and random effects of square, period, and cow. Baseline values were used as covariates. During the challenge period, FR decreased DMI by 48% and ECM yield overtime (25.4 vs. 28.0 kg/d) compared with CTR and RS (both $P < 0.05$). Overall, FR increased plasma NEFA compared with CTR and RS (160 vs. 82.4 mEq/L; $P < 0.05$). In addition, FR increased plasma total proteins (7.57 vs. 7.36 g/dL), albumin (3.69 vs. 3.43 g/dL), and γ-glutamyltransferase (37.9 vs. 31.8 U/L) at d 19, and decreased alkaline phosphatase (41.4 vs. 49.1 U/L) at d 21 compared with CTR (all $P < 0.05$). Responses to the RS treatment were intermediate between CTR and FR for the last parameters mentioned. Treatments did not affect plasma concentrations of lipopolysaccharide binding protein, endotoxin, serum amyloid A, interleukin 1-β, and serum coloration (all $P > 0.20$). Treatments did not affect fecal pH 4 h post-feeding. However, RS decreased fecal pH 8 h post-feeding compared with CTR and FR (6.21 vs. 6.80; $P < 0.05$). Lack of responses on inflammatory biomarkers measured in this experiment suggests the models tested did not affect intestinal barrier function, and therefore, a more intense challenge may be needed to induce leaky gut in healthy post-peak cows. Further, evaluating these insults earlier in lactation is of scientific and practical interest.

Key Words: challenge model, gut health, intestinal barrier function

T36 Associations between the general condition and the selling price of culled dairy cows sold at 3 Ontario auction markets in a defined time period. A. Moorman*[1,2], T. F. Duffield[1,2], M. A. Godkin[3], J. Rau[4], and D. B. Haley[1,2], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, ON, Canada,* [3]*Ontario Ministry of Agriculture, Food and Rural Affairs, Elora, ON, Canada,* [4]*Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

The purpose of this study was to determine the general condition of culled dairy cows sold at auction markets in Ontario, Canada, and how this related to the sale price of the cow. Culled dairy cows were visually observed and evaluated during weekly sales at licensed Ontario livestock auction markets. Data were collected on 4460 dairy cows sold at 3 Ontario auction markets over 16 weeks. Observers recorded the general condition of dairy cows entering the sales ring, by recording each cow's hock injury score, body condition score (BCS), gait score, and tail score in accordance with the Dairy Farmers of Canada 2017 proAction Animal Care standards. Observers also recorded each cow's sale-ring bodyweight (in pounds), breed, and the sale price paid ($/pound). Results were then later converted to metric units. Standardized

data collection sheets were used to record observations and data were directly entered into an excel spreadsheet. The spreadsheet data were then imported into statistical analysis software (SAS) the frequency of each variable, and its relation to price paid, was assessed. Results showed that 27.2% of the culled cows scored had a medium to severe hock injury, 40.7% had a BCS ≤ 2, 72.7% had an abnormal gait, and 12.5% had docked tails. Culled cows with a BCS ≤ 2 sold for $0.20 less per kg compared with those with a BCS > 2, which equated to an average loss of $117 per cow ($P < 0.001$). Cows with an abnormal gait sold for $0.04 less per kg compared with culled cows with a normal gait, which equated to an overall average loss of $32.45 per cow ($P < 0.001$). The main issues identified with culled cows in this study were the high prevalence of suboptimal body condition and lameness, which translated into a reduced sale price for the cow compared with cows with good body condition and a normal gait.

Key Words: culled cow, cattle transport, auction market

T37 Milk yield relative to supplement intake and rumination time differs by health status for fresh cows milked with automated systems. M. T. M. King*[1], K. J. Sparkman[1], S. J. LeBlanc[2], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to determine associations of subclinical ketosis (SCK) and blood β-hydroxybutyrate (BHB) with milk yield, supplemental feed consumption, and rumination time of cows in herds using automated milking systems (AMS). We also examined ratios of milk yield relative to supplement intake and rumination time. We monitored 605 cows from 9 AMS herds, testing blood BHB concentrations 1 ×/wk for the first 3 wk of lactation. Milk yield, supplement intake, and rumination data were collected from the AMS computer on each farm for the first 28 DIM for each cow. For analyses, only multiparous cows from 8 herds were included (n = 172 total) and were matched, within farm, to include an equal number of cows in each of 4 health status groups (n = 43 per group): SCK− (BHB ≥ 1.2 mmol/L at ≥1 of 3 tests, with no other disorder in the first 30 DIM), SCK+ (BHB ≥1.2 mmol/L at ≥1 of 3 tests, with another health disorder), HLT− (BHB always <1.2 mmol/L, with no other disorder), or HLT+ (BHB always <1.2 mmol/L, with a health disorder). Multivariable mixed-effect linear regression models were used to make comparisons by health status. Linear and logistic regressions were used to analyze associations of BHB concentrations and risk of SCK, respectively. Milk yield ($P < 0.001$) and rumination time ($P < 0.001$) varied by health status (HLT+: 32.8 kg/d, 477 min/d; SCK+: 35.1 kg/d, 431 min/d; SCK−: 39.5 kg/d, 484 min/d; HLT−: 37.2 kg/d, 500 min/d), but there was no difference in supplement intake by health status group (averaging 4.2 kg/d). As a result, milk yield relative to supplement intake ($P < 0.001$) and rumination time ($P < 0.001$) differed by health status, and both were associated with BHB and risk of SCK ($P < 0.05$). Cows in SCK- had the highest milk yield and ratio of milk yield:supplement intake, but SCK+ cows had the lowest rumination time and the highest milk yield:rumination ratio. These results highlight the differences in milk production (per day and relative to supplement consumption or rumination time) associated with blood BHB and health status, and the potential to modify supplementation in AMS to reduce negative energy balance.

Key Words: robotic milking, energy balance, subclinical ketosis

T38 Young calves that will suffer at least one episode of diarrhea in the first 30 days of life have a different fecal microbiome than those that will not incur diarrhea. F. Correa[1], F. Fàbregas[2], V. Aragón[1], and À. Bach*[2,3], [1]*IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Barcelona, Spain,* [2]*Department of Ruminant Production, IRTA, Barcelona, Spain,* [3]*ICREA, Barcelona, Spain.*

Diarrhea is the most common affliction of young calves during the first weeks of life. Reasons for diarrhea are multifactorial, but we hypothesized that the composition of the microbiota inhabiting the gastrointestinal tract may act as a protective barrier or as a facilitator for colonization of the intestine by pathogenic bacteria or viruses. For a period of 2 mo, all healthy calves that entered a commercial contract heifer operation were enrolled in a sampling scheme consisting of collecting fecal samples from the rectum at arrival and at 30 d of life. Calves were checked daily by a veterinarian. After completing all samplings, calves were classified as HEALTHY if did not have diarrhea, or as DIA if they experienced at last one case of diarrhea. Calves from DIA and HEALTHY categories were randomly selected and their fecal samples processed to recover DNA. Then, the 16sRNA gene was sequenced (Illumina MiSeq 2X250) individually using barcoded primers. One animal from DIA and 3 from HEALTHY were excluded from sequencing due to poor DNA recovery. Thus, data presented herein correspond to 18 (7 HEALTHY and 11 DIA) Holstein female calves (44.2 ± 2.9 kg of initial BW). The Quantitative Insights Into Microbial Ecology software package was used to define operational taxonomic units (OTUs) and compare the community structures and taxonomical profiles of calves. The diversity within samples (α) and between groups (β) were estimated, and a discriminant analysis of OTUs at the genus level was performed between HEALTHY and DIA calves at arrival. First cases of diarrhea occurred at 19.3 ± 8.6 d of age. Alpha diversity increased with age ($P < 0.05$), but at arrival, calves in the HEALTHY category had greater ($P < 0.05$) α diversity than DIA calves at arrival. Based on the discriminant analysis, HEALTHY calves, in general, had greater abundance of *Collinsella*, *Lactobacillus*, *Lachnospiraceae*, *Subdoligranulum*, and *Megasphaera*, and lower abundance of *Anaerostipes*, *Feacalibcaterium*, and *Ruminococcus* than DIA calves. Management and nutritional practices that promote this microbiome pattern before 10 d of life may represent a potential alternative to minimize diarrhea.

Key Words: gut health, sequencing, scours

T39 On-farm selection of adult fecal microbiome for transplantation into neonatal dairy calves as an enhancer for growth and development. F. Rosa*[1], E. Trevisi[2], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD, US,* [2]*Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

The objective of this study was to evaluate the effects of early life fecal microbial transplantation (FMT) from adult healthy donors into neonatal dairy calves on growth and health performance as well as effects on inflammation and metabolism. The selection of the adult donor was based on health and production records at the Dairy Research and Training Facility (DRTF) at South Dakota State University as well as fecal samples testing negative for *Mycobacterium paratuberculosis*, *Salmonella*, and *Cryptosporidium*. The final selected donor from the DRTF farm was a fifth lactation Holstein dairy cow, among the highest milk yield (~11,090 kg/lactation) of the herd, no treatment records for any disease or metabolic disorder, and negative for all pathogens mentioned above. Sixteen healthy newborn Holstein calves (n = 8/trt) housed in individual hutches were used in a randomized complete block design from birth to 7 wk of age. Calves were fed 2.8 L/d of antibiotic-free milk replacer 2 × /d during wk 1 to 5, 1 × /d in wk 6, and weaned at d 42.

Antibiotic-free starter pellets and water were fed ad libitum. Treatments were a baseline nutritional program (CON) or calves subjected to 1 × /d inoculations with 25 g of fecal donor material (FMT) mixed in the milk replacer from 8 to 12 d of age. Individual intakes of milk and pellets were measured daily. Fecal and respiratory scores were recorded daily. Body weight (BW) and withers height (WH) were recorded weekly. Blood samples were collected weekly for metabolic and inflammatory profiling during the experiment. Data were analyzed using the MIXED procedure of SAS. There was a trend ($P = 0.09$) for greater BW (50.8 vs 52.7 kg ± 0.7) in FMT calves. Similarly, there was a trend for greater WH ($P = 0.13$) in FMT (82.6 vs 83.8 kg ± 0.49) calves. Starter intake was not affected ($P = 0.86$). Fecal scores were not affected ($P = 0.23$) by FMT inoculation. Although mild improvements were observed in BW and WH by FMT inoculation, these are suggestive that neonatal dairy calves may benefit from this approach to enhance gut health and immunity, which might be explained by biomarkers profiling in blood.

Key Words: calves, growth, microbiota

T40 Evaluation of the potential enrichment of RNA from immune cells during isolation of fecal RNA from neonatal dairy calves. F. Rosa* and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

The fecal RNA is a novel approach to study the biological adaptations of the gastrointestinal tract (GI) of neonatal dairy calves through gene expression analysis. Our objectives in this study was to determine the potential enrichment of RNA from immune cells during isolation of fecal RNA from dairy calves, by a comparative transcriptomic profiling of genes specific for polymorphonuclear leukocytes (PMNL) and macrophages (*MPO*) and GI tract epithelial cells (*FABP2*) and cytokeratin 8 (*KRT8)*. Fecal and blood samples were simultaneously taken from 8 neonatal Holstein calves with less than 3 wk old. The total RNA was isolated from 200 mg of feces, using a Trizol based method along with the RNeasy Plus Mini Kit (Qiagen), following the manufacturer's instructions with some modifications. The overall RNA quantity for all fecal samples was 378.8 ± 192.3 ng/µL and purity (260/280 ratio) was 2.0 ± 0.1 determined via Nanodrop. Using the same RNA isolation method, PMNL was isolated from 100 mL of total blood, and the overall RNA quantity for all PMNL samples was 55.6 ± 32.7 ng/µL, and the purity was 1.9 ± 0.1. The standard curve was composite from all samples including cDNA from fecal and PMNL. The internal control genes used in this experiment were *GOLGA5*, *OSBPL2*, *SMUG1*, *B2M*, *ACTB*, *GAPDH*, and *RPS9*. Normalized gene expression data were log-transformed before statistical analysis using the Proc Mixed of SAS (SAS 9.4). The mRNA expression of *KRT8* was greater ($P < 0.01$) in fecal RNA than in PMNL, and a trend ($P = 0.08$) was observed for greater expression of *FABP2* in fecal RNA than PMNL. In contrast to *KRT8* and *FABP2*, the mRNA expression of *MPO* was not detectable on fecal RNA, and this was reflected in a lack of amplification over the standard 40 cycles of the RT-qPCR. However, the *MPO* was amplified on PMNL samples. Our results indicate that RNA isolated from fecal samples contain a low amount of immune cells such as PMNL and further confirms that the signal observed in genes related to inflammation in fecal samples. However, these results need to be validated between normal and diarrhea samples.

Key Words: fecal RNA, immune cell, inflammation

T41 Pre- and post weaning performance and health of dairy calves fed milk replacers supplemented with egg antibodies, direct-fed microbials, neomycin sulfate and oxytetracycline. D. M. Ziegler*[1], H. Chester-Jones[1], B. E. Ziegler[2], A. K. Manthey[2], and J. L. Olson[3], [1]*University of Minnesota, Waseca, MN,* [2]*Hubbard Feeds Inc., Mankato, MN,* [3]*Milk Products, Chilton, WI.*

One hundred seven (2 to 5 d old) individually fed Holstein heifer calves (38.5 ± 0.67 kg) from 3 commercial dairies were randomly assigned to 1 of 4 milk replacer treatments (MR) supplemented with egg antibodies (EA), direct-fed microbials (DFM), or neomycin sulfate and oxytetracycline (NT) to evaluate pre- (d 1–42) and post weaning (d 43–56) calf performance and health. The study was conducted between May and August 2017. Treatments included (1) all-milk protein, non-medicated MR 20% CP:20% Fat fed at 0.28 kg in 2 L of water 2× daily from d 1 to d 35 and 1x daily from d 36 to weaning at d 42, (CON); (2) MR as in CON supplemented with NT at a rate of 22 mg/kg BW/d for 14 d, (MRNT); (3) MR as in CON supplemented with a blend of egg antibodies and DFM containing multiple strains of *Lactobacillus* organisms and lactic acid producing bacteria at a rate of 40 g/d for 14 d then 20 g/d for 14 d, (MREA); 4) MR as in CON supplemented with an additive blend of serum and plasma proteins, Bio-Mos, essential oils, and multiple DFM strains at a rate of 40 g/d for 14 d then 20 g/d for 14 d, (MRAB). Calf starter (18% CP as fed) and water were offered free choice from d 1 to 56. Data were analyzed using the PROC mixed procedures of SAS and repeated measures analyses applied where appropriate. There were no differences in pre- (d 1–42), post weaning (d 43–56), and overall (d 1–56) gains averaging 0.49, 1.06, and 0.63 kg/d respectively ($P > 0.05$). Pre- (d 1–42), and post weaning (d 43–56), calf starter intake was similar across treatments averaging 13.1 and 28.1 kg total intake, respectively. There were no differences in health costs, gain/feed, or hip height gain across treatments ($P > 0.05$). The number of days fecal scores were 3 ≥ (d 1–42, 1 = normal, 4 = very loose, watery) were similar across treatments, averaging 1.61, 1.82, 1.27, and 1.99 d respectively ($P > 0.05$). Under conditions of this study, feeding calves a MR blended with EA and DFM, NT or other additives did not affect growth compared with calves fed a non-medicated MR.

Key Words: calf performance, milk replacer, egg antibody

T42 Pre- and post-weaning performance and health of dairy calves fed milk replacers supplemented with an organic direct-fed microbial or neomycin sulfate and oxytetracycline. D. M. Ziegler*[1], H. Chester-Jones[1], T. Marubashi[2], and R. Shimizu[2], [1]*University of Minnesota, Waseca, MN,* [2]*Calpis America Inc., Peachtree City, GA.*

One hundred six (2 to 5 d old) individually fed Holstein heifer calves (39.0 ± 0.63 kg) from 3 commercial dairies were randomly assigned to 1 of 4 milk replacer treatments (MR) supplemented with an organic direct-fed microbial (DFM), neomycin sulfate and oxytetracycline (NT) or combination of (DFM and NT) to evaluate pre- (d 1–42) and post weaning (d 43–56) calf performance and health. The study was conducted between March and June 2017. Treatments included (1) all-milk-protein, non-medicated MR 20% CP:20% fat fed at 0.28 kg in 2 L of water 2x daily from d 1 to d 35 and 1x daily from d 36 to weaning at d 42, (CON); (2) MR as in CON supplemented with NT at a rate of 22 mg/kg BW/d for 14 d, (MRNT); (3) MR as in CON supplemented with 4 g daily of a DFM containing *Bacillus subtilis* strain C-3102 (1.2×10^9 cfu/g, d 1–42 ; MRDFM); (4) MR as in MRNT supplemented with DFM as in MRDFM, (MRNTDFM); Calf starter (18% CP as fed) and water were offered free choice from d 1 to 56. Data were analyzed using the mixed procedures of SAS and repeated measures applied where appropriate. There were no differences in pre- (d 1–42), post-weaning (d 43–56), or overall (d 1–56) gains averaging 0.54, 1.00, 0.65 kg/d respectively ($P > 0.05$). Pre- (d 1–42) and post-weaning (d 43–56)

calf starter intake was similar across treatments averaging 16.0 and 26.9 kg total intake. There were no differences in the number of days fecal scores were 3 ≥ (d 1–42, 1 = normal, 4 = watery) averaging 1.23, 1.11, 1.49, and 1.63 d, respectively ($P > 0.05$). Fecal sample analyses from a subset of calves on each treatment showed no difference ($P > 0.05$) in Enterobacteriaceae levels across treatments. Health costs were similar across treatments. Under conditions of this study, calves fed a MR supplemented with a DFM, NT or combination of DFM and NT did not affect growth or health costs compared with calves fed a non-medicated MR.

Key Words: calf performance, milk replacer, direct-fed microbial

T43 Pre- and post-weaning performance and health of dairy calves fed milk replacer supplemented with direct-fed microbials or neomycin sulfate and oxytetracyline. D. M. Ziegler*[1], H. Chester-Jones[1], B. E. Ziegler[2], A. K. Manthey[2], and J. L. Olson[3], [1]*University of Minnesota, Waseca, MN,* [2]*Hubbard Feed, Inc., Mankato, MN,* [3]*Milk Products, Chilton, WI.*

One-hundred thirty-four (2 to 5 d old) individually fed Holstein heifer calves (38.6 ± 0.63 kg) from 3 commercial dairies were randomly assigned to 1 of 5 milk replacer treatments (MR) supplemented with direct-fed microbials (DFM) or neomycin sulfate and oxytetracycline (NT) to evaluate pre- (d 1–42) and post weaning (d 43–56) calf performance and health. The study was conducted between December and February 2017. Treatments included (1) all-milk protein, non-medicated MR 20% CP:20% fat fed at 0.28 kg in 2 L of water 2× daily from d 1 to d 35 and 1× daily from d 36 to weaning at d 42, (CON); (2) MR as in CON supplemented with NT at a rate of 22 mg/kg BW/d for 14 d, (MRNT); (3) MR as in CON with a DFM containing *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24 included in the MR providing 0.5 g/d, (MRDFM1); (4) MR as in CON with a DFM containing multiple *Bacillus subtilis* strains (5×10^9 cfu/g) included in the MR providing 1.0 g/d (MRDFM2); (5) MR as in CON supplemented with 5 g per feeding of a DFM containing a single *Bacillus subtilis* strain (5.0×10^9 cfu/g; MRDFM3); Calf starter (18% CP as fed) and water were offered free choice from d 1 to 56. Data were analyzed using the PROC mixed procedures of SAS and repeated measures analyses applied where appropriate. There were no differences in pre-weaning (d 1–42), post-weaning (d 43–56), and overall (d 1–56) gains averaging 0.59, 1.12, and 0.72 kg/d respectively ($P > 0.05$). Pre-weaning (d 1–42) and post-weaning (d 43–56) calf starter intake was similar across treatments averaging 21.2 and 32.8 kg total intake, respectively. There were no differences in gain/feed, hip height gain, daily fecal scores, or health costs ($P > 0.05$). The number of days fecal scores were 3 ≥ (d 1–42, 1 = normal, 4 = very loose, watery) were similar across treatments averaging 3.04, 2.56, 2.48, 3.01, and 2.64 d respectively ($P > 0.05$). Under conditions of this study, calves fed a MR supplemented with a DFM or NT in MR did not improve growth, fecal scores or health costs compared with calves fed a non-medicated MR.

Key Words: calf performance, milk replacer, direct-fed microbial

T44 Identification and characterization of calf management clusters among dairy herds from Trenque Lauquen, Argentina. F. D. Llera[4], C. Vissio[3,1], P. Turiello*[1], A. Herrero[2], and A. Larriestra[1], [1]*Facultad de Agronomía y Veterinaria UNRC, Río Cuarto, Córdoba, Argentina,* [2]*Facultad de Ciencias Veterinarias, UBA, Buenos Aires, Buenos Aires, Argentina,* [3]*CONICET, Río Cuarto, Córdoba, Argentina,* [4]*INTA, Trenque Lauquen, Buenos Aires, Argentina.*

A cross-sectional study was conducted to describe and typify calf health and production management among dairy herds from Trenque Lauquen County, Argentina. A comprehensive survey about calf health care and nutrition management was carried out in 46 randomly selected dairy herds with 60 to 400 cows, 66% of the county dairy herd population belonging to that segment. The questionnaire was divided into topics and subjected to correlation analysis to reduce collinear variables. A multiple correspondence method was used to select the most discriminant management factors, and those factors were used in a hierarchical cluster analysis to typify the herds. The overall survey response rate was 83%. The most discriminant factors were pre-fresh management, colostrum and health management, record keeping, workers profile, and producers' management and performance perceptions. We identified 2 clusters (C1 = 22 herds and C2 = 24 herds) including both individual and collective rearing systems with differences regarding several aspects. Farms in C1 were better managed in comparison with those included in C2. Most of C1 producers vaccinated cows at dry-off and had a pre-fresh pen, administered colostrum artificially, disinfected calves navels and fed them more than 4 L of milk daily, and hired calf managers with higher education level. The dairy farm clusters found and described in this study would contribute to a better understanding of the calf management variation between herds focusing on the strengths and weaknesses of each cluster, and it would also contribute as a research input for extension programs on calf management.

Key Words: calf health, cluster, calf management

T45 Effects of a fully acidified dietary cation-anion difference diet fed at 2 different concentrations of dietary calcium inclusion prepartum on uterine health of Holstein cows after parturition. K. T. Ryan*[1], K. M. Glosson[1], X. Zhang[2], S. S. Bascom[3], A. D. Rowson[3], and F. C. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Institute of Animal Nutrition, Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Sichuan Agricultural University, Chenhdu, Sichuan, China,* [3]*Phibro Animal Health Corp., Teaneck, NJ.*

Feed management during the transition period can play an important role in the cow's reproductive tract recovery and function following parturition. The objective of this study was to determine the effects of feeding a fully acidified dietary cation-anion difference (DCAD) diet prepartum to Holstein cows (n = 76) at 2 different concentrations of dietary calcium (Ca) inclusion on uterine environment postpartum. Multiparous Holstein cows were enrolled at 50d before expected calving and followed until 75 DIM. Treatments began at 28d before expected calving and were CON (n = 23), a positive DCAD diet with low dietary Ca (0.4% DM); LOW (n = 22), a fully acidified DCAD diet (urine pH = 5.7) with low dietary Ca (0.4% DM); HIGH (n = 25), a fully acidified DCAD diet (urine pH = 5.7) with high dietary Ca (2.0% DM). Uterine health was evaluated at 4, 7, 10, 13, 15, 17, and 30 DIM by evaluating vaginal discharge for signs of metritis via Metricheck in 2 ways: a MC score (0–3, where 3 is a discharge containing ≥50% purulent material) and by smell score (0 or 3, where 0 is no smell and 3 is a putrid smell). Swabs of the endometrium were collected at 15 and 30 DIM, streaked onto slides, stained, and scanned using whole image scanning. Polymorphonuclear leukocytes (PMN) were counted and a percentage was calculated from these slides. Data collected were analyzed using PROC MIXED in SAS. Contrasts included CONT1 (CON vs average of LOW and HIGH) and CONT2 (LOW vs HIGH). Cows in CON (1.82 ± 0.11) tended to have a lower ($P = 0.06$) MC score than the average of cows in LOW and HIGH (2.07 ± 0.12) and cows in LOW tended ($P = 0.11$) to have higher MC score than cows in HIGH (1.97 ± 1.08). There were

no differences observed between treatments in the proportion of PMN cells present in the uterus. In conclusion, the uterine health of cows fed LOW appeared to not recover as well as cows fed CON or HIGH after parturition.

Key Words: DCAD, metritis, uterus

T46 Effects of fully acidified dietary cation-anion difference diet fed at 2 different concentrations of dietary calcium inclusion prepartum on inflammatory related blood metabolites. K. T. Ryan*[1], K. M. Glosson[1], X. Zhang[2], S. S. Bascom[3], A. D. Rowson[3], and F. C. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Institute of Animal Nutrition, Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Sichuan Agricultural University, Chenhdu, Sichuan, China,* [3]*Phibro Animal Health Corp., Teaneck, NJ.*

Inflammatory related blood metabolites are key indicators of stress that dairy cows can experience during the transition period. The objective of this study was to determine the effects of feeding a fully acidified dietary cation-anion difference (DCAD) diet prepartum to Holstein cows (n = 76) at 2 different concentrations of dietary Ca on circulating inflammatory related blood metabolites both pre- and postpartum. Multiparous Holstein cows were enrolled 50d before expected calving and followed until 75 DIM. Treatments began 28d before expected calving and were CON (n = 23), a positive DCAD diet with low dietary Ca (0.4% DM); LOW (n = 22), a fully acidified DCAD diet (urine pH = 5.7); HIGH (n = 25), a fully acidified DCAD diet (urine pH = 5.7) with high dietary Ca (2.0% DM). Plasma lipopolysaccharide binding protein (LBP) and plasma serum amyloid A (SAA) were assessed at −30, −21, −7, 15, and 30 d relative to calving using commercially available kits. Data were analyzed using PROC MIXED in SAS (v9.4). Contrasts included CONT1 (CON vs. the average of LOW and HIGH) and CONT2 (LOW vs. HIGH). Prepartum LBP and SAA concentrations increased for all treatments as cows approached calving ($P < 0.001$ and $P < 0.001$, respectively). Prepartum concentrations of LBP and SAA were 5.21, 5.01, 8.65 ± 0.47 µg/mL, and 20.28, 31.18, 41.11 ± 0.9 µg/mL for d −30, −21, and −7, respectively. Cows fed LOW had a higher LBP concentration prepartum than cows fed HIGH (7.33 vs. 5.84 ± 0.47 µg/mL, respectively; $P = 0.03$). Postpartum LBP and SAA concentrations decreased for all treatments over time ($P = 0.006$ and $P = 0.02$, respectively). Postpartum concentrations of LBP and SAA were 9.09, 7.76 ± 0.37 µg/mL, and 37.49, 34.57 ± 0.82 µg/mL for d 15, and 30, respectively. In conclusion, cows in HIGH had a lower circulating LBP concentration than cows in LOW prepartum. However, postpartum LBP, and both pre- and postpartum SAA concentrations were not statistically different in both CONT1 and CONT2. Cows in all treatments follow a general increase of LBP and SAA concentrations approaching calving and a decrease of LBP and SAA concentrations postpartum.

Key Words: DCAD, inflammatory, LPS binding protein (LBP)

T47 Impact of intravaginal probiotic treatment prepartum on metritis prevalence and fertility. A. M. L. Madureira*, T. A. Burnett, S. Genís, and R. L. A. Cerri, *University of British Columbia, Vancouver, BC, Canada.*

The aim of this study was to determine the impact of the use of intravaginal probiotics prepartum on the prevalence of metritis postpartum and overall fertility. Holstein cows were enrolled 3 weeks before their expected calving date from 2 farms (Farm A: 322; Farm B:290). Each cow received a 2 mL dose of a combination of 3 lactic acid bacteria, washed with approximately 2 mL of a sterile solution, into the vaginal canal twice weekly until parturition. Metritis diagnoses were carried out at d 6 and d 12 postpartum. Vaginal discharge and rectal temperature were assessed and scored on a scale from 1 to 4, where 1 = clear and 4 = fetid, purulent discharge. Metritis was classified as cows having a vaginal discharge score of 4, with or without the presence of fever, on at least one exam. Cows were bred after a 60d voluntary waiting period primarily via automated activity monitors; cows not found in estrus were enrolled on timed AI protocol to receive 1st breeding before 100DIM. Pregnancy diagnosis was carried out at 31 ± 3 d post-AI. Data analyzed by ANOVA was carried out using the GLIMMIX procedure and survival analysis using the PHREG procedure of SAS. The overall prevalence of metritis was 28.6%. Probiotic treatment reduced the prevalence of metritis; however, there was an interaction with farm (Farm A: Treatment - 18.2 ± 0.04 Control - 28.2 ± 0.03; Farm B: Treatment - 33.5 ± 0.04 Control - 30.0 ± 0.04%; $P = 0.03$). Pregnancy at 1st AI was not affected by the probiotic treatment, however, less metritic cows became pregnant at first AI than non-metritic cows (22.1 ± 0.04 vs 32.2 ± 0.03%; $P = 0.04$). There was an interaction between metritis and treatment on hazard of pregnancy (HP) at 300DIM, where treatment did not affect the HP for non-metritic cows, however, for metritic cows, the HP was in increased for cows on the probiotic treatment (hazard ratio = 1.42; $P = 0.03$). Farm also affected HP, but no interaction between treatment and farm was found. In conclusion, vaginal probiotic treatment has the potential to decrease the prevalence of metrtitis and is associated with increased fertility, however, this seems subjective to farm effects.

Key Words: probiotic, metritis, fertility

T48 Investigation of antibiotic alternatives to improve health and growth of veal calves. J. A. Pempek*, E. M. Holder, K. L. Proudfoot, M. Masterson, and G. G. Habing, *Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.*

Veal calves are inherently susceptible to disease early in life, resulting in frequent antimicrobial use. Recent regulations mandate calves no longer be fed medically important antibiotics continuously in milk or milk replacer. Thus, improvements in antimicrobial stewardship necessitate alternative therapies to improve calf health and growth, while reducing the need for antimicrobials that are important to human health. This study investigated the effect of 2 alternative therapies, lactoferrin (an iron-binding protein found in colostrum) and cinnamaldehyde (an essential oil of the cinnamon plant) on growth, disease incidence, and mortality risk in special-fed veal calves. On the day of arrival to the growing facility (3 to 7 d of age), calves (n = 80 per treatment) were randomized to 1 of 3 treatments: 1) control (no supplement), 2) lactoferrin (1 g/d in milk replacer for 7 d), or 3) cinnamaldehyde (1 g/d in milk replacer for 21 d). Body weight was measured on the day of arrival (d 0), 21, and 42 d post-arrival. Health assessments were performed twice weekly through 21 d, and mortality records were obtained through 6 wk post-arrival. A repeated measures ANOVA was used to compare growth between treatment groups, and a poisson regression model (PROC GENMOD, SAS) was used to test differences between groups in the frequency of diarrhea (fecal score ≥2 with and without depression and temperature) and disease through 3 wk post-arrival. Body weight and average daily gain were similar ($P > 0.05$) between treatments. Neither lactoferrin nor cinnamaldehyde had an effect on diarrhea incidence. However, the risk of navel inflammation was lower for calves that received cinnamaldehyde compared with calves in the control group (RR: 0.68; 95% CI: 0.47–0.99; $P = 0.04$). Mortality through 6 wk post-arrival was low, with 4, 1, and 0 deaths from control, lactoferrin, and cinnamaldehyde treatment groups, respectively. Additional research is needed to investigate various doses

of these alternative therapies on calf health and growth, in addition to different routes of administration.

Key Words: calf diarrhea, lactoferrin, cinnamaldehyde

T49 A multi-site randomized field trial to evaluate the influence of lactoferrin on health of dairy calves with diarrhea. J. A. Pempek*, L. R. Watkins, C. E. Bruner, and G. G. Habing, *Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.*

Neonatal calf diarrhea remains the most common cause of mortality and antimicrobial use in dairy calves. Reduced overall antimicrobial use necessitates research on viable alternative therapies for calf diarrhea. Lactoferrin, an iron-binding protein found in colostrum, has been shown to improve growth and reduce mortality in pre-weaned heifer calves. The objective of this study was to investigate the effect of lactoferrin on the health of pre-weaned dairy heifer calves with diarrhea. This randomized controlled field trial was conducted on 5 commercial dairy farms in Ohio. In total, 485 calves (≤21 d of age) were enrolled after first diagnosis of diarrhea (fecal score ≥3 defined as loose to watery), and randomly assigned to receive an oral dose of lactoferrin (3 g lactoferrin powder dissolved in 30 mL water) or water (control) once daily for 3 consecutive days. Health assessments were conducted on the day of enrollment and 1, 2, 3, 7, 14, 21, 28, and 35 d post-enrollment. A poisson regression model was used to test differences between treatments in frequency of disease through 35 d post-enrollment. Median calf age at enrollment was 11 d, and ranged from 1 to 21 d of age. On the day of enrollment, 51.3% (123/240) and 52.2% (128/245) of calves in the control and lactoferrin treatment groups, respectively, were diagnosed with severe diarrhea (fecal score = 4). The frequency of diarrhea through 35 d post-enrollment did not differ between control and lactoferrin treatment groups (RR: 1.01, 95% CI: 0.93–1.08; $P = 0.87$). Further, depression (depression score ≥2 defined as moderate to severe depression) was not different for calves supplemented with lactoferrin compared with calves in the control group (RR: 0.85, 95% CI: 0.59–1.22; $P = 0.37$). This study suggests supplementing lactoferrin to dairy calves with diarrhea does not influence the frequency of diarrhea or depression 5 wk post-diagnosis; however, additional analyses of secondary outcomes and future research may uncover longer-term benefits of supplemental lactoferrin on morbidity and mortality in dairy heifer calves.

Key Words: calf diarrhea, lactoferrin, antimicrobial alternatives

T50 Two variants of galectin-8 secretion and expression in bovine whole blood. E. Eluka-Okoludoh*, E. Asiamah, K. Ekwemalor, B. Mulakala, S. Adjei-Fremah, and M. Worku, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Galectins (Gal) are glycan-binding proteins. Galectin-8 modulates inflammatory cell adhesion, migration, proliferation, and apoptosis. The objective of this study was to determine the transcription and secretion of Gal-8 variants in blood from high (H) and low (L) SCC cows. HSCC is an early indicator of mastitis (SCC > 200,000). Clinically healthy Holstein-Friesian cows from the North Carolina A&T State University Dairy Unit were used for this study (n = 10). Whole blood was collected from the jugular vein of 5 HSCC and 5 LSCC cows based on Dairy Herd Index (DHI) records. Plasma was extracted from blood. Total protein concertation was determined using the bicinchoninic acid assay. Gal-8 secretion was determined using a cow Gal-8 specific ELISA. Polymorphnuclear leukocyte (PMNL) were isolated from blood, and total RNA extracted. RNA was reverse transcribed to cDNA using Oligo(dT) primer. Cow specific primers for Gal-8 (LGALS8_1 and LGALS8_2) were designed, using NCBI Primer-Blast and Primer3, for real-time PCR. GAPDH and β-actin were used as internal controls. The Livak method was used to calculate fold change and transcript abundance. ELISA data were analyzed using PROC ANOVA model in SAS version 9.4. The average total plasma protein concentration for LSCC and HSCC was 5,289 ng/mL, and there was no difference between the 2 groups ($P > 0.05$). Gal-8 was secreted in plasma. The average concentration of Gal-8 was higher ($P = 0.003$) in LSCC (2.88 ng/mL) compared with HSCC (1.99 ng/mL) cows. Both variants of LGALS8 were detected in PMNL. LGALS8_1 expression was 3.2 folds higher in LSCC compared with HSCC cows; LGALS8_2 expression was 3.6-fold higher in LSCC compared with HSCC cows. Our results suggest a possible association between SCC levels and Gal-8. Thus, further studies are needed to better understand the role of galectin-8 in dairy cows.

Key Words: galectin, somatic cell, bovine

T51 Impact of *Saccharomyces cerevisiae* fermentation product (SCFP) on oxidative status and immune response of transition dairy cattle. S. E. Sivinski*[1], K. E. Olagaray[1], L. K. Mamedova[1], J. M. McIntosh[1], B. A. Saylor[1], J. E. Shaffer[1], J. A. Sauls[1], I. Yoon[2], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*Diamond V, Cedar Rapids, IA.*

Holstein cows (n = 64) were used to evaluate effects of dietary SCFP (NutriTek, Diamond V, Cedar Rapids, IA) on oxidative status and immune response during the transition period. Control (CON; n = 30) or SCFP (n = 34) TMR were fed from −29 ± 5 to 42 d relative to calving (45% NDF, 14% starch prepartum; 32% NDF, 19% starch postpartum). Blood samples were collected during wk −4, −2, 1, 2, and 5 relative to calving. Oxidative status was evaluated in plasma by retinol and tocopherol concentrations, glutathione peroxidase activity (GPx), and Trolox equivalent antioxidant capacity (TEAC). Innate immune response was evaluated by PMA-stimulated oxidative burst capacity (OBC) and glycogen content of polymorphonuclear cells (neutrophils; PMN) isolated from blood. Ovalbumin was administered with adjuvant on d 7 and 21 postpartum, and adaptive immune response was evaluated by serum anti-ovalbumin IgG content on d 28 and 35. Mixed models were used to assess effects of treatment, time, parity, and all interactions. Treatment did not affect oxidative or immune parameters (all $P > 0.05$). There were no treatment × wk interactions except for plasma tocopherol concentration, which tended to be greater in SCFP cows during wk 2 ($P = 0.06$). There was a tendency for a treatment × parity interaction for anti-ovalbumin IgG concentrations, which tended to be greater in SCFP vs. CON primiparous cows ($P = 0.08$). Relationships among oxidative and immune measures were explored with regression techniques. In general, individual antioxidant measures weakly predicted TEAC ($R^2 < 0.20$), but GPx became a stronger predictor during wk 2 ($R^2 = 0.31$; $P < 0.01$). Blood glucose showed weak but positive associations with PMN glycogen content postpartum ($R^2 = 0.14$; $P \leq 0.001$), but not prepartum ($R^2 < 0.01$). PMN glycogen did not predict OBC at any time point ($R^2 < 0.05$; $P > 0.10$). In this cohort of transition cows with low disease incidence, SCFP generally did not affect oxidative or immune parameters, antioxidant measures weakly predicted TEAC, and PMN function was not related to glycogen content.

Key Words: yeast culture, transition, immunity

T52 Effects of metritis on incidence of postpartum disorders and days in the hospital in Holstein dairy cows. F. S. Lima*[1], A. Vieira-Neto[2], and J. E. Santos[2], [1]*Department of Veterinary Clinical*

Medicine, University of Illinois, Urbana, IL, [2]Department of Animal Sciences, Gainesville, FL.

Objectives were to compare the incidence of disease and days spent in the hospital for cows diagnosed with metritis treated either with ampicillin trihydrate (AMP) or ceftiofur hydrochloride (CEFT) and matched control herdmates without metritis. Holstein cows with fetid, reddish/brownish watery vaginal discharge were diagnosed with metritis. Cows with metritis were blocked by parity and type of metritis (with or without fever) and assigned randomly to receive 11 mg/kg of AMP (n = 259) or 2.2 mg/kg of CEFT (n = 269) daily for 5 d. A cohort of healthy cows (NOMET, n = 268) with the same days postpartum and parity was selected as controls. Postpartum disorders (displacement of abomasum, fever, mastitis, and pneumonia) were monitored for the first 60 d postpartum. Data were analyzed using GLIMMIX procedure of SAS. Cows with metritis tended to have a higher incidence ($P = 0.09$) of postpartum disorders than cows without metritis (41.3% vs. 29.5%). Multiparous cows had a higher incidence ($P < 0.001$) of postpartum disorders than primiparous cows (44.9% vs. 28.9%). The incidence postpartum disorders was greater ($P = 0.01$) in AMP (45.6%) cows than in CEFT (37.2%) and NOMET (29.5%) cows. There were no interactions among treatment and parity. Cows with metritis spent more days in the hospital than ($P < 0.01$) cows without metritis (6.0 vs. 2.7). The number of days in hospital was greater ($P < 0.01$) in AMP (8.6) cows than in CEFT (3.4) and NOMET (2.7) cows. Multiparous cows spent more days in the hospital ($P < 0.01$) than primiparous cows (5.8 vs. 4.1). Among the individual disorders, mastitis was increased in AMP (34.0%) when compared with CEFT (24.5%) and NOMET (19.0%). There was no interaction between treatment and parity. Metritis increased the number of days in the hospital and tended to increase postpartum disorder. Cows treated with AMP had more postpartum disorders, cases of mastitis and a greater number of days in the hospital than CEFT cows.

Key Words: metritis, postpartum disorder, mastitis

T53 Comfort or stress in the housing environment: Effects on milk quality, milk production, and immune function of dairy cattle. M. Borchers*[1], A. Bradtmueller[1], and J. Bewley[2], [1]*University of Kentucky, Lexington, KY,* [2]*CowFocused Housing, Bardstown, KY.*

The purpose of this study was to identify the effects of long-term high comfort, low stress (HC) and low comfort, high stress (LC) housing environments milk quality, milk production, and immune function of Holstein dairy cattle. Thirty healthy, confirmed pregnant, lactating Holstein dairy cattle were selected from the University of Kentucky Dairy Research Herd and assigned to high comfort, low stress (n = 15) or low comfort, high stress (n = 15) environments for 28 d. After 28 d, cows were crossed-over to the alternate housing for an additional 28 d. Milk samples were collected every 7 d for SCC and culture, and milk production data were collected from the AfiLab (Afimilk, S.A.E. Afikim, Kibbutz Afikim, Israel). Blood samples were collected at the study's beginning, midpoint, and end. Ten cows were selected from each sequence (10 HC to LC and 10 LC to HC) and blood samples stimulated with *Escherichia coli* 0111:B4 endotoxin, or left unstimulated as controls. Samples was lysed, RNA extracted, reverse transcribed, and cytokines quantified using PCR (IL-6, IL-8, IL-10, IFN-γ, and TNF-α). Individual cytokines, energy corrected milk, SCS, and subclinical infection prevalence were analyzed as dependent variables using mixed linear models in SAS (SAS Institute; Cary, NC). Housing type (HC or LC), sequence, and baseline values for each predicted variable served as fixed effects in each model. Cytokine models included treatment (endotoxin or unstimulated blood). Cow was a repeated subject in all models. All 2 and 3-way interactions were considered and non-significant ($P \geq 0.05$) interactions removed using stepwise backward elimination. Housing type significantly affected ECM (LSmean ± SE; HC, 35.4 ± 0.4 vs. LC, 31.9 ± 0.9 kg) but not subclinical infection prevalence, SCS, or cytokine activity. Future analysis will include behavioral information from each cow and weather data. Stress in the housing environment did not affect immune function indicators but did decrease milk production.

Key Words: cow health, cow comfort, wellbeing

T54 Blackberry pomace—A novel feed supplement for transition dairy cows. K. Swanson*, S. Akers, K. Estenson, R. Wilson, M. Keller, and G. Bobe, *Oregon State University, Corvallis, OR.*

Blackberry pomace (BBP) is a by-product generated from juice processing that contains skins, pulp, and seeds of the original fruit. Currently, BBP is disposed in landfills. As alternative, we propose BBP as a novel antioxidant-rich feed supplement for dairy cows during the transition period. Thus, the objective of this study was to evaluate BBP as antioxidant-rich feed supplement in transition dairy cows. Using a randomized block design, 24 multiparous dairy cows (blocked by breed) were given 0 (Control), 57 (Low BBP), or 114 g/d (High BBP) of dried BBP as a top dressing to their fed total mixed ration (TMR). Supplementation started 28 d before predicted calving date and ended 28 d post calving. Blood and milk samples were collected on d 0, 1, 3, 7, 14, 21, and 28 postpartum. Besides markers of metabolism, inflammation, and antioxidant status, we measured at d 7, 14, 21, and 28 postpartum serum concentrations of estradiol and progesterone as indicators of follicular and luteal activity, respectively. In addition, we recorded reproductive events after calving. Reproductive data were analyzed using PROC MIXED, PROC GLIMMIX, and PROC LIFEREG in SAS version 9.4. Fixed effects were breed (Holstein, Jersey) and supplementation (Control, Low BBP, High BBP). For repeated-measures, the variance-covariance matrix was modeled using heterogeneous compound symmetry. High BBP cows showed first heat 12 d earlier ($P = 0.02$), were first bred 15 d earlier ($P = 0.04$), and were open for 73 d less ($P = 0.002$) than Control cows, as more cows tended to be confirmed pregnant from their first breeding ($P = 0.06$). Moreover, High BB cows resumed earlier follicular activity than Control cows, as Control cows did not reach serum estradiol concentrations similar to those of High BBP cows at d 7 until d 28 (treatment x time effect: $P = 0.08$). Furthermore, High BBP cows tended to have higher serum progesterone concentrations than Control cows ($P = 0.08$), as High BBP cows reached serum progesterone concentrations indicative of luteal activity at d 21, one week ahead of Control cows. Our results suggest that supplementation with 112 g/d of BBP may accelerate resumption of ovarian activity and improve reproductive performance of dairy cows.

Key Words: blackberry pomace, reproduction, transition cow

T55 Phenotypic relationship between body weight changes and milk composition in dairy cows. A. Fleming*[1], F. Miglior[1,2], and C. Baes[1], [1]*CGIL, University of Guelph, Guelph, ON, Canada,* [2]*Canadian Dairy Network, Guelph, ON, Canada.*

In the initial stage of lactation, dairy cattle experience negative energy balance, as the energy expenditure of milk production exceeds the amount of energy the cow is able to take up through eating. In this state, there is increased body fat mobilization by the cow, with consequences in milk composition, particularly in fatty acid profile and possibly increased ketone bodies in both blood and milk. On-farm technologies allow novel monitoring techniques and increase the overall amount of data available to producers and researchers. The objective of this study is to analyze the pallet of automatically collected data sources currently recorded at the University of Guelph's Livestock Research and Innovation Centre (LRIC) and relate them to changes in milk composition. The automatically collected data sources currently recorded at LRIC include, but are not limited to, automatically recorded cow weights via a walkover parlour scale, automatic body condition scoring via 3-dimensional imaging, as well as individual feed intake events. Moreover, milk testing is performed weekly, with the mid-infrared spectral data also obtained for every test. Major milk fatty acid groups were predicted using previously developed calibration equations, and predicted milk beta-hydroxybutyrate contents also obtained. Changes in cow body weight in the initial stage of lactation were examined, along with milk composition. The results of this study improve the understanding of the phenotypic relationship between body weight changes and milk composition in early-lactation dairy cattle. Furthermore, we explore phenotypes associated with energy balance with implications for cow health and future genetic selection strategies.

Key Words: milk composition, body weight

T56 Preliminary exploration of the relationship between automated rumen sensor data and feed intake in lactating dairy cows. C. J. Siberski*, M. R. O'Neil, J. E. Koltes, and H. A. Ramirez-Ramirez, *Iowa State University, Ames, IA.*

Feed efficiency is an economically important trait in the dairy industry as feed costs account for a large proportion of cost of production. Unfortunately, technologies to measure feed intake are cost prohibitive for use on commercial dairies. Thus, identification of portable technologies that collect measurements as indicators of feed intake or efficiency could be of great value to the industry. The objective of the current study was to explore the use of automated rumen sensor data as possible indicators of feed intake in lactating dairy cattle between 40 and 80 DIM. Wireless ruminal monitors (smaXtec Animal Care. Wastiangasse, Austria) were used to collect pH and temperature every 10 min on 17 cows. pH probes were delivered by mouth on the first day of lactation and began automated data collection at that point. Daily feed intake and weekly body weights were also recorded. Data were filtered in an attempt to remove faulty readings based on constant decline in pH readings overtime (drift), resulting in a total of 14 cows remaining in the data set. Hourly mean pH and temperature values were calculated for 40 to 80 DIM on each cow. Data were analyzed using PROC MIXED in SAS where feed intake was modeled including fixed effects for rumen pH or temperature, and metabolic body weight (MBW) as well as random effects of animal and time. The MBW was calculated as body weight taken to the 0.75 power ($BW^{0.75}$). Rumen temperature was significantly associated with feed intake ($P < 0.016$), while there was a slight tendency for an association of rumen pH ($P < 0.13$). In both models, MBW was significantly associated with feed intake ($P < 0.0001$). Further analyses are warranted to determine the relationship of these rumen traits to milk production and composition, and other measures of efficiency. Additional studies are merited to validate these findings to determine if either of these novel traits could be a suitable indicators of feed efficiency.

T58 Inbreeding depression in a Hereford beef cattle population using the pedigree and genomic information. P. Sumreddee*[1], S. Toghiani[1], E. H. Hay[2], S. E. Aggrey[1], and R. Rekaya[1], [1]*University of Georgia, Athens, GA,* [2]*USDA/ARS/LARRL, Miles City, MT.*

Increase in inbreeding leads to loss in fitness and reduction in production traits. Although its effects are clear, measuring inbreeding is often complicated by the lack of complete and error free pedigrees. Availability of high-density marker panels provide an alternative tool to assess inbreeding. Additionally, inbreeding depression seems to be affected not only by the animal level of inbreeding but also by its parent's inbreeding. The aim of this study was to determine the effects of the animal and its parent level of inbreeding on growth and female fertility in a small population of Hereford cattle. Inbreeding levels were assessed based on pedigree (Fped), genomic relationship matrix (FGRM), and runs of homozygosity (FROH). The pedigree consisted of 10,476 animals spanning over 48 generations. Genomic-based inbreeding was evaluated using 788 animals genotyped for 30,810 single nucleotide polymorphisms (SNPs). Traits analyzed included growth (birth weight, weaning weight, yearling weight, and average daily gain (ADG, kg/day) and female fertility traits (age at first calving and heifer pregnancy status). The phenotypic data consisted of records on growth and fertility traits on 3,995 animals. Linear regression analyses were used to assess the effects of inbreeding. The percentage average inbreeding was 29.01 (SD = 5.75), 14.80 (SD = 10.53) and 22.91 (SD = 5.14) for Fped, FGRM, and FROH, respectively. This high inbreeding was expected given the small size and the level of isolation of the population used in this study. An increase of 1% in animal inbreeding resulted in a decrease of 107, 36, and 110 kg in yearling weight for Fped, FGRM, and FROH, respectively. Similarly, ADG was reduced by 0.19 to 0.60 kg. Inbreeding increased significantly the age at first calving but not the pregnancy status. Joint estimates of animal (FA) and its sire's (FS) or dam's (FD) inbreeding effects showed a clear effect of FD on the progeny performance. The sire inbreeding has limited to no effects. The high inbreeding level of the studied population seems to have affected both production and fertility traits.

Key Words: genomic relationship, inbreeding depression, runs of homozygosity

T59 Diet digestibility measured from fecal samples and associations with phenotypic and genetic merit for milk yield and composition. E. K. Panunzi[1,2], K. J. Harvatine[1], Y. Ying[1], and C. D. Dechow*[1], [1]*Pennsylvania State University, University Park, PA,* [2]*University of Pennsylvania, Philadelphia, PA.*

Dry-matter intake is costly to obtain and may not provide insight into mechanisms of feed efficiency. Our objectives were to evaluate feed digestibility via fecal sampling and determine associations with phenotypic and genetic merit of milk yield and composition. Fecal samples were obtained from 115 Holsteins cows in March 2012 with 48

resampled in August. Samples were dried, ground to 1 mm, and placed in Ankom filter bags to determine % neutral detergent fiber (NDF%) or % indigestible NDF (iNDF%) for morning (n = 32 in March; n = 48 in August), afternoon (n = 18; March), evening (n = 16; March), and composite (n = 86; March) sample periods. Sample disappearance following placement into a cannulated rumen for 12 d determined iNDF%. An Ankom A200 analyzer was used to determine fecal NDF%; TMR samples were evaluated using the same procedures. Total-tract DM digestibility (TTDM%) was calculated as 1 - (TMR iNDF%/Feces iNDF%); total-tract NDF digestibility (TTNDF%) was calculated as 1 – [(TMR iNDF% × Feces NDF%)/(Feces iNDF% × TMR NDF%)]. TTDM% and TTNDF% were merged with phenotypes and estimated breeding values for milk yield, fat yield and %, and protein yield and %. The average composite TTDM% and TTNDF% in March was 65.8% ± 2.4 and 42.9% ± 2.7, respectively. Correlations of TTNDF% among sample periods were significant ($P < 0.05$), ranging from 0.56 to 0.92; correlations among sample periods for TTDM% ranged from 0.30 (not significant) to 0.94. August TTDM% (59.3% ± 4.4) and TTNDF% (38.4% ± 4.0) were lower and August TTNDF% was significantly correlated with March morning (0.72). Significant and unfavorable correlations of TTNDF% with milk (−0.27) and protein (−0.30) yields were observed, whereas higher milk-fat % was positively correlated with TTDM% and TTNDF% (0.26 and 0.44, respectively). Likewise, genetic merit for higher milk (−0.34) and protein (−0.30) yield was unfavorably correlated with TTNDF%, and increased genetic merit for milk-fat % was associated with higher TTNDF% (0.39) and TTDM% (0.27). Fecal sampling could provide a cost effective indicator of feed efficiency and higher milk-fat % was associated with improved digestibility.

Key Words: feed efficiency, feces, digestibility

T60 Development of AFLP breed identification markers for Pakistani Cholistani cattle. M. Moaeen-ud-Din* and G. Bilal, *Laboratories of Animal Breeding and Genetics, Faculty of Veterinary and Animal Sciences, PMAS Arid Agriculture University, Rawalpindi, Rawalpindi, Punjab, Pakistan.*

It is important to identify livestock breeds for breeding programs. The identification issue of livestock can be resolved by using molecular identification tools that acceptable to preserve and maintain pure breeds worldwide. The application of molecular identification methodology is more important for developing nations such as Pakistan, where uncontrolled crossbreeding has become a common practice and import of exotic animals and germplasm is ever increasing. This is always keeping local breeds at risk that is also an FAO concern. Therefore, the current study was designed to develop standard molecular markers for Cholistani cattle to ascertain their purity for breeding purpose. In this study, 50 and 48 unrelated males were sampled for each Cholistani and Crossbred cattle respectively. Candidate molecular markers present in Cholistani but absent in Crossbred and vice versa were detected using amplified fragment length polymorphism method. Eleven markers were developed and were converted to single nucleotide polymorphism markers for high throughput genotyping. The allele frequencies in both breeds were determined for discrimination ability using PCR-AFLP. The probability of identifying Cholistani breed was 0.981 and probability of misjudgment was 0.313 using single selected markers. However, probability for judgment and misjudgment with 2 markers and combined with 3 markers was 0.740 and 0.362 and 0.995 and 0.562, respectively. The identified markers can ascertain breed identification and facilitate the breed improvement program through genetic selection in the country.

Key Words: molecular marker, breed identification, Cholistani

T61 Body weight and body condition score variations in Romane ewes: Intraflock variability in their dynamics and magnitude over multiple production cycles. T. Macé[1], D. Hazard[2], F. Carrière[2], S. Douls[2], C. Robert-Granié[1], and E. González-García*[3], [1]*GENPHYSE UMR1388, Université de Toulouse, INRA, ENVT, Castanet-Tolosan, France,* [2]*INRA La Fage UE321, Roquefort-sur-Soulzon, France,* [3]*INRA SELMET (Systèmes d'Élevage Méditerranéens et Tropicaux), Montpellier, France.*

Breeding for resilience requires a better understanding of intra-flock variability and the related mechanisms responsible for robustness traits. Among such traits, the animals' ability to cope with feed fluctuations by mobilizing or restoring body reserves (BR) is a key mechanism in ruminants. The objective of this work was to characterize individual variability in BR dynamics in productive Romane ewes reared in extensive conditions. The BR dynamics profiles were characterized by combining individual longitudinal measurements of BW and BCS over several production cycles. Historical data, including up to 2,628 records per trait distributed in 1,146 ewes, underwent cluster analysis. Two to 4 trajectories were observed for BW depending on the cycle, while 3 trajectories were always found for BCS, whatever the cycle. Most trajectories suggested that BR dynamics were similar but that the level of BR differed between ewes. Nevertheless, some trajectories suggested that both BR dynamics and levels were different for a proportion of ewes. The distribution of the ewes between the trajectories was explained by the ewe's age, litter size, and individual variability. Many individual trajectories remained the same throughout a ewe's life, whatever the age, parity, or litter size. Our results demonstrate the relevance of using BW and BCS for characterizing the diversity of BR mobilization-accretion profiles in sheep in a long timespan perspective. This is probably the first report of proven intra-flock variability in the adaptive capacities of grazing sheep. Nevertheless, further research efforts are needed to complete the characterization of BR dynamics by including other physiological (e.g., a set of plasma metabolites and hormones) and zootechnical parameters to obtain new insights into the complex mechanisms determining individual robustness.

T304 Genome-wide association study on health and reproductive traits in US Holstein cattle. F. Brito*[1], G. Rosa[1], P. Pinedo[2], J. Santos[3], G. Schuenemann[4], R. Bicalho[5], R. Chebel[3], K. Galvao[3], R. Gilbert[5,9], S. Rodriguez-Zas[6], C. Seabury[7], J. Fetrow[8], and W. Thatcher[3], [1]*University of Wisconsin, Madison, WI,* [2]*Colorado State University, Fort Collins, CO,* [3]*University of Florida, Gainesville, FL,* [4]*The Ohio State University, Columbus, OH,* [5]*Cornell University, Ithaca, NY* [6]*University of Illinois, Urbana-Champaign, IL,* [7]*Texas A&M University, College Station, TX,* [8]*University of Minnesota, Saint Paul, MN,* [9]*Ross University, Basseterre, St. Kitts and Nevis, West Indies.*

This study was carried out to identify genomic regions associated with health and reproductive traits in Holstein cows, including lameness (LS), metritis (MET), mastitis (MAT), resumption of cyclicity (CY), pregnancy 60 d after first AI (P60), and a reproductive index (IndexP60). Phenotypic information was collected on 11,733 cows calving during warm and cool seasons in 16 farms located in 4 US regions: Northeast (4 herds), Midwest (6 herds), Southeast (1 herd), and the Southwest (5 herds). From those, 2,505 cows were selectively genotyped using the Illumina Bovine HD SNP Chip. Genotypes were edited to remove SNP located on sex and mitochondrial chromosomes, unknown map position, call rate <0.95, minor allele frequency <0.01, and deviation from HWE ($P < 10^{-10}$), resulting on 592,788 SNPs retained for further analyses. GBLUP and genome-wide association studies (GWAS) were

Table 1 (Abstr. T304). Estimates of heritability (h^2), number of significant SNP hits (*P*-value < 1e-5) and genes located within 20 kb upstream and downstream of the SNP

Trait	h^2 (SE)	No. of hits	Genes
Lameness at 40 DIM	0.030 (0.048)	21	RIMS2, U6
Metritis	0.063 (0.026)	3	FHIT, OPCML
Mastitis	0.047 (0.042)	1	—
Resumption of cyclicity	0.070 (0.026)	5	STAC
Pregnancy 60 d after first AI	0.110 (0.024)	49	ABI3BP, FOXJ3, GC-201, NBEA, NPFFR2, SLC7A8, and TRIM71
Reproductive index	0.126 (0.029)	38	FOXJ3, GC, NPFFR2, and TRIM71

performed using a linear model for IndexP60 and logistic models for the other traits. The AI-REML algorithm was used to estimate variance components, and marker effects were evaluated using the *score* test. Although heritability estimates were low for all traits, some interesting peaks were found in the GWAS analyses (Table 1). The GWAS analyses indicated that 16 genes on bovine chromosomes 1, 3, 6, 10, 11, 12, 14, 15, 22, and 29 were associated with the traits of interest. From those, chromosomes 3 and 6 presented common regions for P60 and IndexP60, and chromosome 22 presented common regions for CY, LS, P60 and IndexP60. The chromosomal regions identified have confirmed several previous findings that may contribute to variation in fertility and health traits in dairy cattle.

Key Words: dairy cattle, fertility, health traits

T305 Genetic and functional relationships among reproductive traits in US Holstein cows. F. Brito*[1], G. Rosa[1], P. Pinedo[2], J. Santos[3], G. Schuenemann[4], R. Bicalho[5], R. Chebel[3], K. Galvao[3], R. Gilbert[5,9], S. Rodriguez-Zas[6], C. Seabury[7], J. Fetrow[8], and W. Thatcher[3], [1]*University of Wisconsin, Madison, WI,* [2]*Colorado State University, Fort Collins, CO,* [3]*University of Florida, Gainesville, FL,* [4]*The Ohio State University, Columbus, OH,* [5]*Cornell University, Ithaca, NY,* [6]*University of Illinois, Urbana-Champaign, IL,* [7]*Texas A&M University, College Station, TX,* [8]*University of Minnesota, Saint Paul, MN,* [9]*Ross University, Basseterre, St. Kitts and Nevis, West Indies.*

Knowledge of causal relationships among phenotypic traits in dairy cattle can contribute to more efficient genetic selection and management decisions. Thus, this research investigated genetic and functional relationships among retained fetal membranes (RP), metritis (MET), clinical endometritis (CE), resumption of cyclicity (CY), pregnancy on d 60 after first AI (P60), and lameness (LS). The data set comprised information on 11,733 Holstein cows from 16 farms located in 4 regions of the US: Northeast (4 herds), Midwest (6 herds), Southeast (1 herd), and Southwest (5 herds). A directed acyclic graph (DAG) describing causal relationships between the traits was inferred using a Bayesian implementation of the Inductive Causation (IC) algorithm. Structural equation models (SEM) were subsequently fitted conditionally on the inferred DAG. Estimates of direct heritability for P60, RP, MET, LS, CE and CY were 0.20 ± 0.07, 0.09 ± 0.02, 0.10 ± 0.04, 0.15 ± 0.03, 0.10 ± 0.03 and 0.35 ± 0.04, respectively, with estimates of genetic correlation among these traits ranging from −0.82 ± 0.07 (MET and CY) to 0.70 ± 0.13 (RP and MET). The IC algorithm retrieved 6 directed relationships: RP- > MET, RP- > CE, LS- > CE, CE- > P60, CY- > P60 and CY- > CE. Except for the directed edge CE- > LS, the signs of the causal relationships were all on the same direction (i.e., same sign) of the corresponding genetic correlations. The inferred DAG indicates that RP and LS are key variables upstream the causal network affecting P60. As such, they should be considered target traits to improve reproductive performance in dairy cattle. The network indicates also that CE is an intermediate variable through which RP and LS affect P60. The moderate magnitude of heritability estimate for P60 suggests that genetic improvement can be obtained through direct selection on P60. Moreover, genetic progress for P60 can be aided by indirect selection on intermediate phenotypes in the network. The causal network provides also information on potential targets for management interventions to improve P60 in dairy herds, such as RP, LS, CE and CY.

Key Words: causal inference, dairy cattle, reproductive performance

T62 Rheological and wear behaviors of full-fat cheese. F. Z. B. Seighalani* and H. Joyner, *School of Food Science, University of Idaho, Moscow, ID.*

Determination of food rheological and wear behaviors is important for understanding both processing behaviors and texture attributes. Information on cheese wear rates and wear patterns, which are interrelated to textural properties, may be applicable to slicing and shredding operations in cheese industries, as different wear behavior could relate to ease of cheese slicing and shredding. The objective of this study was to determine the rheological and wear behavior of full-fat cheeses with 52% and 54% fat content. Wear measurements were performed at different sliding speeds (30, 50 and 70 mm/s) and temperatures (5, 15, and 25°C) at a normal force of 0.5 N. Large amplitude oscillatory shear and strain sweep tests were used to measure the rheological properties of the cheeses. Wear track depth significantly increased at higher sliding speeds and higher temperatures in both cheeses. However, the cheese at 54% fat content showed a significantly lower mass loss compared with the cheese at 52% fat content. Cheeses with higher fat content had greater storage and loss modulus values under small strains and greater extent of nonlinear viscoelastic behavior under large strains. Lissajous curves showed that the cheeses had greater deformation at higher strain amplitudes and frequencies. In general, cheeses with higher viscoelastic moduli had lower wear rate. The results of this study provided a more fundamental understanding of the effect of fat content on relationships between cheese rheological and wear behaviors.

Key Words: cheese, rheology, wear behavior

T63 Effects of composition, processing, and recovery of buffalo milk solids on the yield of mozzarella cheese. D. C. Sales[1], A. H. N. Rangel*[1], L. H. F. Borba[1], S. A. Urbano[1], A. F. Brito[3], J. G. B. Galvão Jr.[2], H. Tonhati[5], E. G. Silva[1], A. R. Freitas[4], and D. M. Lima Jr.[6], [1]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [2]*Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil,* [3]*University of New Hampshire, Durham, NH,* [4]*Empresa Brasileira de Pesquisa Agropecuária, São Carlos, SP, Brazil,* [5]*Universidade Estadual Paulista Julio de Mesquita Filho, Jaboticabal, SP, Brazil,* [6]*Universidade Federal de Alagoas, Arapiraca, AL, Brazil.*

The aim of this study was to identify the effects of buffalo milk composition, processing factors, and recovery of whey (RW) constituents on the mozzarella cheese yield (MCY). For this study, we used 30 lots of mozzarella from a milk processor in Taipu, Rio Grande do Norte, Brazil. For each batch, 01 samples of raw milk and whey were collected for analysis of constituents by infrared spectroscopy. Milk somatic cells count was estimated by SOMATICELL and log-transformed to yield SCS. MCY (%) was calculated by the ratio between cheese (kg)/milk (kg) mass. RW was calculated by the ratio between whey constituents relative to milk constituents. The relationship between different variables and MCY was evaluated using a polynomial regression model at a significance level of 5%. Adjusted R^2 was used for model selection. All regression coefficients associated with each explanatory variable were significantly different from zero (Table 1). The models explained on average 99% of the variation in the data set. In conclusion, the regression models showed that the concentration of milk components, SCS, age and acidity of the starter culture, time between the curd cuts, and percentage of lost whey constituents affected the yield and manufacturing efficiency of mozzarella cheese.

Key Words: buffalo milk, processing, somatic cell count

T64 Sensory acceptance of Coalho cheese from Zebu cow milk. I. L. S. Oliveira[1], A. H. N. Rangel*[1], R. C. Madruga[3], M. F. Bezerra[1], Y. M. O. Silva[1], R. D. S. Gomes[1], J. S. Bezerra[1], E. O. Moura[1], L. F. C. Trindade[1], and J. G. B. Galvão Jr.[2], [1]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [2]*Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil,* [3]*Associação Brasileira dos Criadores de Zebu, Parnamirim, RN, Brazil.*

The aim of this study was to evaluate the sensorial acceptance of Coalho cheese produced from milk of Gir (CCGI), Guzerá (CCGU) and Sindi (CCSI) cow breeds throughout 46 d of shelf life under refrigeration at 5°C. The appearance, aroma, texture and taste attributes of the cheeses were evaluated according to a 9-point hedonic scale ranging from 1

Table 1 (Abstr. T63). Coefficients of estimated polynomial regression equations, without intercept

Variable	X	*P*-value	X^2	*P*-value	X^3	*P*-value	R^2_{adj}
Fat, %	1.8950	<0.0001	– 0.1796	0.009			0.9950
Total protein, %			0.8620	<0.0001	– 0.1379	0.0490	0.9952
Casein, %			1.4176	<0.0001	– 0.2910	<0.0001	0.9952
Lactose, %			0.6229	<0.0034	– 0.0854	<0.0323	0.9950
Total solids, %			0.0530	<0.0006	– 0.0021	<0.0174	0.9949
Solids-non-fat, %			0.1156	<0.0019	0.1156	<0.0504	0.9951
SCS	11.477	<0.0001	– 6.5964	<0.0001			0.9952
Starter culture acidity, °D	0.1142	<0.0001	– 0.00064	<0.0001			0.9931
Starter culture age, h	0.4330	<0.0001	– 0.0111	<0.0001	0.00008	<0.0001	0.9888
Time between curd cuts, min	0.1971	<0.0001	– 0.0019	<0.0001			0.9929
Fat RW, %	2.4973	<0.0001	0.4035	<0.0001	0.0206	<0.0001	0.9950
Casein RW, %	0.3575	<0.0001	– 0.0063	<0.0001			0.9950
Lactose RW, %	0.0952	<0.0001	– 0.00046	<0.0134			0.9951
Total solids RW, %	0.2109	<0.0001	– 0.0023	<0.0080			0.9950

(Disliked extremely) to 9 (Liked extremely), by 60 untrained testers on the first, 25th and 46th days of storage. Data were submitted to ANOVA using the Statistica 7.0 program and the differences between the means were evaluated by the Tukey test at a significance level of 5%. The results showed that the sensory scores ranged from 6.32 (Liked slightly) to 7.85 (Liked moderately). The Coalho cheeses from milk of different cow breeds presented similar appearance throughout the storage period. The aroma on the first day of storage obtained lower sensory scores (Liked slightly) possibly due to volatile compounds which develop over time. Regarding the texture, CCGU and CCSI reached similar sensorial acceptance ($P > 0.05$) throughout the evaluation period, while the CCGI received a lower score (7.05) at 46 d of storage ($P < 0.05$) than on the first day (7.85). For the flavor attribute, the cheeses received better ($P < 0.05$) sensory scores (Liked moderately) on the first day of shelf life. In comparing the respective storage periods, we observed that the cheeses obtained from the milk of the different breeds studied are similar in appearance, aroma, and flavor ($P > 0.05$), while only the texture of the CCGU (7.71) differed ($P < 0.05$) from the CCSI (6.91) at 46 d of storage. The results found indicate that the storage time of Coalho cheeses prepared with cow milk from Gir, Guzerá, and Sindi breeds reduced consumer acceptance of flavor, but the sensorial characteristics were pleasant for consumption until 46 d of shelf life.

Key Words: sensorial analysis, shelf life, storage

T65 Sensorial acceptance of pasteurized fluid milk and Coalho cheese from milk with two levels of somatic cell counts. J. S. Bezerra[1], A. H. N. Rangel*[1], L. Murmann[1], J. G. B. Galvão Jr.[2], E. P. E. Silva[1], Y. M. O. Silva[1], C. S. Macedo[1], A. L. Vasconcelos[1], R. D. S. Gomes[1], and L. H. F. Borba[1], [1]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [2]*Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Norte, Ipanguaçu, RN, Brazil.*

The adverse effects from high somatic cell count (SCC) in milk include changes in the sensory quality of dairy products. In this sense, the objective of this study was to evaluate the sensorial characteristics of pasteurized bovine milk and Coalho cheese produced from milk with 2 levels of SCC. Two batches of milk were selected: low SCC (<100,000 cells/mL) and high SCC (400,000 cells/mL < SCC <500,000 cells/mL). Milk and cheese samples were evaluated by the triangle test, the acceptance test using a 9-point hedonic scale ranging from 1 (Disliked extremely) to 9 (Liked extremely), and by the consumption index. The sensorial tests were performed by 100 untrained volunteers. The evaluated attributes were appearance, aroma, flavor, texture, and overall acceptance. The triangle test was measured by counting the correct answers and the statistical difference was based on the chi-squared test. The acceptance test was submitted to ANOVA at 5% of significance. For pasteurized milk, the triangle test indicated a significant difference ($P < 0.001$), and all analyzed attributes presented a statistically significant difference ($P < 0.05$) on the hedonic scale except for appearance. Statistical difference ($P < 0.05$) was observed in relation to intent to consume, in which the evaluators reported that they would "often drink" (5.26) the milk with low SCC, while they would "occasionally drink" (4.58) the milk with high SCC. Regarding the Coalho cheese, a significant statistical difference ($P < 0.001$) was evidenced for the triangle test, in which 87 evaluators correctly chose the different sample. The Coalho cheese obtained from milk with low SCC had a higher preference in relation to that of high SCC ($P < 0.05$). The flavor attribute presented the greatest difference between the samples with mean scores ranging between 6 (Liked slightly) and 7 (Liked moderately). Regarding the intent to consume, there was greater availability for consumers to acquire Coalho cheese made from low SCC milk observed. Therefore, pasteurized milk and Coalho cheese made from milk with low SCC had a better sensorial acceptance.

Key Words: hedonic scale, triangle test, quality

T66 Sodium analysis equivalency of dry ashing and microwave assisted digestion of mozzarella cheese. D. Grossbier* and T. Schoenfuss, *University of Minnesota, Minneapolis, MN.*

Mineral analysis of dairy products is a labor and time-intensive process. Pretreatment using combustion or atmospheric wet ashing can take in excess of 24 h to complete. Twenty-four samples of low moisture part skim mozzarella (LMPS) cheese were digested by both a microwave accelerated digestor (Mars 6, CEM, Matthews, NC), and the dry ashing standard method. The analysis was done in duplicate. Sodium was quantified on a Perkin-Elmer AAnalyst 100 (Perkin-Elmer Inc., Waltham, MA) atomic absorption spectrometer. Dry ashing sodium were 450 to 908 mg/100g which encompass the range of commercial samples found in a previous study, including reduced sodium LMPS. A bias was observed with the quantification of microwave digested samples, with a mean of 14 mg/100g higher in sodium. The International Dairy Federation method for sodium in dairy products proposes that the standard deviation of reproducibility for freeze-dried cheese is 70 mg/100g. A 2 one-sided test (TOST) analysis showed that the confidence intervals were within the acceptable differences for standard method variation ($P = 0.029$). Based on this analysis, we conclude that the methods are equivalent. Microwave accelerated digestion substantially reduces analysis time and can be completed in 2 h.

Key Words: mozzarella, sodium, cheese

T67 Influence of using different proportions of cow and goat milk on the properties of Chanco cheese. R. A. Ibáñez*[1], S. Wyhmeister[1], C. Geldsetzer-Mendoza[1], M. Medel-Marabolí[2], M. A. Fellenberg[1], and E. Vargas-Bello-Pérez[3], [1]*Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Universidad de Chile, Santiago, Chile,* [3]*University of Copenhagen, Copenhagen, Denmark.*

Chanco cheese is a variety produced in Chile from cow milk and made by stirred-washed curd processing with a ripening period of 45 d. It has a semi-soft consistency, creamy body and contains several small eyeholes. In recent years, the manufacture of traditional cheese varieties using a mixture of cow and goat milk has gained popularity in the dairy industry, because consumers are willing to purchase and taste products with diverse appearance, flavor and texture development. In this context, differences are attributed to the composition of raw materials. Goat milk, when compared with cow milk, has no β-carotene, it contains increased levels of medium chain fatty acids (C6:0, C8:0 and C10:0), and has lower α_{S1}:β-casein (CN) ratio, contributing to cheeses with reduced yellowness, typical goat flavor, and brittle texture, respectively. To our knowledge, there is no information if the quality of Chanco cheese is affected by using this alternative technique. This study aimed to evaluate the effect of increasing the proportion of goat milk on the composition, texture and sensory properties of Chanco cheese. Cheesemilks were blended to 4 different cow:goat milk ratios (100:0, 67:33, 33:67 and 0:100), maintaining a protein-to-fat ratio of 0.9:1.0. These treatments (20-kg scale) were subjected to a stirred-curd, direct salted cheese-making protocol and ripened for 45 d at 12°C. Cheeses varying in the proportion of cow and goat milk had similar composition, pH values and primary proteolysis ($P > 0.05$) among treatments. However, an increase in the proportion of goat milk led to cheeses with reduced levels and degradation extent of α_{S1}-CN ($P < 0.05$), influencing on a weaker texture ($P < 0.05$) when analyzed by uniaxial compression test;

increased whiteness and reduced yellowness ($P < 0.05$), when CIELAB color was measured; and higher levels of medium chain fatty acids ($P < 0.05$). Sensory analysis also indicated that increasing levels of goat milk led to cheeses with whiter appearance and goat notes ($P < 0.05$). These results suggest that blending goat into cow milk leads to Chanco cheeses with modified appearance, texture, and flavor.

Key Words: Chanco cheese, cow milk, goat milk

T68 Textural characteristics of caprine milk Cheddar cheeses supplemented with microencapsulated and regular ferrous sulfate. A. Siddique*, R. Paswan, and Y. W. Park, *Fort Valley State University, Fort Valley, GA.*

Iron deficiency anemia is a widespread epidemic around the world, whereby dietary supplementation with Fe has been recommended. Iron fortification on qualities of bovine milk and its dairy products have been extensively studied, while such reports on caprine milk counterparts are almost non-existent. The objective of this study was to compare textural characteristics of non-fortified caprine control cheese (CC) with those of 2 types of iron-fortified caprine milk cheeses. Three batches of 3 types of caprine milk Cheddar cheeses [CC, regular ferrous sulfate (RFS) and large microencapsulated ferrous sulfate (LMFS) fortified] were manufactured at the Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, GA. For each batch, the cheeses were subdivided and made in 3 subgroups as CC, RFS and LMFS cheeses, vacuum packed in 3" × 5" plastic pouches and stored at 4 and −18°C for 0, 2 and 4 mo. Iron salts were fortified during milling by adding 8.23 g RFS and 9.03g LMFS per 9 kg cheese, respectively, resulting in 16% Fe in both Fe added cheeses. Textural characteristics of all cheese samples were determined using a texture analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY). Results showed that all textural properties of RFS and LMFS fortified cheeses were higher than those of CC cheese. LMFS fortified cheeses showed highest textural values (g force: gram force for crushing samples) among 3 cheeses, showing the values of hardness (5044), springiness (80.3), gumminess (3208), chewiness (3816). The hardness, resilience, cohesiveness, gumminess and chewiness except cohesiveness were significantly ($P < 0.01$) affected by cheese type. Storage period had significant effect on hardness ($P < 0.01$) and springiness ($P < 0.05$). The 2-way interaction of cheese type × storage period showed significant influence on hardness, springiness, gumminess, and chewiness properties. It was concluded that Fe fortification in caprine cheeses elevated springiness, chewiness and gumminess compared with control cheeses, where these properties had inverse relationships with hardness of the caprine milk cheeses.

Key Words: caprine cheese, textural property, iron fortification

T69 Volume of whey expelled and whey composition as influence by salting, wet and dry season. O. D. Oshibanjo*, K. L. Kazeem, and D. O. Akintola, *University of Ibadan, Ibadan, Oyo Nigeria.*

The effect of salting and season of the year on the volume of whey expelled during cheese making and whey composition from pure Holstein cow milk were investigated which was carried out in 2 phases. The first phase was carried out during the late dry season (March to April) and the second phase during the early rainy season (May to July). The cows were fed on controlled feeds and were allowed to graze on the paddock. First, 1000 mL of milk at 30 - 33.5°C was poured into the aluminum pot, and 1.5 mL of *Calotropis procera* extract (juice) was added and mixed with the milk. The mixture was heated to coagulation temperature of 71°C to 74°C. In each case, an identical experiment was set up but with the addition of 8 g sodium chloride salt which was replicated thrice in 2 batches. The salt was evenly sprinkled and thoroughly mixed before the commencement of heating. Time and temperature change were closely monitored and controlled. The mixtures were left undisturbed. The soft cheese made in each case was weighed and recorded as the wet yield. The volume of whey obtained from each set was measured. Samples for fat, lactose, protein, minerals, and nitrogen determinations were taken and pH of the samples was determined immediately. The collected parameters were subjected to a factorial arrangement ($2 \times 2 \times 4$) in a completely randomized design. The result obtained from the average volume of whey expelled during cheese making from the unsalted milk was 619.5 mL and 600 mL during the last and early seasons, while 636 mL and 610 mL were expected from salted cheese during the late dry season and early rainy season respectively. When these means were subjected to statistical analysis, there was no significant difference due to season or salting ($P > 0.05$). Besides, there was no significant difference in whey composition such as whey fat content and whey total solid for seasons and salting, while there was a significant difference in seasonal effect on casein and protein. The result of this study showed that both additions of salt and seasonal does not influence the volume of whey expelled from cheese making and its composition.

Key Words: cheese, whey, salt

T70 The effect of high hydrostatic pressure on the texture, appearance, and shelf life of Camembert type cheese. D. Batty*, D. Berry, L. Meunier-Goddik, and J. Waite-Cusic, *Oregon State University, Corvallis, OR.*

Surface ripened cheeses, such as Camembert, are limited in shelf life due to the fast ripening and eventual texture and color degradation. High hydrostatic pressure (HPP) has been shown to be effective at extending the shelf life of food products with minimal quality defects. The objective of this study was to determine the effect of HPP on the quality characteristics of Camembert type cheese. Three varieties of Camembert type cheese (traditional, stabilized, and a hybrid) were manufactured. The cheese was HPP treated (550 MPa for 10 min at 25°C) at either 3 (treatment A; before surface mold growth) or 10 d (tratement B; after surface mold growth) post manufacture. The cheeses were analyzed for composition, texture by penetrometry, and color. Among the 3 cheese types, average moisture content ranged from 53.2 to 56.6%, protein ranged from 15.8 to 17.8%, fat ranged from 23.0 to 23.6%, and salt ranged from 1.1 to 1.3%. After treatment A the firmness of the cheese significantly increased ($P < 0.05$). The stabilized variety increased the most (2.05 N to 7.41 N) and the traditional increased the least (1.04 N to 1.91 N) compared with the control (untreated cheese) after 21 d of maturation. For treatment B, the firmness was less than the control cheeses for the stabilized variety (1.42 N) and the traditional variety (0.84 N) after 21 d of maturation. The color of the rind was significantly ($P < 0.05$) affected by the HPP for both treatments, especially the L* value, indicating browning of the rind. For treatment B, L* value decreased most compared with the control for the hybrid variety (85.69 to 77.44), next was the traditional variety (87.08 to 80.18), and least for the stabilized variety (81.15 to 79.24) after 21 d of maturation. This study demonstrates that time of HPP impacts the textual properties and appearance of Camembert type cheeses. However, earlier treatment time can increase the firmness of Camembert cheese during maturation, providing some evidence that the shelf life could be increased with an early treatment time.

Key Words: color, firmness, HPP treatment time

T71 Identification of bacteria responsible for off-odor development in non-fat chocolate milk. D. Batty*, L. Meunier-Goddik, and J. Waite-Cusic, *Oregon State University, Corvallis, OR.*

Chocolate milk typically has higher microbiological counts than unflavored milk and is susceptible to off-flavor development before the end of targeted shelf life. It has been suggested that the addition of multiple ingredients (chocolate, sucrose, stabilizers, and vitamin A and D) contributes to expedited growth of spoilage organisms in chocolate milk. The objective of this study was to isolate and identify the organisms responsible for off-flavor development in nonfat chocolate milk. Nonfat chocolate milk was obtained from regional fluid milk processors (3 plants). At the end of shelf life (18 d) off-odors had developed and the milk was serial diluted and spread plated on standard and differential media including tryptic soy agar, De Man, Rogosa and Sharpe agar, skim milk agar, spirit blue agar, chocolate milk agar, and MacConkey agar. Isolates were streaked on chocolate milk agar (standard methods agar with 10% vol/vol chocolate milk). Five causative isolates were selected based on the development of off odors (band-aid, fishy, and bready) after growing on chocolate milk agar. Selected isolates were inoculated in triplicate at varying levels (1–4 log cfu/mL) into UHT nonfat chocolate milk to confirm off-odor development. Off-odors developed after 5–14 d with 7°C storage. Whole genome sequencing was performed on isolates and species were determined by 16S rRNA and *rpo*B sequence comparisons in the NCBI database. "Band-aid" odor was associated with *Psychrobacillus psychrotolerans*, bready odor with *Paenibacillus odorifer*, and fishy odor with *Acinetobacter guillouiae*. Knowing the identification of these spoilage organisms in chocolate milk can help fluid milk processors monitor for these microorganisms. Determining their prevalence and point of entry can help control the risk of an off-flavor spoilage event through targeted processing parameters and sanitation methods.

Key Words: shelf life, quality

T72 Lactose oxidase: An enzymatic preservative of raw milk. S. Lara* and S. D. Alcaine, *Cornell University, Ithaca, NY.*

The lactoperoxidase system (LPS) is an antimicrobial system naturally present in milk that has been used as a preservative to inhibit microbial growth in raw milk when refrigeration is not viable. Traditionally, the Food and Agriculture Organization (FAO) has recommended the addition of sodium percarbonate and thiocyanate for LPS activation in such instances. Lactose oxidase oxidizes lactose to lactobionic acid, producing H_2O_2 that could act as an antimicrobial or potentially activate the LPS. The aim of this research was to determine if LO can be added as a preservative to inhibit microbial growth in raw milk when refrigeration is limited. A solution of LO was added to raw milk at a concentration of 0.12 g/L both individually (LOX) and combined with NaSCN at 14 mg/L (LOX-T), while the FAO method (FM) consisted of adding 14 mg/L of NaSCN and 30 mg/L of sodium percarbonate. A total of 4 treatments were evaluated including the control (C), all began within 2 h after milking. A total aerobic bacterial growth curve was determined for each treatment, with samples stored at 21°C and sampled 10 times over 24 h. The experiment was carried out in triplicate, and a Buchanan model was applied to each growth curve to calculate the lag phase and growth rate during exponential phase. A one-way ANOVA and Tukey's test were performed to determine differences in counts among treatments. The lag phase for LOX-T (11.3 h) was significantly longer than C (4.0 h) and LO (5.4 h), but no different from FM (9.4 h). The average growth rate was 0.48 h^{-1}, and no significant difference was determined among treatments. The total bacterial count after 24 h for LOX-T (5.3 cfu/mL) was significantly lower than C (7.2 cfu/mL), LOX (7.1 cfu/mL), and FM (6.1 cfu/mL) ($P < 0.0001$). Overall, LOX-T shows the most potential for preservation of raw milk stored under abused temperature and its inhibition effect is comparable to the method of reference (FM). Results from this study suggest that LO is an alternative source of H_2O_2 that enhances the microbial inhibition achieved by the LPS. LO could be used to create enzyme-based preservation technologies for applications where cold chain access is limited.

Key Words: raw milk, lactose oxidase, preservation

T73 *Lactobacillus wasatchensis* does not survive pasteurization of milk prior to cheesemaking. I. Bowen*[1], D. McMahon[1], and C. Oberg[2], [1]*Utah State University, Logan, UT,* [2]*Weber State University, Ogden, UT.*

Lactobacillus wasatchensis can be part of the nonstarter lactic acid bacteria microbiota of cheese and has been associated with unwanted gas production during storage. Our objective was to determine whether *Lb. wasatchensis* can survive pasteurization of milk and this allows its entry into the cheese manufacturing process as previous studies suggested they could survive at a low level. To determine if *Lb. wasatchensis* can survive high temperature short time (HTST) pasteurization using a plate heat exchanger, *Lb. wasatchensis* WDC04 was grown in MRS-ribose broth and then inoculated into 30-L batches of raw whole milk at levels of 10^8 cfu/mL. The inoculated milk was then processed in the university creamery with the HTST pasteurizer set at 69, 72, 74, and 76°C, a holding time of 15 s, with water preceding and following the milk. Samples of milk were collected at triplicate times previously observed to not be diluted with water and the numbers of *Lb. wasatchensis* counted on MRS-ribose agar after anaerobic incubation for 5 d at 25°C. No survival of *Lb. wasatchensis* was observed at any of the temperatures, including the sub-pasteurization treatment of 69°C for 15 s. Since there was at least an 8-log destruction of *Lb. wasatchensis* during normal pasteurization, or even sub-pasteurization heat treatment of milk at 69°C (156°F), its presence as part of the NSLAB microbiota of cheese appears to be through other environmental contamination rather than directly from the incoming pasteurized milk.

Key Words: late gas defect, lactobacilli, cheese

T74 Isolation of a *Lactobacillus wasatchensis*-like isolate from an aged European Cheddar cheese showing late gas defects. I. Martineau*[1], M. Culumber[1], C. Oberg[1], and D. McMahon[2], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

Lactobacillus wasatchensis WDC04, a non-starter lactic acid bacterium (NSLAB), was isolated from aged Cheddar cheese that had late gas formation. Subsequent studies have demonstrated *Lb. wasatchensis* can produce gas in culture and in experimental cheeses. *Lactobacillus wasatchensis* has been identified in cheeses with late gas defects from geographically dispersed locations within the United States. Recently, a sample of a European Cheddar cheese with late gas defects was examined. NSLABs from the cheese were isolated on de Man, Rogosa and Sharpe agar supplemented with 1% ribose (MRS-R) anaerobically for 7 d at 30°C. Colonies with similar morphology to *Lb. wasatchensis*

were selected for 16S rRNA gene sequencing, API 50 carbohydrate panels, and were observed for gas production in MRS-R broth with Durham tubes. All of the isolates selected had identical 16S rRNA gene sequences. The 16S rRNA gene had 99% sequence identity to *Lb. hokkaidonensis* strain LOOC260 and 97% sequence identity with *Lb. wasatchensis* WDC04. Unlike *Lb. wasatchensis*, the new isolate showed acid production with several carbohydrates in addition to ribose, including D-xylose at 24 h, and methyl-β,D-xylopyranoside, glucose, and maltose at 48 h. This carbohydrate profile also distinguishes this organism from *Lb. hokkaidonensis* and other related species including *Lb. suebicus, Lb. vaccinostercus,* and *Lb. oligofermentans.* This isolate also produced gas in MRS-R broth. This isolate appears to be another member of this closely related group of lactobacilli and, like *Lb. wasatchensis,* may cause late gas defects in aged cheeses. Further genetic and physiologic characterization will provide more evidence for the geographical distribution, diversity, and environmental reservoirs for this group of organisms.

Key Words: *Lactobacillus,* cheese, late gas defect

T75 Suppressing growth of *Lactobacillus wasatchensis* WDC04 using organic acids. I. Green*[1], C. Oberg[1], M. Domek[1], and D. McMahon[2], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

Lactobacillus wasatchensis is a slow growing, non-starter lactic acid bacterium (NSLAB) that causes late gas defect in aging cheese and results in significant economic losses to producers. During cheese aging, organic acids can be produced by other NSLAB cultures or purposefully added to cheese during manufacture. Organic acids are often used as food preservatives, can occur naturally in foods, and generally do not affect flavor or product quality. Selected organic acids, in their naturally occurring concentration ranges in Cheddar cheese, were investigated for their ability to inhibit *Lb. wasatchensis* WDC04. Five organic acids (lactic, formic, propionic, citric, and acetic) produced by NSLAB organisms were tested. They were each added at their minimum, median, and maximum concentrations, as found naturally in aged Cheddar cheese, to individual wells of a 48 well plate containing MRS broth with 1% ribose (MRS + R) inoculated with *Lb. wasatchensis* WDC04. Growth rates were determined on a Tecan Infinite 200 PRO spectrophotometer over 40 h. All tests were done in triplicate and a one-sided *t*-test used to determine significance ($P < 0.05$) between treatments and the control (no added organic acid). Initially, tests were done at pH 7.0 with several organic acids exhibiting some inhibition. Tests were then run at pH 5.0 to determine if the organic acids were more effective at a pH of aged cheese. Both formic and citric acid showed significant inhibition of *Lb. wasatchensis* WDC04, especially at pH 5.0. Formic acid was the most inhibitory of all 5 organic acids with the maximum concentration (100 m*M*) showing the greatest inhibition. Addition of citric acid at the minimum (12 m*M*) and median (13.5 m*M*) concentrations also produced inhibition. Use of selected organic acids at concentrations normally found in Cheddar cheese could be a potential antimicrobial measure to prevent or reduce late gas defect in aging cheese.

Key Words: lactobacilli, cheese, organic acids

T76 Inhibition of *Lactobacillus wasatchensis* by bio-protective lactic acid bacteria. S. Overbeck*[1], C. Oberg[1,2], M. Culumber[1], and D. McMahon[2], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

Lactobacillus wasatchensis, a non-starter lactic acid bacterium (NSLAB), is an important cause of late gas defect in aging cheese. Controlling its growth may be possible by incorporating bio-protective lactic acid bacteria (BP-LAB) cultures into the cheese during manufacture. Potential BP-LAB strains were tested for their inhibitory ability against *Lb. wasatchensis*. Quantification of inhibition between BP-LAB cultures and *Lb. wasatchensis* was done using the spot test with the agar-flip method then measuring inhibition zones over time. MRS agar with 1% ribose (MRS-R) was inoculated with each BP-LAB and incubated anaerobically at 35°C for 48 h to form a spot colony. Inoculated agar was flipped over and a *Lb. wasatchensis* strain swabbed on the exposed surface, then plates were incubated anaerobically at 25°C for 72 h. All tests were done in triplicate allowing calculation of averages and standard deviations for inhibition zones. Inhibition zones using the spot test for the 5 most inhibitory BP-LAB cultures were *Lactobacillus rhamnosus* LB3 (28 mm), *Lactobacillus paracasei* P-210 (32 mm), *Lactobacillus brevis* ATCC 13648 (34 mm), *Lactobacillus casei* F19 (33 mm), and *Lb. paracasei* LILA (32 mm). Quantification of possible synergistic inhibition by co-BP-LAB strains was tested by mixing 1 mL each of 2 different BP-LAB strains, and then repeating the agar-flip protocol. Four cocultures were tested LB3/ P-210, LB3/P-220, P-200/P-210, and P-200/P-220. No significant increases in inhibition zones were observed when BP-LAB cultures were paired versus individual strains. Results confirm selected BP-LAB strains can inhibit growth of *Lb. wasatchensis.* Addition of selected BP-LAB cultures during cheesemaking could control late gas defect during cheese aging.

Key Words: lactobacilli, late gas defect, bio-protective culture

T77 Potential protective effect of bifidobacteria isolated from Egyptian yogurt against carbapenem-resistant *Acinetobacter baumannii*. M. Motawee*[1], S. M. A. Sharaf[2], and N. M. Saleh[2], [1]*Nutritional Evaluation and Food Science Department, National Organization for Drug Control and Research, Giza, Egypt,* [2]*Microbiology Department, National Organization for Drug Control and Research, Giza, Egypt.*

Acinetobacter baumannii is a major worldwide pathogen involved in healthcare-associated infections and is one of the most common causes of ventilator-associated pneumonia in the United States. There is growing concern with its ability to acquire resistance to multiple drugs and carbapenem-resistant *A. baumannii* (CRAB) are a focus since second-line treatments for *A. baumannii* infections are less defined in their efficacy and toxicity. We isolated various lactic acid bacteria (LAB) from Egyptian yogurt that were then tested for their ability to produce bacteriocins that would inhibit growth of CRAB. Tracheal aspirate isolates were collected from patients at Egyptian hospitals and 85 isolates were recognized as *A. baumannii* by PCR detection of bla_{OXA-51}. Eighty-two isolates had multiple drug resistance based on antimicrobial susceptibility testing. These CRAB isolates were tested for known drug-resistance genes, and 38% tested positive for bla_{OXA-24} while only one isolate carried bla_{OXA-58} indicating other genes may also be involved in providing drug-resistance. Cell-free extracts were prepared from the 25 LAB isolates and tested for inhibition of all 85 CRAB isolates using an agar diffusion bioassay. Four LAB isolates identified as *Lactobacillus casei, Lactobacillus plantarum, Bifidobacterium bifidum* and *Streptococcus thermophilus* had the most inhibitory effect, and inhibited growth of 61, 54, 71 and 60 of the CRAB isolates, respectively. The *B. bifidum* supernatant (BbV1) was purified using ion-exchange chromatography after which inhibition of CRAB isolates reached 97%. The BbV1

active agent was heat stable and based upon amino acid content it was a bacteriocin-like compound. Our finding demonstrated that *B. bifidum* BbV1 isolated from Egyptian yogurt produces an antimicrobial compound that is inhibitory against *Acinetobacter* infection in vitro. Further studies will be aimed at in vivo use of a probiotic yogurt containing *B. bifidum* BbV1 in animal and human clinical trials to determine its application in the treatment of CRAB infections.

Key Words: *Acinetobacter baumannii*, lactic acid bacteria, drug resistance

T78 A selective medium for the enumeration and differentiation of *Lactobacillus delbrueckii* ssp. *bulgaricus*. N. Nwamaioha*, R. Gyawali, T. Zimmerman, and S. A. Ibrahim, *North Carolina A&T State University, Greensboro, NC.*

Lactobacillus delbrueckii ssp. *bulgaricus* has been widely used for decades in the production of fermented products, especially yogurt and some cheeses. Several studies have linked the consumption of this species of bacteria to a reduced risk of colorectal cancer, the common cold, and inflammatory bowel disease. Consequently, due to the high demand for yogurt products in the United States, there is a need to isolate new strains of *Lactobacillus bulgaricus* to meet the increased yogurt production. In this study, we developed and evaluated Modified Reinforced Clostridial Medium (mRCM) for the differential enumeration of *L. delbrueckii* ssp. *bulgaricus*. Our results showed that supplementing Reinforced Clostridial Medium (RCM) with 0.025% $CaCl_2$, 0.01% uracil and 0.2% Tween 80 (mRCM) significantly ($P < 0.05$) enhanced the growth rate of *L. bulgaricus* RR and ATCC 11842 strains as measured by the optical densities of these strains after 12 h incubation at 42°C. The bacterial populations (plate count) of the RR and ATCC 11842 strains were 0.76 and 0.77 log cfu/g higher in mRCM than in de Man Rogosa and Sharpe (MRS) and RCM media, respectively. Conversely, other bacterial species (*Bifidobacterium*, *L. rhamnosus*, and *L. reuteri*) were significantly inhibited in the mRCM medium. The addition of aniline-blue dye to mRCM (mRCM-BLUE) improved the selectivity of *L. bulgaricus* in mixed lactic bacterial cultures compared with MRS and lactic agar (LA) with regard to colony appearance and morphology. mRCM-BLUE performed better than conventional media in culturing, enumerating, and differentiating *L. bulgaricus*. Thus, mRCM-BLUE, could be used as a selective medium to enhance the growth and differentiation of *L. bulgaricus* to meet the increasing demand for this beneficial species of bacteria.

Key Words: selective medium, *Lactobacillus bulgaricus*, yogurt

T79 A survey of live microorganisms in fermented foods. S. D. Rezac*[2,1], M. Heermann[1], C. R. Kok[1], and R. Hutkins[1], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*University of Illinois Urbana-Champaign, Urbana, IL.*

The popularity of fermented foods and beverages is due to their enhanced shelf-life, safety, functionality, sensory, and nutritional properties. The latter includes the presence of bioactive molecules, vitamins, and other constituents present in the initial food substrates. Many fermented foods also contain live microorganisms that may provide gastrointestinal and other health benefits. The number of organisms in fermented foods depends on how they were manufactured and processed, as well as conditions and duration of storage. In this review, we surveyed studies in which lactic acid and other relevant bacteria were enumerated from the most commonly consumed fermented foods, including cultured dairy products, cheese, fermented sausage, fermented vegetables, and soy-fermented foods. Most of the reported data were based on retail food samples, rather than experimentally produced products made on a laboratory scale. Results indicated that many of these fermented foods contained 10^{5-7} lactic acid bacteria per mL or gram, although there was considerable variation based on region and sampling time. In general, cultured dairy products consistently contained higher levels, up to 10^9/g. Collectively, the findings from this survey revealed that many fermented foods are a good source of live lactic acid bacteria, including species that reportedly provide human health benefits.

Key Words: fermented foods, cultured dairy products

T80 Fermented carbonated dairy beverage: Microbial and physicochemical characteristics. K. Miley*[1] and K. Aryana[2,1], [1]*Louisiana State University, Baton Rouge, LA,* [2]*Louisiana State University Agricultural Center, Baton Rouge, LA.*

Carbon dioxide tends to penetrate bacterial cell membranes and influence bacterial cell growth by acidification of the interior of bacterial cells, displacement of oxygen and interference with cellular enzymes. The objective was to study the influence of carbonation on microbial and physicochemical characteristics of a fermented carbonated dairy beverage. Pasteurized milk was tempered to 32°C and inoculated with *Lactococcus lactis*. Inoculated milk was poured into bottles and incubated at 32°C until pH 4.6. Coagulum was broken by agitation. Coagulum was plain or flavored with blueberry puree. Carbon dioxide was incorporated at 0 (control) and 40 psi and products were stored at 4°C. Product was tested weekly over 3 wk for *Lactococcus lactis*, coliform and yeast and mold counts, pH, titratable acidity and carbon dioxide content. Entire experiment was replicated 3 times. On d 1 of manufacture, the *Lactococcus lactis* counts in all 4 products were steady at 6 logs. At 3 wk of storage, the *Lactococcus lactis* counts in the carbonated products were one log lower than their non-carbonated counterparts. *Lactococcus lactis* counts in the plain product with no carbonation was the highest while the counts in flavored product carbonated at 40 psi was the lowest at 3 wk. All products had no detectable coliform and yeast and mold counts over the 3 wk. Volume of dissolved carbon dioxide in the plain and flavored carbonated products held steady over the 3 wk of storage at 4.3 g/L. Combination of carbonation and flavoring lowered *Lactococcus lactis* counts at 3 wk in the carbonated drinkable cheese product.

Key Words: carbonated drinkable cheese, *Lactococcus lactis*, count

T81 High concentrations of cinnamon are needed to lower the counts of *Lactococcus lactis* R 604. M. Hannan*[1] and K. Aryana[2,1], [1]*Louisiana State University, Baton Rouge, LA,* [2]*Louisiana State University Agricultural Center, Baton Rouge, LA.*

Cinnamon offers several health benefits such as lowering blood sugar, lowering cholesterol, reducing inflammation and treating yeast and bacterial infections. Cinnamon has antimicrobial effects. In the manufacture of health beneficial, flavored and cultured dairy products it would be important that cinnamon does not kill the health beneficial culture bacteria. The objective was to study the influence of various amounts of cinnamon powder on the viability of *Lactococcus lactis.* Cinnamon was incorporated into M17 broth at various concentrations of 0 (control), 0.01, 0.1, 1, 4, and 10 g per liter of broth. Broths were sterilized and inoculated with freshly thawed *Lactococcus lactis* R 604. The inoculated broths were aerobically incubated at 30°C for 24 h. Samples were drawn at 0 and 24 h, serially diluted and plated using M17 agar. Pour plates were incubated aerobically at 30°C for 72 h. Surviving colonies were counted. Entire experiment was replicated 3 times. Data were analyzed

using PROC ANOVA of SAS and means were separated using Fisher's Least Significant Difference test. Mean counts of 0 (control), 0.01, 0.1, 1, 4, and 10 g of cinnamon were 5.38, 5.28, 5.25, 5.28, 4.66 and 3.86 log cfu's / mL. There was no significant difference in counts between control (0), 0.01, 0.1 and 1 g of cinnamon use. Counts significantly lowered with the use of 4 g of cinnamon, while the least counts were obtained with the use of 10 g of cinnamon. At low concentrations, cinnamon did not influence counts but at higher concentrations cinnamon decreased counts of culture bacteria.

Key Words: cinnamon, antimicrobial, *Lactococcus lactis*

T82 A yogurt-based activity designed to improve understanding of biochemistry concepts. T. Zimmerman and S. Ibrahim*, *North Carolina A&T State University, Greensboro, NC.*

The goal of this laboratory activity was to strengthen our Food and Nutritional Science students' knowledge of biochemistry concepts and the relationship between these concepts and food science. In order to accomplish this, we designed a laboratory experience that teaches biochemistry concepts using yogurt as a model, and we linked those concepts to food safety, an important area of food science. The objective of this laboratory exercise was thus to reinforce the following: (1) enzyme activity; (2) the role enzymes play in fermentation; (3) fermentation and associated biochemical pathways; and (4) fermentation and the role it plays in food safety. The duration of the laboratory activity was 5.2 h over 4 d. The students employed a colorimetric method to measure the lactase activity of different strains of LAB found in different commercial yogurts and to relate the activity to bacterial lactic acid production, fermentation, and food safety. Students were assessed using a pre- and posttest and by rubrics assessing their laboratory reports and class performance. The Intrinsic Motivation Inventory (IMI) was used to assess intrinsic motivation. The data collected from the assessments demonstrated that the students successfully completed the learning objectives and were motivated during the activity. This exercise could be used as a template for how to introduce complex concepts in biochemistry and molecular biology in a simplified way using food, particularly yogurt, as a model for Food Science students. However, because food is a concept that everyone can relate to, the activity should be suitable for other science majors as well as non-science majors.

Key Words: yogurt, biochemistry, microbiology

T83 Raw milk microbiome of naturalized Brazilian breeds Curraleiro Pé-Duro and Pantaneiro. N. R. Soares[1], M. C. Sola[2], C. Gebara*[1], G. V. Barancelli[3], O. F. Zaccaroni[4], M. C. S. Fioravanti[4], E. S. Nicolau[1], and C. S. Minafra-Rezende[1], [1]*Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil,* [2]*Unified Higher Education Institute, Objetivo Faculty, Goiânia, Goiás, Brazil,* [3]*Department of Agro-Industry, Food and Nutrition, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil,* [4]*School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil.*

Raw milk microbiota is complex and dependent of animal-environment ecosystem. Brazilian cattle breeds Curraleiro Pé-Duro and Pantaneiro represent important local genetic resources, given their socioeconomic impact and potential use in animal husbandry. Although they are not consider as specialized dairy breeds, is relevant to know the microbiota of raw milk, as potential for local exploration.The aim of this study was to identify the microorganisms present on raw milk from naturalized Brazilian breeds Curraleiro Pé-Duro and Pantaneiro through microbiome. Raw milk samples were aseptically collected from Brazilian breeds Curraleiro (6 animals) and Pantaneiro (5 animals) and submitted to molecular analysis. However, the samples were previously cultured on BHI broth, which is a standard protocol for food research, since naturally occurring fastidious microorganisms are hardly recovered in conventional assay in raw milk. Microbial community was determined through sequencing of amplicons from the V3 and V4 hypervariable region of the 16S rRNA gene that was amplified by PCR and optimized by Illumina TruSeq platform. A total of 740,907 quality-checked sequences were formed (394,710 from Curraleiro and 346,197 from Pantaneiro). Main genera identified on raw milk were *Bacillus,* present on 90.9% of samples and representing 65.97% of identified sequences for Curraleiro and 67.75% for Pantaneiro. Other identified genera and its respective sequences for Curraleiro (C) and Pantaneiro (P) samples were *Enterococcus* (10.50% C, 19.63% P), *Enterobacter* (10.19% C, 7.29% P), *Staphylococcus* (8.07% C, 0.08% P), *Streptococcus* (1.25% C, 0.16% P), *Pseudomonas* (1.20% C, 0.11% P), *Lactococcus* (0.02% C, 0.33% P) and *Salmonella* (0.24% P). Despite raw milk microbiota complexity, including pathogens, our hypothesis is that exist a balance between these microorganisms, once these animals presents low mastitis incidence and produce milk with low SCC. Results also highlight relevance of microbiome for understanding milk bacterial ecosystem and its importance for animal health as well as for technological and safety characteristics of milk.

Key Words: milk microbiota, *Bacillus* sp., molecular analysis

T84 Sensory evaluation of whey fermented beverages with buttermilk and Brazilian Cerrado fruit. R. T. Pfrimer[1], L. Damasceno[1], C. F. Cardoso[2], T. V. de Almeida[1], J. C. R. S. More[1], E. Arnhold[3], E. S. Nicolau[1], and C. Gebara*[1], [1]*Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil,* [2]*School of Agronomy, Federal University of Goiás, Goiânia, Goiás, Brazil,* [3]*School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil.*

Sustainable food production is encouraged by United Nations' 2030 Agenda, which has a plan to eradicate hunger and malnutrition. Hence, the food production that uses co-products and regionals fruits becomes attractive. Consumers have been looking for functional products with new flavors. The aim of this study was to develop 7 formulations of fermented beverages with whey and buttermilk, which are co-products of dairy industry and if discarded incorrectly results on environmental damages. However, whey and buttermilk possess functional, nutritional and technological properties besides its health benefits. Cagaita (*Eugenia dysenterica*) pulp was used to flavoring the beverages. It is an appreciated fruit of Brazilian Cerrado with functional properties and produced by local family farmers. The sensory profile of the samples was evaluated by 100 untrained panelists and the attributes evaluated were appearance, color, flavor, texture and overall impression, using a 9-point hedonic score, where 1 = dislike extremely and 9 = like extremely. The purchase intention was measured on a 5-point score, where 1 = definitely would not buy and 5 = definitely would buy. The result was evaluated by ANOVA and adjust.fdr mean comparison test ($P < 0.05$). The formulations with higher milk concentration (50%) and lower visual syneresis presented higher notes ($P < 0.05$) for appearance and texture. The formulations with higher pulp concentration (20%) obtained higher notes ($P < 0.05$) for aroma. All formulations presented scores between 6 and 9 ("like slightly" to "like extremely") for color and flavor. All beverages were well accepted by consumers and the scores were about 7 ("like moderately") for overall impression and scores about 4 ("I probably would buy") for purchase intention. Beverage with 50% milk 44% whey/buttermilk and 6% pulp was best accepted by consumers and had better cost-benefit. The estimated cost for production of 180 mL of this formulation was US$0.32. This product is an example of proposal made by the 2030 Agenda, and can be classified as sustainable, functional, and attractive product.

Key Words: innovative product, dairy coproduct, 2030 Agenda

T85 Development and characterization of whey fermented beverages with buttermilk and Cagaita pulp (*Eugenia dysenterica*). L. Damasceno*[1], R. T. Pfrimer[1], C. F. Cardoso[2], R. M. do Carmo[1], C. S. Minafra-Rezende[1], E. Arnhold[3], C. S. Prado[1], E. S. Nicolau[1], and C. Gebara[1], [1]*Food Research Center, School of Veterinary Medicine and Animal Science Federal University of Goiás, Goiânia, Goiás, Brazil,* [2]*School of Agronomy, Federal University of Goiás, Goiânia, Goiás, Brazil,* [3]*School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil.*

The United Nations' 2030 Agenda has the objective to promote sustainable development, eradicating hunger and ending all forms of malnutrition. Industry coproducts, such as whey and buttermilk, can be used to increase functional, nutritional and technological aspects of foods and contribute to sustainable development. The use of exotic fruits with functional characteristics has also gained attention on food market, and cagaita, a Brazilian Cerrado fruit, has gained attention due its antioxidants properties. The aim of this work was the development and characterization of different formulations of whey-fermented beverages with buttermilk and cagaita pulp (*Eugenia dysenterica*). Using simplex-centroid mixture design, 7 formulations were defined with different concentrations of whey/buttermilk mix (W/B) between 30- 44%, milk (M) between 36 and 50% and cagaita pulp (C) between 6 and 20%. Physico-chemical and microbiological characteristics were determined by official methods. The results were evaluate by ANOVA and Pearson correlation test and mean values were compared by Scott-Knott's test ($P < 0.05$). Beverages presented moisture between 80.50 and 82.81%, ash between 0.47 and 0.67%, protein between 1.59 and 2.00%, lipids between 1.18 and 2.70%, carbohydrates between 13.37 and 15.32% and total energy value between 72.75 kcal and 85.01 kcal, pH 3.96 and 4.47, acidity was between 0.36 and 0.58%. Milk concentration shows positive correlation with protein and lipids whereas W/B mixture shows negative correlation, therefore beverages with higher W/B and lower M had the lowest protein and lipids. The higher W/B and C, the higher moisture of beverages. All beverages had LAB content above 6 $\log_{10}$cfu/mL and, for *Salmonella* spp. and coliforms, the beverages were within the Brazilian law standard and for food safety. The developed products presented expected physico-chemical and microbiological characteristics and were safe for consumption. Besides, these beverages are low cost dairy foods, by using dairy industry coproducts and still have functional characteristics conferred by whey, buttermilk and cagaita pulp.

Key Words: fermented milk, functional ingredients, exotic fruit

T86 Bronopol-based preservatives evaluation on somatic cell count and chemical composition of raw milk samples. S. Dâmaso*[1], T. V. de Almeida[1], T. L. Batistoti[1], J. R. de Sousa[1], R. T. Pfrimer[1], L. Damasceno[1], E. Arnhold[2], I. A. Nunes[1], E. S. Nicolau[1], and C. Gebara[1], [1]*Food Research Center, School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil,* [2]*School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, Goiás, Brazil.*

Milk quality evaluation is essential for dairy industry and each country establishes its own directives, based on international standards, to evaluate and standardize milk parameters. In Brazil, all milk produced must be monthly submitted to analysis in one of 12 laboratories of Brazilian Network for Milk Quality (BNMQ). Due to the Brazilian large territorial extension, conservation by refrigeration and preservatives of samples are necessary, because samples will be analyzed after several days of being collected. Among substances used for milk preservation, IDF recognizes only boric acid, potassium dichromate, bronopol and sodic azide. We evaluated all 3 kinds of bronopol-based preservatives available on Brazilian market to use on milk samples destined to analyses on laboratories of BNMQ. Preservative tablets have the following composition: 8 mg bronopol, 0.30 mg natmicin and 10 mg inert carrier (P1); 8 mg bronopol, 12.5% natmicin, 20.76% yellow dye, NaCl, PVP solution (P2); 8 mg bronopol, 0.4 mg natmicin, 0.5 mg yellow dye (P3), according to manufacturers. Raw milk samples were collected in vials containing different microtablets of bronopol-based preservatives, stored at 4°C and analyzed during 14 d for Somatic Cell Count (SCC) and chemical composition through flow citometry and FTIR spectroscopy

respectively. The entire experiment was repeated 8 times. Results were evaluated by ANOVA and Tukey's test mean comparison ($P < 0.05$). All results obtained were according to standards for raw milk. There were no differences in fat, protein, casein, lactose, total solids, milk urea-nitrogen and SCC between preservatives.The 3 bronopol-based preservatives were used during 14 d of refrigerated storage and did not affect milk evaluated parameters. However, in practice P3 leads to analytical disorders, considering tablets dissolution time and interruptions on equipment operation, making its usage more difficult. Therefore, we recommend evaluation of bronopol-based preservatives and its convenience before electronic analysis.

Key Words: bronopol, milk preservation, electronic analysis

T87 Proteomic analysis on whey proteins of Guanzhong goat milk. Y. Sun*[1], C. Wang[1], X. Sun[1], and M. Guo[2,1], [1]*Department of Food Science, College of Food Science and Engineering, Jilin University, Changchun, Jilin, China,* [2]*Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT.*

Guanzhong goat is one of the major dairy goats in China and its milk is also a major milk supply for the Chinese dairy industry. Whey protein has a balanced nutritional value and certain biological functions. To explore the proteomics of Guanzhong goat whey, whey fraction was separated from goat mature milk, and analyzed using iTRAQ-coupled LC-MS/MS. A total of 368 whey proteins were identified and quantified in the serum fraction, of which only 7% belong to goat, whereas 57% pertain to sheep, and 36% are homologous to bovine. The identified proteins were categorized according to their biological processes, cellular components, and molecular function based on their gene ontology annotation. The main biological processes involved were response to regulation of biological functions, metabolic process, and response to stimulus. The most general cellular components was extracellular (37%), and the most common molecular functions was protein binding, representing 46%. The data could provide important information for understanding the composition and unique biological properties of goat milk.

Key Words: milk proteomics, whey protein, Guanzhong goat

T88 Preservation of lactase activity in a gastric environment. J. F. Flanagan*, J. Simmons, J. R. Boone, C. Elkins, and K. Clinger, *David Lipscomb University, Nashville, TN.*

Approximately 70% to 75% of the world's adult population cannot fully utilize dairy due to varying degrees of lactase deficiency. With the high incidence of lactose intolerance in the population there is a need for reliable utilization of oral lactase enzyme. In a 2010 literature review, the effectiveness of prehydrolyzed milk products or lactase was questioned. Problems with study design, source of enzyme, and dosage were cited. The aim of our study was to explore the effects of pH and temperature on lactase kinetics to determine a more effective delivery method. Four antacids were investigated as modifiers of simulated stomach acid. The effects of antacid pretreatment on lactase preservation in acid (HCl) were evaluated at 4°C and 37°C using a spectrophotometric assay. This assay measured the appearance of o-nitrophenol from the hydrolysis of o-nitrophenyl-β-galactoside (ONPG) as a substrate. Using the same model, data from simultaneous addition of antacid with lactase to an acidic solution were generated. Glucose production from lactose was monitored with a handheld glucometer to determine the effectiveness of exogenous lactase under varying in vitro conditions. Three of 4 antacids were found to raise the pH of an acidic medium to within a sufficient working range (approx. 5.5–7.5) at all tested volumes. Sodium bicarbonate ($NaHCO_3$) was observed to neutralize acid fastest. Lactase enzyme was found to hydrolyze lactose approximately 1.6 times faster at 37°C compared with 4°C. Using an ONPG assay, $NaHCO_3$-containing antacids proved effective at enzyme preservation when added to acid simultaneously with lactase. When added to 100mL of 0.1M HCl, a combined lactase/$NaHCO_3$ preparation in whole milk (240mL) yielded glucose concentrations of 202 ± 23.5 mg/dL at 5mins and 520 ± 30.7 mg/dL at 10 min. In contrast, an antacid-free preparation yielded values less than the minimum detectable concentration of the glucose meter, 20 mg/dL. In our pursuit to find an effective lactase preparation and delivery method, we suggest a lactase-sodium bicarbonate pairing to allow for immediate lactase-lactose interaction within the stomach while still avoiding any acid-mediated enzyme degradation.

Key Words: lactase, antacid

T89 Changes in structure and antioxidant activity of β-lactoglobulin by ultrasound and enzymatic treatment. S. Ma[1], C. Wang[1], and M. Guo*[2,1], [1]*Department of Food Science, College of Food Science and Engineering, Jilin University, Changchun, Jilin, China,* [2]*Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont,, Burlington, VT.*

Effects of ultrasound (20–40% amplitudes at 45–55°C) and enzymatic (pepsin and trypsin) treatment on structure and antioxidant activity of β-lactoglobulin were studied. Changes in structure of β-lactoglobulin were investigated using spectroscopy techniques and changes in antioxidant activity were measured by chemical and cellular-based assays. Ultrasound treatment had considerable impact on the structure of β-lactoglobulin and increased the susceptibility of β-lactoglobulin to both pepsin and trypsin proteolysis. Intrinsic fluorescence intensity of β-lactoglobulin was increased by ultrasound and then decreased after following enzymatic treatment. Compared with control, the β-lactoglobulin after ultrasound and enzymatic treatments showed significantly higher oxygen scavenging activities in Caco-2 cells models, ABTS (2,2'-Azinobis-3-ethylbenzthiazoline-6-sulphonate) radical scavenging activity and oxygen radical absorbance capacity ($P < 0.05$). Results indicated that ultrasound treatment increased the proteolysis of β-lactoglobulin by both pepsin and trypsin and improved the antioxidant activity of the protein and its proteolytic products.

Key Words: β-lactoglobulin, ultrasound treatment, proteolysis

T90 Evaluation of the potential of bovine immunoglobulins and glycomacropeptide to serve as sources of peptides with dipeptidyl-peptidase IV inhibitory activity. I. M. E. Lacroix*[1], B. Anderson[2], D. E. Otter[3], and R. D. Bremel[2], [1]*Wageningen University & Research, Wageningen, the Netherlands,* [2]*ioGenetics LLC, Madison, WI,* [3]*Centre for Dairy Research, University of Wisconsin-Madison, Madison, WI.*

The consumption of dairy proteins, particularly those from whey, has been shown in interventional studies to have beneficial effects on glucose metabolism. It has been proposed that the antidiabetic properties of whey protein may be attributable to its content of bioactive peptides which, upon being released during digestion, can modulate hormones, enzymes and/or organ systems involved in glycemia regulation. In particular, recent studies have suggested that milk protein-derived peptides can inhibit the activity of the dipeptidyl-peptidase IV (DPP-

IV) enzyme, a target in the management of type 2 diabetes. The aim of this study was to evaluate the potential of bovine immunoglobulins (Ig) and glycomacropeptide (GMP) to serve as precursors of peptides with DPP-IV inhibitory activity. Ig and GMP were first submitted to in vitro gastrointestinal (GI) digestion and the resulting digests assessed for their effect on DPP-IV activity. While undigested proteins showed no effect on DPP-IV, both digests were able to inhibit the enzyme, the Ig digest displaying the greatest potency (IC_{50} = 0.6 and 1.0 mg/mL, for the Ig and GMP digests, respectively). Ultrafiltration of the digested proteins to further characterize them revealed peptide fractions with varying degree of effectiveness against DPP-IV, none being, however, more potent than the whole digests they originated from. Findings from this research show that DPP-IV-inhibiting peptides can be generated during GI digestion of Ig and GMP and suggest that the consumption of these milk constituents could have a beneficial effect on blood glucose regulation.

Key Words: immunoglobulin, glycomacropeptide, dipeptidyl-peptidase IV inhibitor

T91 Physicochemical modifications of MFGM proteins during temperature processing of milk. F. Yu*, J. Ortega-Anaya, and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

MFGM as a complex mixture of proteins and phospholipids, abundant in the buttermilk and can be used in pharmaceutical and functional food applications. The proteins in the MFGM have been shown to have bioactive properties that are beneficial for human health. Two of these proteins, lactadherin (PAS 6/7) and Butyrophilin (BTN), have gained attention due to their antiviral, anticoagulant, anti-inflammatory properties, as well as the ability to control milk fat globule secretion. The protein aggregation and conformational changes that induced by temperature changes are important factors to determine the protein functionality. The objective is to study the temperature effects on buttermilk and the effect on physicochemical properties of PAS 6/7 and BTN. Cream obtained from local raw milk was churned in our laboratory and different heat treatments (4°C, LTLT, HTST, and 90°C for 10 s) were applied before and after churning to additionally assess the temperature effects on the proteins. This procedure was repeated by triplicate to assess variability. After each treatment, size exclusion methodologies were used to recover enriched protein extracts and dynamic light scattering analysis (DLS) was used to obtain particle size and the oligomeric state in solution. The zeta-potential was measured to determine charge changes. Fractionation of proteins by membrane filtration (100 and 30 kDa) showed the proteins suffered aggregation and interaction with whey proteins (SDS-PAGE); however, instead of associating with larger proteins under 4°C, whey proteins started to associate among themselves under HTST. To overcome this, gel filtration chromatography with Sephacryl S200 HR was used to maintain proteins native state. Before DLS and zeta-potential analysis, the isolated fraction was analyzed by native- and SDS-PAGE to identify PAS 6/7 and BTN, and assess the native oligomeric state and interaction with whey proteins. All results were analyzed by ANOVA. The results show the effect of temperature on MFGM, where maximum complexity was found in heating before churning, and indicate the need to better understand the effect of heat treatment on minor components of milk.

Key Words: milk fat globule membrane (MFGM), protein, thermal

T92 Localization of milk gangliosides in emulsion monolayers that resemble the milk fat globule membrane outer leaflet. L. M. Real Hernandez* and R. J. Flores, *The Ohio State University, Columbus, OH.*

Currently, the localization of sialic acid containing glycolipids in the outer leaflet of the milk fat globule membrane (MFGM) is stated to be in regions of the MFGM not occupied by microdomains, highly ordered lipid structures formed from the interactions of sphingomyelin (SM) and cholesterol (CH) compounds. This contrasts with many reports in the literature describing the presence of gangliosides in microdomains in the outer leaflet of living cell membranes. Gangliosides are bioactive sialic acid containing glycolipids found in many dairy products. The outer leaflet of the MFGM is derived from the outer leaflet of the membrane of mammary cells, so one would hypothesize that MFGM microdomains could contain gangliosides. The uncertainty behind the mechanisms behind the mismatch in current data between ganglioside localization in MFGMs and living cells creates some skepticism of the currently reported data on this topic. In this work, oil-in-water emulsions were created where the predominant lipids present in the outer leaflet of the MFGM (phosphatidylcholine [PC], SM, and CH) were used as emulsifiers, and varying amounts of the predominant gangliosides (GM3 and GD3) in milk were added to observe their location within the MFGM-like emulsification monolayer. Localization was determined using a FV3000 fluorescent confocal microscope. Localization of non-microdomain forming lipids was done with the use of various fatty acid tail labeled PC probes added to the initial emulsifier mixtures before the creation of the emulsion. The commonly used rhodamine-phosphatidylethanolamine (Rho-PE) probe was not used in this work since PE is not significant present in the outer leaflet of the MFGM. Ganglioside localization was observed with the use of a wheat germ agglutinin probe coupled to an Alexa Fluor dye. Current preliminary data suggests that ganglioside localization is dependent on the emulsifier composition, which suggests that ganglioside localization might not be universal to all MFGMs.

Key Words: gangliosides, milk fat globule membrane (MFGM), confocal microscopy

T93 The potential of milk production and consumption for improving nutrition of smallholder dairy households in Ethiopia. H. Didanna*[1], A. Wossen[2], T. Worako[1], and B. Shano[1], [1]*Wolaita Sodo University, Sodo, Ethiopia,* [2]*Addis Ababa University, Addis Ababa, Ethiopia.*

Evidences on potential of milk consumption in preventing malnourishment vis-à-vis market-oriented/intensifying smallholder dairy-producing areas are scant. Hence, this study explored the consumption habits of fresh bovine milk in the dairy-producing households. Data were collected from a survey of 200 dairy households and key informant interviews. The results revealed that the amount of self-consumed fresh milk per farm and day by producer families varied from 0.5 to 5 L per day. The majority consumed and traded milk at the same time. The practice of treating milk before consumption differed significantly across production systems. Eighty-four percent of the dairy producers boiled milk before consumption, and 8.5% of the respondents did not consume fresh but rather fermented/sour milk (*ergo*) as most of them had symptoms of lactose intolerance. Based on United States Department of Agriculture recommendations, the daily requirement is 10–15 cups if on average 5 of the family members are drinking milk. Hence, there was a lack of 1.40–2.85 L of milk, which is insufficient to satisfy the nutrition requirement from dairy foods. However, there are ample experiences of dairy farming, local availability, milk production, and culture of milk

consumption. There is scope to improve nutrition through consuming sufficient quantities of milk by the milk-producing households and balancing the staple foods (teff and wheat) in the area. Improving milk productivity will increase the levels of milk consumption, which in turn would have great potential as a cost-effective and sustainable household food production strategy for malnourished children.

Key Words: bovine milk, intensive milk production systems, smallholder dairying

T94 Acid-induced gel properties of dry-heated low-heat nonfat dry milk. K. S. Alan* and K. Schmidt, *Kansas State University, Manhattan, KS.*

Different foods, such as bread incorporate high heat nonfat dry milk (HH) to their formulation to improve their texture and mouthfeel. Exposing low heat nonfat dry milk (LH) to a radio frequency dielectric heat (RFDH) treatment can enhance the acid-gelling properties of dairy proteins to a point where they can provide or exceed the gelling properties of HH. The aim of this study was to apply a RFDH treatment to LH to improve its gelling properties while maintaining solubility and color. A RFDH unit was used to heat LH to 85°C and held for 3 or 6 h (85/3, 85/6). HH, which was not RFDH-treated was the control and used to compare the gelling properties against the RFDH-treated samples. As powders, samples were evaluated for solubility (NSI), soluble aggregate formation, and color. As GDL acidified gels, evaluations included water holding capacity (WHC), syneresis, firmness, and cohesiveness. Three replications were conducted and evaluated using one-way ANOVA. Significant means of the RFDH-treated LH were compared against HH using the Dunnett's method. All tests were determined at a $P \leq 0.05$. Results showed that the gels made from 85/3 and 85/6 maintained good solubility, ~4% and 2.6% greater than the HH, respectively. However, the RFDH treatment increased the yellowness by 6.5% and 13.4%, respectively. Based on the ΔE values, this color change was not visible for consumers. The 85/3 and 85/6 gels were ~20% and 30% more firm and ~7% and 8% more cohesiveness, respectively compared with the HH gel. However, the WHC was significantly lower (~15% and 13%) and the syneresis was significantly higher (~90% and 163%), respectively when compared with the HH gel. The formation of soluble aggregates during the dry heat treatment may contribute to the improved gel firmness and cohesiveness observed in the present study. These results suggest that RFDH treating LH at 85°C for 3 h can alter proteins to have similar firmness and cohesiveness as the HH gels without drastically affecting their solubility and color. The LH that was RFDH-treated at 85°C for 6 h exhibited improved firmness and cohesiveness of the acid-induced gels, but it had lower quality parameters such as increased syneresis while exhibiting more yellowness vs. the 85/3 sample.

Key Words: radiofrequency dielectric heating, acid-induced gels, low-heat nonfat dry milk

T95 Power ultrasound as a tool to modify texture properties of protein enriched acid milk gels. A. O. Körzendörfer*, J. Hinrichs, and S. Nöbel, *University of Hohenheim, Institute of Food Science and Biotechnology, Stuttgart, BW, Germany.*

There is still a rising demand for fermented milks like Greek yogurt and skyr. Nutrition-conscious consumers appreciate the high protein content up to 10%, whereas products commonly contain no fat. The consistency is creamy and thick without the need for stabilizers. Typically, the milk is acidified in tanks forming a gel that is finally broken up by stirring and concentrated by separation. This process generates large amounts of acid whey that is undesired due to environmental concerns. A novel approach to avoid acid whey is to concentrate the milk before fermentation, however, resultant gels are firm so that stirring in the vat and further processing is difficult. It is also challenging to produce a smooth texture. We hypothesize that power ultrasound during fermentation is a tool to soften the gel as sound waves cause cavitation and strong shear forces in the fluid. Skim milk was concentrated to a protein content of 10% by reverse osmosis and heated to 95°C for 5 min. Milk was fermented in jars (140 g) at 44°C until pH 4.6. During acidification, samples were treated with a sonotrode (20 kHz, 200 W). The sonication was performed at different pH (5.5–5.1) for 100 s in pulsation to prevent temperature rise. Immediately after fermentation, gels were stirred using a rheometer with a vane geometry. The maximum torque required to break the set gel was recorded. Gel firmness was measured by penetration tests. Afterward, gels were processed into stirred products and analyzed (water-holding capacity, rheology, particle size). Short-term sonication at pH 5.2 reduced the maximum torque required to break the gel and the firmness by 21% ($P < 0.001$) and 26% ($P < 0.001$), respectively. Treatments at higher pH values did not result in a softening of the gel. Samples sonicated at pH ≤ 5.2 exhibited a reduced water-holding capacity. Furthermore, the Sauter mean diameter was decreased from 40.8 ± 2.0 µm (control) to 30.1 ± 1.8 µm due to sonication at pH 5.2, and the maximum shear stress was lowered by 14%. We conclude that power ultrasound affects the network structure by softening the gel and offers the potential to improve Greek yogurt production.

Key Words: strained yogurt, Greek yogurt, gelation

T96 Physical chemical and sensory evaluation of yogurt enriched with tamarind pulp. M. R. L. dos Santos*, J. V. M. Oliveira, M. A. D. Silva, J. S. Queiro, and M. R. F. Neto, *Instituto Federal Goiano Campus Ceres, Ceres, Goiás, Brazil.*

This work aimed to evaluate physical chemical and sensory properties of yogurt enriched with tamarind pulp. Tamarind is a tropical fruit very appreciated in Brazil but its use is underestimate. Three formulations of yogurt with tamarind pulp (2, 4, and 6%) were prepared and analyzed to determine pH, humidity, ash level, and titratable acidity. The sensory evaluation used a 9-point structured hedonic scale with 50 non-trained tasters. The results were analyzed by ANOVA and the Tukey test at 5% of significance to verify the interactions between the averages. For pH, the sample 6% of tamarind pulp presented lowest value (4.35) compared with sample 2% (4.50) and sample 4% (4.44). For humidity and ash, there were no statistical differences with averages of 76.53% ± 3.41 and 0.47 ± 0.05%, respectively. To sensory evaluation, there were no significant differences between analyzed parameters. The lowest score was to sample 2% (7,71) and the highest was to sample 6% (8.89), but all formulations got notes higher than 7.0 indicating the acceptance of this treatments. The higher score (8.72 ± 0.38) was obtained by 6%, followed by 4% with 8.64 ± 0.52 and 2% of tamarind pulp with 8.45 ± 0.36. All formulations attending to Brazilian legislation and were accepted by tasters showing that this product could be commercialized in the Brazilian market.

Key Words: dairy product, quality control, food composition

T98 Pectin and whey protein concentrate reduces acid whey generation in Greek style yogurt. R. Gyawali*, T. Zimmerman, and S. A. Ibrahim, *North Carolina A&T State University, Greensboro, NC.*

The production of Greek and Greek style yogurt (GSY) generates large amounts of an environmentally harmful waste product known as acid whey. Because disposal of this waste product is an expensive process, the Greek yogurt industry is searching for a solution to decrease the production of acid whey. The purpose of this study was thus to investigate the effects of pectin and whey protein concentrate (WPC) on the generation of acid whey during GSY production. Acid whey production was measured by calculating the water holding capacity (WHC). First, pectin (0.05%) and WPC (1%) were added to skim milk for the production of GSY. The yogurt mixes were then heated at 90°C for 10 min, inoculated with 3.0% of starter culture, incubated at 40°C for 4 h (pH ~4.6), and then refrigerated overnight at 5°C. A control yogurt sample was prepared without the addition of these ingredients. The yogurt made with pectin and WPC had a significantly higher WHC ($P < 0.05$) and lower syneresis than the control. The WHC of yogurt with both pectin and WPC was ~56%, which was 23% higher than the control (33%). Similarly, yogurt supplemented with both pectin and WPC exhibited 15% less susceptibility to syneresis compared with the control. Native PAGE analysis revealed an interaction between pectin and the WPC. Pectin hinders the formation of large oligomeric aggregates of whey protein, which correlates with an increase in WHC and a decrease in syneresis. Our results demonstrated that ingredients such pectin and WPC could be used as additives to lower the generation of acid whey in the production of Greek style yogurt.

Key Words: acid whey, Greek yogurt, pectin

T99 Effect of hydrocolloids on the water-holding capacity of Greek style yogurt. S. A. Ibrahim*, R. Gyawali, and T. Zimmerman, *North Carolina A&T State University, Greensboro, NC.*

Greek style yogurt (GSY) has become popular in the United States and now accounts for more than one-third of total yogurt sales. The popularity of GSY has resulted in a concomitant increase in the production of an unwanted byproduct known as acid whey that cannot be readily utilized nor disposed of easily. Hydrocolloids help bind the water and are promising additives that could be useful in reducing the quantity of acid whey in the production of GSY. In this study, we investigated the effect of hydrocolloids on acid whey production of GSY. Nonfat yogurt samples were manufactured using hydrocolloids (gums and proteins). Gum arabic (GA), Inulin (IN), and Pectin (PE) at 0.01, and 0.05% (wt/vol), whey protein concentrate (WPC), whey protein isolate (WPI) at 0.5 and 1.0% (wt/vol) were mixed slowly into milk at 50°C with agitation. Milk without supplementation served as a control sample. The yogurt mixes were heated at 90°C for 10 min, inoculated with 3.0% starter culture, incubated at 40°C for 4 h (pH 4.6) and, then refrigerated overnight at 4°C. The next day, each sample was centrifuged (1300 *g*, 10 min) and acid whey production was measured by calculating the water holding capacity (WHC). An ANOVA of the data was performed using a completely randomized design and the Tukey test was used to determine statistically different groups. Our results showed that yogurt prepared with gum pectin and whey proteins significantly reduced acid whey production compared with the control sample ($P < 0.001$). The highest WHC was 39.71 ± 0.51, 50.23 ± 0.23, and 48.86 ± 0.24% in yogurt with pectin 0.05%, WPC 1.0%, and WPI 1.0%, respectively compared with the control (34.95 ± 0.97%). Our results demonstrate that hydrocolloids such as pectin and whey protein can reduce acid whey and could have industrial applications for the production of GSY.

Key Words: hydrocolloid, acid whey, Greek style yogurt

T100 Comparison of natural sweeteners in low carbohydrate whey protein bars. H. M. Keefer* and M. A. Drake, *North Caroline State University, Raleigh, NC.*

Protein bar consumption by Americans has increased in recent years as has an interest for natural non-nutritive sweeteners. Each sweetener has unique temporal properties that can influence sensory properties. The objective of this study was to characterize the temporal sensory properties of low carbohydrate whey protein bars with different sweeteners using 3 temporal methods: Time Intensity (TI), Temporal Dominance of Sensations (TDS), and Temporal Check-All-That-Apply (TCATA). A category survey of commercial protein bars (n = 12) was conducted to identify a target sweet taste intensity. Subsequently, protein bars were formulated with whey protein isolate (WPI), fiber syrup, shortening, and each sweetener. Iso-sweet concentrations for each sweetener (sucralose, sucrose, fructose, stevia, monk fruit) in WPI bars were established using magnitude estimation scaling (n = 8 panelists, 3 replications) followed by confirmation by alternative forced choice tests (n = 40). Sweetener blends were subsequently created with reduced bitter and metallic tastes. Temporal sensory profiling (TI, TDS and TCATA) was conducted on protein bars with each sweetener and sweetener blend by a trained panel (n = 8). Consumer acceptance testing was conducted on selected sweeteners in bars. Data were analyzed by appropriate univariate analyses. Protein bars sweetened with fructose or sucrose were characterized by initial intense sweetness that quickly faded. Sucralose displayed a sweet taste profile that was most similar to fructose or sucrose, but differed by metallic taste and lingering sweetness after expectoration. Monk fruit and stevia were slower in sweet taste onset ($P < 0.05$) and were characterized by bitter and metallic aftertastes and lingering sweetness. These sweeteners were characterized by initial dominant sweet taste, then by bitter and metallic tastes by TDS and TCATA. Sucralose and a blend of monk fruit with fructose were the most similar to sucrose sweetened bars ($P > 0.05$), and these bars were preferred by consumers ($P < 0.05$). Knowledge of the temporal properties of non-nutritive sweeteners and the effects of the food matrix on sweeteners are important to understand how sugar reduction and/or sweetener replacement will affect the sensory properties of protein bars.

Key Words: protein bar, sweetener, flavor

T101 Contamination and spatial distribution of Pb, As, and Cd contents in Chinese cow raw milk. X. Zhou[1,2], X. Qu[1], N. Zheng[1], C. Su[1], J. Wang*[1], and H. Soyeurt[1], [1]*Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Statistics, Informatics and Applied Modeling lab, Agrobiochem Department, Gembloux Agro-Bio Tech, University of Liège, Liège, Belgium.*

Due to environmental pollution, heavy metals such as Pb, As, and Cd may contaminate raw milk and can involve serious systemic health problems if they are consumed in excessive concentrations. This study investigated the spatial distribution of Pb, As, and Cd in raw milk produced in the 10 main milk producing areas in China. The contents of Pb, As, and Cd in 996 raw milk samples [i.e., 100 milk samples per area except for 2 area (n = 97, n = 99)] were measured by ICP-MS after

microwave-assisted acid digestion. Non-parametric Kruskal-Wallis test were performed to study the differences of Pb, As, and Cd between areas. Spearman correlations were calculated to assess the relationships between the studied heavy metals. Then, the spatial distribution of Pb, As, Cd was studied by ordinary kriging estimates within the studied areas. Cross-validation was used to assess the robustness of the distribution map. Mean values of Pb, As, and Cd were 1.75, 0.31 and 0.06 μg/L of milk, respectively. Levels of Pb in 1.20% (12/996) of collected samples were above the maximum residue limit (MRL) imposed by the European Union (0.02 mg/kg). All samples were below the Chinese MRL (i.e., 0.05 mg/kg for Pb, 0.1 mg/kg for As). High coefficient of variation were obtained within area suggesting a large variability of those metal contents in milk within regions. This shows the need to conduct a reflection about the best way to collect samples if this kind of pollution in milk want to be studied on a long period. Pb-Cd, As-Cd, Pb-As showed positive significant correlations in 9, 6, and 5 areas, respectively. Correlation values ranged between 0.20 and 0.60. However, these correlations changed between areas suggesting different pollution origins. Based on the ordinary kriging estimates, Pb, As, and Cd showed different spatial patterns following the studied area. Based on the cross-validation, the root mean square error was not closed to the average standard error in some areas. This leads potentially to wrong predictions. The high density of sample collection may lead to this result. Further studies could implement a more appropriate sample collection to clarify the relationships between the contamination of raw milk by heavy metals and the herd environment.

Key Words: heavy metals, milk, spatial distribution

T102 Aptamer-based fluorescence-quenching assay for detection of aflatoxin M_1 in milk samples. Q. Qiao[1,2], F. Wen[1,3], L. Chen[1,3], J. Cheng[2], H. Zhang[1,3], S. Li[1,3], N. Zheng[1,3], and J. Wang*[1,3], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Agricultural University, Hefei, China,* [3]*Milk and Milk Product Inspection Center of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Aflatoxin M_1 (AFM_1), one of the most toxic of the mycotoxins, is a global concern for feed and food contamination. A simple and fast aptasensor for the detection of AFM_1 was developed based on structure-switching signaling aptamer. The principle of the aptasensor is based on fluorescent signal change because of the formation of an AFM_1/aptamer complex. To construct the aptasensor, AFM_1 aptamers were modified with FAM and its complementary DNA (cDNA) was modified by TAMRA quenching group. Without adding AFM_1, AFM_1 aptamers hybridized with cDNA, resulting in quenching of the aptamer fluorescence due to the proximity of the fluorescent group of aptamer to the quenching group of cDNA. After adding AFM_1, the structure switch of AFM_1 aptamer was induced according to the formation of AFM_1/aptamer complex. The changes in the structure of the aptamer released the cDNA, resulting in fluorescence recovery of the aptamer, which enabled the quantitative detection of AFM_1 by monitoring the fluorescence enhancement. Under optimized conditions, this assay exhibited a linear response to AFM_1 in the range of 5–100 ng/mL with a detection limit down to 1.7 ng/mL. The assay was also applied to 2 brand infant formula rice flour samples spiked with a dilution series of AFM_1, obtaining satisfactory recoveries from 96.4 to 103.6% and 95–102.8%, respectively. The results demonstrated that this detection technique had a significant potential for high-throughput, and quantitative determination of mycotoxin levels in dairy products.

Key Words: aflatoxin M_1, aptasensor, fluorescent

T103 Modulation of intestinal epithelial permeability in differentiated Caco-2 cells exposed to aflatoxin M_1 and ochratoxin A individually or collectively. Y. N. Gao[1,2], J. Q. Wang*[1,2], C. C. Luo[1,2], and N. Zheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Milk Product Risk Assessment Laboratory of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Although aflatoxin M_1 (AFM_1) is the only mycotoxin with an established maximum residue limit (MRL) in milk worldwide, it's common to find the co-occurrence of AFM_1 and ochratoxin A (OTA) in milk. The gastrointestinal tract (GIT) is the first tissue barrier to come into contact with food contaminants, such as mycotoxins, and intestinal epithelial cells are most affected. The GIT barrier is constituted by intercellular tight junction (TJ) proteins that localize to the apical domain of epithelial cells. Aflatoxin M_1 (AFM_1) and ochratoxin A (OTA) are mycotoxins commonly found in milk; however, their effects on intestinal epithelial cells have not been reported. In the present study, we show that AFM_1 (0.12 and 12 μ*M*) and OTA (0.2 and 20 μ*M*) individually or collectively increased the paracellular flux of lucifer yellow and fluorescein isothiocyanate (FITC)-dextrans (4 and 40 kDa) and decreased transepithelial electrical resistance values in differentiated Caco-2 cells after 48 h of exposure, indicating increased epithelial permeability. Immunoblotting and immunofluorescent analysis revealed that AFM_1, OTA, and their combination decreased the expression levels of tight junction (TJ) proteins and disrupted their structures, namely, claudin-3, claudin-4, occludin, and zonula occludens-1 (ZO-1), and p44/42 mitogen-activated protein kinase (MAPK) partially involved in the mycotoxins-induced disruption of intestinal barrier. The effects of a combination of AFM_1 and OTA on intestinal barrier function were more significant ($P < 0.05$) than those of AFM_1 and OTA alone, yielding additive or synergistic effects. The additive or synergistic effects of AFM_1 and OTA on intestinal barrier function might affect human health, especially in children, and toxin risks should be considered.

Key Words: mycotoxins, intestinal epithelial cells, permeability

T104 Quantitative PCR coupled with sodium dodecyl sulfate and propidium monoazide for detection of viable *Staphylococcus aureus* in milk. L. Dong[1,2], H. Liu[1,2], L. Meng[1,2], N. Zheng[1,2], and J. Q. Wang*[1,2], [1]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Milk Product Risk Assessment Laboratory of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Staphylococcus aureus has been frequently reported as an agent leading to outbreaks of disease in raw milk. The conventional quantitative PCR (qPCR) are unable to differentiate DNA of viable *Staph. aureus* from dead ones. The aim of this study was to use sodium dodecyl sulfate (SDS) and propidium monoazide (PMA) coupled with lysostaphin to detect viable *Staph. aureus*. The cell suspensions were treated with SDS

and PMA before DNA extraction, because SDS could enhance DNA intercalating ability to dead cells of PMA. The lysostaphin was applied to improve the effectiveness of DNA extraction. The reliability and specificity of this method were further determined by the detection of *Staph. aureus* in spiked milk. The results showed that there were significant differences between the SDS-PMA-qPCR and qPCR when a final concentration of 200μg/mL of lysostaphin was added in DNA extraction ($P < 0.001$). A standard curve with a good linear relationship ($R^2 = 0.9983$, amplification efficiency = 96%) was obtained when lysostaphin was applied at a final concentration of 200 μg/mL. The viable *Staph. aureus* could be effectively detected when SDS and PMA concentrations were 100 ppm and 40 μM, respectively. Compared with conventional qPCR, the SDS–PMA–qPCR assay coupled with lysostaphin was more specific and sensitive. Therefore, this method could detect the number of viable *Staph. aureus* accurately.

Key Words: propidium monoazide, sodium dodecyl sulfate, *Staphylococcus aureus*

T105 Occurrence of tetracyclines, quinolones, lincomycin and streptomycin in milk in China's market. B. Du[1,2], F. Wen[1], Y. Zhang[1], N. Zheng[1], S. Li[1], F. Li[2], and J. Wang*[1], [1]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*College of Pastoral Agriculture Science and Technology,Lanzhou University, Lanzhou, China.*

Veterinary drugs are generally used to prevent and treat diseases in dairy farming. However, abuse or improper use may lead to veterinary drugs residues remaining in milk, which may cause serious side effects on consumers, such as allergic reactions, drug resistance, and toxicity. Therefore, maximum residue limits (MRL) for veterinary drugs have been set by many authorities in the world, including China to guarantee the safety. Rapid screening methods for multiple residues have been widely used in routine test to find the positive ones for further confirmation. In this study, we examined 148 samples of ultra-high temperature (UHT) milk and 50 samples of pasteurized milk collected from the market in China in 2016, using ELISA-based visualization microarray chip technique to assess contamination with tetracyclines, quinolones, lincomycin, and streptomycin. Results showed that the detection rates of tetracyclines, quinolones, lincomycin, and streptomycin in UHT milk samples were 4.7, 3.3, 2.7, and 15.5%, respectively, and in pasteurized milk samples were 16.0, 4.0, 2.0, and 14.0%, respectively. The maximum concentrations of the tetracyclines, quinolones, lincomycin, and streptomycin in all liquid milk samples were 9.06, 4.06, 7.66, and 8.92 μg kg^{-1}, respectively, which is lower than MRLs set by China, the European Union (EU) and the Codex Alimentarius Commission (CAC).

Key Words: milk, veterinary drugs, detection

T106 Development of a rapid detection method of lactoperoxidase in milk. W. Du[1,5], Y. Zhang[1,2], N. Zheng[1,2], F. Li[5], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*Milk Product Risk Assessment Laboratory of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [4]*Milk and Milk Product Inspection Center of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [5]*State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu, China.*

Lactoperoxidase (LPO) is one of the most heat resistant enzymes in milk, and is used as an indicator of heat load of dairy products, especially for overpasteurization of milk. The aim of this study was to develop a rapid detection method of LPO activity of overpasteurization milk, which could be used to qualitatively determine the degree of heat load of milk in practice. Based on the principle of enzymatic reaction, LPO activity was determined by color change using potassium iodide and p-phenylenediamine assay. LPO could oxidize iodide ions to iodide when hydrogen peroxide existed in milk, the LPO activity could be assayed according to the color reaction between iodide and starch. Similarly, p-phenylenediamine could also be oxidated in this way. Samples for the tests were raw and different heat treated milk of 72.5°C, 75°C, 80°C, 85°C, 90°C, 95°C, 100°C, 105°C, 110°C, 115°C, and 120°C for 15 S. Three kinds of reagent solutions (starch soluble, potassium iodide, and hydrogen peroxide) were mixed together by shaking. P-phenylenediamine solution and hydrogen peroxide would be mixed in another assay to determine the LPO activity. The results showed that there were noticeable color changes from blue to light blue for raw and heat treated milk from 72.5°C to 80°C, and the color of heat treated milk above 80°C did not change. It was inferred that LPO lost its enzyme activity in the heat-treated milk above 80°C for 15 S. This study contributed new information to developing a LPO activity assay kit for overpasteurization milk test in practice.

Key Words: milk, lactoperoxidase, enzyme activity

T107 Identification and proteolytic activity quantification of *Pseudomonas* spp. isolated from different raw milks at storage temperatures. L. Meng[1,2], H. Liu[1,2], L. Dong[1,2], N. Zheng[1,2], and J. Wang*[1,2], [1]*Key Laboratory of Quality & Safety Control for Dairy Products of Ministry of Agriculture, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Milk Product Risk Assessment Laboratory of China Ministry of Agriculture (Beijing), Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

The largest proportion of commercial milk products is derived from cows worldwide. Milks from other ruminants are also important culturally and economically. *Pseudomonas* is frequently linked to milk spoilage under different storage temperatures. Therefore, the aim was to measure proteolytic activity of *Pseudomonas* spp. isolated from different raw milks under low storage temperatures. Raw milk samples of cows (n = 87), goats (n = 50), buffalos (n = 25), camels (n = 25), and yaks (n = 25) were collected from 5 provinces in China. *Pseudomonas* was identified by universal 16S rRNA and *rpoB* gene sequence analyses. Proteolytic activity on milk agar, and quantification via the trinitrobenzenesulfonic acid (TNBS) assay at 2°C, 4°C, 7°C, and 10°C were performed to ascertain proteolytic activity of *Pseudomonas* isolates. The TNBS can react with the released α-amino groups, indicators of protein hydrolysis, with the intensity of the yellow-orange color of reaction products. A total of 143 isolates from cow milk samples, 31 isolates from goat milks, 8 isolates from buffalo milks, 9 isolates from camel milks, and 19 isolates from yak milks were confirmed as *Pseudomonas* spp. Of *Pseudomonas*

isolates, we obtained extracellular peptidase activity from 47 (22.4%) strains at 2°C, 91 (43.3%) at 4°C, 128 (61.0%) at 7°C, and 141 (67.1%) at 10°C. However, proteolytic activity of 79 (37.6%) isolates exceeded 2 μmol of glycine equivalents per mL at 10°C, followed by 61 (29.0%) at 7°C, 28 (13.3%) at 4°C, and 9 (4.3%) at 2°C. The results revealed that a large diversity of *Pseudomonas* spp. were present in different raw milks with the ability to produce peptidases at storage temperatures. Most isolates from cow and yak milks had high preoteolytic activity. In conclusion, our findings highlighted the importance of *Pseudomonas* species as broadly adaptable spoilage organisms in different types of raw milk. Low temperature and short period storage before processing (within 48 h) could reduce peptidase production of *Pseudomonas* spp, but milking hygiene should also be properly controlled.

Key Words: raw milk, *Pseudomonas* spp., proteolytic activity

T108 Application of a handheld infrared meter for determining silage moisture. J. J. Blackburn*[1], R. G. Bonner[2], J. P. Goeser[3], C. I. Vahl[1], and M. J. Brouk[1], [1]*Kansas State University, Manhattan, KS,* [2]*Topcon Agriculture Americas, Fort Atkinson, WI,* [3]*Rock-River Laboratories, Watertown, WI.*

This study compared the accuracy of 3 handheld near infrared spectrophotometer (NIRS) units (Digi-Star Moisture Tracker, Fort Atkinson, WI) and 2 on-farm testing methods for predicting DM to conventional oven drying at 60°C of corn silage. Corn silage samples (1,500 g) were obtained from 4 commercial farms in Kansas and analyzed for DM daily for 20 d. Two calibrations were also tested within each unit: NIRu was the DM predicted from the factory-preset calibration, and NIRc was a bias-adjusted DM prediction based on the average difference oven-dried corn silage and NIRu over the 20-d experiment. The NIRc was determined after the experiment was completed. Each sample was scanned 20 times by each NIRS unit and the average predicted DM was recorded as the DM. This process was replicated 3 times with each NIRS unit. Two duplicate 100-g subsamples were dried by 3 different methods: food dehydrator at 71°C (Nesco, Two Rivers, WI), 71, Koster Tester (Koster Moisture Tester Inc., Brunswick, OH), and 60°C oven. The 60°C oven samples were dried for 48 h and then dried in a 105°C oven for 24 h to obtain the final DM daily. Data were analyzed using PROC MIXED (SAS, version 9.4), with method, method × farm, and method × day as fixed effects and equipment as a random effect. Average Oven DM of corn silage was 36.21% for the 20-d experiment. The 3 NIRu predictions of 35.87 ± 0.5957, 32.43 ± 0.5957, and 32.97% ± 0.5957 were significantly different from the corn silage oven value of 36.21% DM ($P < 0.05$). While all 3 NIRc predictions of 36.20 ± 0.7407, 36.25 ± 0.7407, and 36.20% ± 0.7407 were similar to oven DM value of 36.21% for corn silage ($P > 0.05$). The DM predictions for the food dehydrator and Koster Tester of 38.73 ± 0.7407 and 38.22% ± 0.7407 respectively, were significantly different from oven-dried DM for corn silage ($P < 0.05$). The handheld NIRS units accurately predicted DM content of the corn silages when the factory preset calibrations were corrected for bias, while the food dehydrator and Koster Tester overestimated the DM of the corn silage.

Key Words: corn silage, dry matter, near-infrared

T109 Energy-dispersive X-ray fluorescence (EDXRF) as a comprehensive method for mineral analysis of feeds. R. Ward*[1], D. Ye[2], A. Buman[2], D. Pecard[2], and D. R. Mertens[3], [1]*Cumberland Valley Analytical Services, Waynesboro, PA,* [2]*Bruker AXS Inc., Madison, WI,* [3]*Mertens Innovation & Research LLC, Belleville, WI.*

The objective was to evaluate EDXRF for measuring mineral concentrations in feeds and mixtures. When an X-ray tube radiates a sample, secondary X-ray photons are emitted with specific energies that generate spectral peaks related to individual elements. Heights of peaks can be calibrated to calculate element concentrations. Calibration samples included 20 TMR, 10 corn grain, 10 corn silages, 12 legume and 8 grass forages that were analyzed for Ca, P, Mg, K, Na, Fe, Mn, Zn, and Cu by ICP; S by combustion analysis; and Cl by titration. Typically, samples are finely ground (0.2 mm) and pelleted for EDXRF, but for this evaluation samples were ground through a 1.0 mm screen in a cyclone mill and 5 g of loose powder was placed in a 40-mm cup and covered with 3.0-um Prolene film before scanning for 9 min. The EDXRF instrument was a Bruker S2 PUMA with SPECTRA.ELEMENTS software used for calibration. Elements Na, Mg, P, S, and Cl were measured with 20 kV and no filter, and K, Ca, Mn, Fe, Cu, and Zn were measured with 40 kV and a 500-µm Al filter. Tube current was automatically adjusted. Mineral concentration ranges were typical for feeds and mixtures. High R^2 and low standard deviation of linear regression (SD) in the table indicate excellent agreement between analytical and EXDXRF results at selected peaks. Instrument precision was excellent when the same sample was scanned 10 times. When scanning time was reduced to < 5 min, there was a small loss in precision and accuracy. The results (Table 1) indicate that using EDXRF with routine feed sample preparation offers an excellent alternative for mineral analysis in feeds and mixtures.

Key Words: trace mineral

T110 Effect of corn planting population on phosphorus concentration and extraction in the forage (study 1). G. Ferreira* and C. Teets, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

The objective of this on-farm study was to determine the effects of corn planting population on forage yield, plant phosphorus (P) concentration, and P removal from the soil. The study was performed during the

Table 1 (Abstr. T109).

Element	n	Range (in DM; %)	R^2	SD	Peak (keV)	Precision (%)
Ca	58	0.01 to 2.65%	0.986	±0.07%	3.696	±0.01%
P	57	0.15 to 0.60%	0.959	±0.02%	2.016	±0.00%
Mg	60	0.09 to 0.53%	0.941	±0.03%	1.251	±0.01%
S	53	0.10 to 0.48%	0.974	±0.02%	2.311	±0.00%
K	54	0.35 to 3.91%	0.994	±0.07%	3.302	±0.00%
Cl	51	0.00 to 1.88%	0.993	±0.026%	2.635	±0.00%
Na	60	0.01 to 0.72%	0.942	±0.05%	1.047	±0.01%
Fe	57	20.8 to 1821 ppm	0.994	±26.0 ppm	6.400	3.50 ppm
Mn	58	4.61 to 138.3 ppm	0.963	±7.11 ppm	5.895	±1.04 ppm
Zn	60	13.4 to 133.5 ppm	0.986	±3.47 ppm	8.632	±1.71 ppm
Cu	59	1.23 to 24.9 ppm	0.956	±1.35 ppm	8.044	±0.44 ppm

spring and summer seasons of 2014 and 2015 at 2 commercial dairy farms located in Virginia. The study included 7 cornfields with different growing and harvesting conditions. In each cornfield, corn was planted in plots at a theoretical seeding rate of 55,000, 70,000, 85,000, and 100,000 seeds/ha (55K, 70K, 85K, and 100K, respectively). Each seeding rate had 4 replicates within each cornfield. Corn was planted with no-till planters. For all cornfields, the preceding crop was annual ryegrass that was harvested for silage within 10 d before planting corn. Fertilization was the same for all plots and followed nutrient management plans of each farm. At harvesting time, 10 plants from each plot were cut by hand (15 cm above ground), weighed, chopped, mixed, and analyzed for DM and P concentrations. All variables were analyzed using the MIXED procedure of SAS. The statistical model included the effects of field (fixed; 6 df), planting density (fixed; 3 df), planting density nested within field (fixed; 18 df), and the random residual error. Plant DM biomass decreased linearly when planting density increased (376 vs. 253 g of DM/plant for 55K and 100K, respectively; $P < 0.01$), whereas DM yield increased linearly when planting density increased (19.8 vs. 26.0 Mg/ha for 55K and 100K, respectively; $P < 0.01$). The concentration of P in the fresh forage was highest ($P < 0.01$) for 55K and 70K (0.250% DM) and lowest for 85K and 100K (0.235% DM). The removal of P through the harvested biomass increased linearly ($P < 0.01$) when increasing corn planting density (14.0, 16.8, 19.2, and 23.2 kg P/ha for 55K, 70K, 85K, and 100K, respectively). In conclusion, increasing corn planting density decreased P concentration in the corn plant and increased P removal from the field.

Key Words: phosphorus, corn silage, corn planting density

T111 Effect of corn planting population on phosphorus concentration and extraction in the forage (study 2). G. Ferreira and C. L. Teets*, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

The objective of this on-farm study was to determine the effects of corn planting population on forage yield, plant phosphorus (P) concentration, and P removal from the soil. The study was performed on a 200-cow dairy farm located in Virginia. In 2 cornfields, 2 corn hybrids (conventional and BMR) were planted in plots at a theoretical seeding rate of 60,000, 80,000, and 100,000 seeds/ha (LOW, MID, and HIGH, respectively). Each seeding rate had 4 replicates within each cornfield. Corn was planted with a 6-row no-till planter. Plots were 3 (3) 30.5-m long rows separated by 76 cm. Pre-planting fertilization included 112 kg N/ha. The resulting populations were 64,000, 75,000, and 87,000 plants/ha for LOW, MID, and HIGH, respectively. At V6, half of each plot was fertilized with 51 kg N/ha and the other half was fertilized with 102 kg N/ha. At early-dent stage of maturity, 10 plants from each plot were cut by hand (15 cm above ground), weighed, chopped, mixed, and analyzed for DM and P concentrations. Data were analyzed as a split-plot in a completely randomized design. The model included the fixed effects of field, hybrid, planting density, fertilization, and all their interactions, and the random effects of whole and split-plot errors. Dry matter yield tended to differ among planting populations ($P < 0.06$), being greatest for LOW and HIGH (19.2 Mg/ha) and lowest for MID (17.8 Mg/ha). Dry matter yield was greater ($P < 0.01$) for the conventional than for the BMR hybrid (20.6 and 16.9 Mg/ha, respectively). Doubling N fertilization at V6 increased DM yield ($P < 0.03$) from 18.2 to 19.3 Mg/ha. No interaction was observed between planting population and N fertilization ($P > 0.22$). The concentration of P in the forage differed among planting populations ($P < 0.01$), being greatest for LOW (0.249% DM) and lowest for MID and HIGH (0.226% DM). Total P removed differed among planting populations ($P < 0.01$), being greatest for LOW and HIGH (46 kg P/ha) and lowest for MID (40 kg P/ha). In conclusion, under the conditions of this study, high corn planting population resulted in similar DM yields and P removals to those obtained with low planting population, and the concentration of P was reduced at high planting populations.

Key Words: corn silage, planting population, phosphorus

T112 Effect of bacterial inoculants containing *Lactobacillus buchneri* and/or *Lactobacillus hilgardii* on the fermentation and quality of corn silage. K. G. Arriola*, D. Vyas, D. Kim, M. C. Agarussi, V. P. Silva, J. M. Flores, Y. Jiang, A. A. Pech-Cervantes, and A. T. Adesogan, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective was to determine the effect of *Lactobacillus buchneri* and/or *Lactobacillus hilgardii* inoculation on the fermentation and chemical composition of corn silage. Two corn hybrids harvested at either 34 or 43.8% DM (Experiment 1 or 2) were chopped and ensiled in vacuum-sealed bags (40 × 61cm) for 30 and 90 d. Treatments included (1) deionized water (CON) or inoculants containing (2) *Lactobacillus buchneri* (1.5×10^5 cfu/g, LB); (3) *Lactobacillus hilgardii* (1.5×10^5 cfu/g; LH); and (4) *Lactobacillus buchneri* and *L. hilgardii* (1.5×10^5 cfu/g of each inoculant, LB+LH). Samples collected from d 0, 30, and 90 were analyzed for chemical composition and microbial counts. Aerobic stability was measured at d 30 and 90. Data for each experiment were analyzed separately with the Glimmix procedure of SAS for a completely randomized design. In Experiment 1 (34% DM) d 30 samples, inoculation tended to reduce lactate to acetate ratio (6.5 vs. 6.9; $P = 0.07$) and increase aerobic stability (301 vs. 197 h; $P = 0.07$) and LH and LB+LH reduced the pH compared with CON. In d 90 samples, inoculation increased aerobic stability (488 vs. 400 h) and LAB counts compared with CON (7.93 vs. 6.34 log cfu/g; $P < 0.0001$). Also, LH reduced yeast counts relative to CON and LB+LH reduced lactate concentration relative to CON and LB. In Experiment 2 (43.8% DM) d 30 samples, LH reduced DM loss relative to LB (1.89 vs. 9.07%) and LB increased acetate relative to other treatments; inoculation increased ($P < 0.0001$) LAB counts, and reduced lactate concentration (tendency) and yeast counts compared with CON. In d 90 samples, inoculation increased LAB counts (8.91 vs. 6.96 log cfu/g; $P < 0.0001$) and reduced yeast counts (0 vs. 2.38 log cfu/g; $P = 0.003$) compared with CON. In conclusion, inoculating a corn hybrid harvested at 34% with LB, LH, and LB+LH improved LAB counts and aerobic stability after 30 or 90d of ensiling. However, inoculating a hybrid harvested at 43.8% DM improved LAB counts and reduced yeast counts at d 30 and 90 but did not affect aerobic stability.

Key Words: corn silage, *Lactobacillus buchneri*, *Lactobacillus hilgardii*

T113 Meta-analysis of the effect of *Lactobacillus buchneri* inoculation on dry matter recovery and aerobic stability of silages. K. G. Arriola*[1], A. S. Oliveira[2], Y. Jiang[1], I. M. Ogunade[1], D. Kim[1], H. M. Silva[2], F. X. Amaro[1], A. A. Pech-Cervantes[1], S. C. Kim[3], H. Sultana[1], D. Vyas[1], L. F. Ferraretto[1], and A. T. Adesogan[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Institute of Agriculture and Environmental Sciences, Universidade Federal de Mato Grosso, Sinop, Brazil,* [3]*Division of Applied Life Science, Gyeongsang National University, Jinju, South Korea.*

A meta-analysis of 103 peer-reviewed papers was conducted to examine effects of inoculation with *Lactobacillus buchneri* (LB) or homolactic

and or facultative heterolactic acid bacteria (HAB) in combination with LB (LB+HAB) on silage DM recovery and aerobic stability. Raw mean differences (RMD) between inoculant and control treatment means that had been weighted by inverse variance using random models were compared. Heterogeneity sources evaluated included forage type, application rate (AR; $< 10^4$, 10^5, 10^6 or $>10^7$ cfu/g as fed, bacteria type (LB vs. LB plus each of various HAB), enzyme inclusion, ensiling duration and silo type (laboratory or farm-scale) as covariates. Inoculation increased aerobic stability (RMD = 95 ± 52h, $P < 0.01$, n = 163) and DM recovery (RMD = 0.81 ± 0.75%, $P = 0.04$, n = 112) but high heterogeneity was detected (I^2 statistic = 99.8 and 98.3%, respectively). Enzyme use, ensiling duration or silo type did not affect aerobic stability or DM recovery and AR did not affect aerobic stability. Inoculation did not affect the aerobic stability of silages of tropical grasses (RMD = 12 ± 21 h, n = 6), but tended to increase those of corn (RMD = 84 ± 61 h, $P = 0.06$, n = 66), temperate grasses (RMD = 102 ± 18 h, $P < 0.01$, n = 44), alfalfa (RMD = 71 ± 26 h, $P < 0.01$, n = 3), other legumes (RMD = 24 ± 8 h, $P < 0.01$, n = 5), high moisture grain (RMD = 174 ± 35 h, $P < 0.01$, n = 17) and other crops (RMD = 61 ± 46 h, $P < 0.04$, n = 22). The LB and LB+HAB types similarly increased aerobic stability ($P > 0.10$), except LB plus *L. plantarum* (LP) and *Enterococcus faecium* (EF), which had no effect (RMD = 17 ± 32 h, $P = 0.31$, n = 22). Forage or bacteria type did not affect DM recovery, but unlike higher doses, inoculation with 10^5 cfu/g increased DM recovery (RMD = 1.22 ± 0.66%, $P < 0.01$, n = 88). Inoculation with LB alone or combined with HAB (except LB+LP+EF) markedly improved the aerobic stability of corn, alfalfa and other legumes, temperate grasses and high moisture grain, but not tropical grasses. Inoculation also increased DM recovery when applied at 10^5 cfu/g but not at higher doses.

Key Words: *Lactobacillus buchneri*, aerobic stability, silage

T114 Impacts of silage bacterial additives on forage fiber. P. Drouin*[1], S. Ordaz[2], and P. Sivakumar[3], [1]*Lallemand Specialities Inc., Milwaukee, WI,* [2]*University of Vermont, Burlington, VT,* [3]*Lallemand Inc., Lebanon, NH.*

Silage fermentation involves the metabolic activity of lactic acid bacteria, but classical microbiology and next generation sequencing approaches show that several other microorganisms are also active during different phases of fermentation. Carbohydrate-active enzymes from microorganisms and from the plant cells are released and will bind to plant cell walls to initiate degradation. Inoculation of the forage may modify the succession of the microbial population, which would in turn induce modifications to the quality of the fiber. To test this hypothesis, one ensiling trial with corn (37.5% DM-5.2kg/unit) and one with alfalfa (40.0%DM-5.5kg/unit) were performed. The forages were inoculated with one of 4 inoculants (*Lactobacillus plantarum*, *Pediococcus pensosaceus*, *Lactobacillus buchneri*, and mixed inoculants of *Lactobacillus buchneri* and *Lactobacillus hilgardii*) and compared with a negative control. Six independent repetitions were performed. For both trials, the experimental mini-silos (7-L) were opened after 40 and 120 d of fermentation. The silage digestibility was quantified by measuring undigested neutral detergent fiber (NDF). Fermentation quantification and digestibility data were analyzed by ANOVA (treatment × repetition). The size distribution of particles after grinding at 1 mm on a Wiley grinder was measured using laser reflectance technology and analyzed by PCA and LDA. Lastly, changes in carbohydrates were determined by glycome profiling using antibodies targeting branching and terminal fractions of cell wall polysaccharides. Results from NDF digestibility, particles size distribution, and glycome profiling allowed grouping of treatments according to the type of inoculant. Whatever the forage considered, principal component analysis grouped together samples inoculated with heterolactic strains against inoculation with homolactic strains based on digestibility and fiber strength. Glycome profiling showed that xyloglucan concentration was decreased by the microbiota developed in the presence of the heterolactic strains, while glucuronoxylan profile was mostly affected by the microbiota associated with homolactic strains. Either directly or by orienting succession of microbial communities, silage inoculants contribute to the nutritional quality of silage.

Key Words: forage, silage inoculant, fiber

T115 Pasture base dairy farm intensification: the role of growth strategy (stocking rate vs. individual milk production) and availability of infrastructure. M. Aguerre*[1], M. N. Méndez[1], S. Torterolo[1], and P. Chilibroste[2], [1]*Red Tecnológica Sectorial de Lechería, Montevideo, Uruguay,* [2]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, UdelaR, Paysandú, Uruguay.*

The aim of this work was to evaluate in pasture based commercial dairy farms the effect of different productive strategies linked to different combinations of stocking rate (SR, cow/ha), and individual milk production (IMP, L/cow/day) and the availability and condition of the infrastructure to fed the animals, on productivity, diet structure and margin over feeding cost. Sixteen dairy farms were categorized according to SR and IMP in 4 groups (n = 4/group): high SR and high IMP (1.51 ± 0.02 and 24.8 ± 0.42; HH), high SR and low IMP (1.44 ± 0.02 and 18.3 ± 0.26; HL), low SR and high IMP (1.09 ± 0.02 and 23.8 ± 0.25; LH) and low SR and low IMP (1.00 ± 0.01 and 16.2 ± 0.23; LL). Farms were visited biweekly (June 2016/May 2017) to record SR, IMP and the diet supplied to milking cows. Once in each season the availability and condition (m^2/cow; mud presence and mud deep around the fed path and the rest

Table 1 (Abstr. T115). Productivity, margin over feeding and animals dry matter intake of pasture based dairy systems with high stocking rate (SR) and high individual milk production (IMP) (HH) high SR and low IMP (HL) low SR and high IMP (LH) and low SR and low IMP (LL)

Item	HH	HL	LH	LL	SEM
Productivity (L/ha/yr)	14,819^{a}	9,527^{bc}	11,498^{ab}	5,986^{c}	1,566
Margin over feeding (U$S/ha/yr)	1,998^{a}	1,443^{bx}	1,451^{bx}	1,004^{by}	114.4
DMI (kg DM/cow/d)					
Herbage	6.93^{b}	7.94^{ab}	6.1^{b}	9.61^{a}	0.621
Conserved forage	5.19^{a}	3.37^{abx}	5.81^{ay}	2.34^{b}	0.629
Concentrate	7.12^{ax}	4.88^{aby}	6.82^{a}	3.47^{b}	0.617

Values within a same row differ at $^{a–c}$ $P \leq 0.05$ or ^{x,y} $P \leq 0.10$.

area) were recorded. All response variables were analyzed with a mix model with farm category as fixed effect and season as random effect. The HH strategy resulted in the highest productivity and margin over feeding cost (Table 1). Strategies based on high IMP use higher levels of supplements despite the stocking rate. No significant differences were detected among strategies on the availability and conditions of fed paths and rest areas. The HH system shows better performance than any other combination of SR and IMP on productivity and margin over feeding despite the availability and condition of the fed path and rest area.

Key Words: infrastructure, stocking rate, individual milk production

T116 Effects of forage allowance on production and pasture use efficiency in Uruguay. A. de Moura Zanine[1], G. P. M. Rebuffo[2], G. S. Oliveira*[1], D. de Jesus Ferreira[1], R. M. Araújo Pinho[1], M. O. M. Parente[1], and H. N. Parente[1], [1]*Universidade Federal do Maranhão, Chapadinha, Maranhão, Brazil,* [2]*Universidade Federal do Mato Grosso, Cuiabá, Mato Grosso, Brazil.*

Pasture utilization per unit of area and pasture intake per cow are major factors determining milk production of grazing dairy cows, both being primarily controlled by pasture allowance (PA). This study aimed to evaluate the effects of pasture allowance (PA) on forage production and pasture use efficiency by Holstein cows during autumn. The experiment was conducted at the Mario Antonio Cassinoni Experimental Station (Universidad de La República), Paysandú-Uruguay, during autumn from April 9 to June 3, 2010. Eighteen hectares of pasture with Tall Fescue (*Festuca arundinacea*) intercropped for the second productive year with legumes white clover (*Trifolium repens*) and bird's-foot-trefoil (*Lotus corniculatus*), was utilized in this study. Total area was divided into 4 blocks of 4.5 ha, and each block was divided into 3 grazing paddocks (GP) of 1.5 ha. Thirty-six Holstein cows calving in the autumn were distributed in the blocks considering: calving date, body weight, and body condition score. Treatments consisted of 3 PA as follows: high (HA, 38.4 kg DM/cow/day), medium (MA, 30.3 kg DM/cow/day), and low (LA, 26.8 kg DM/cow/day). Means were compared using Tukey test ($P \leq 0.05$). Forage mass (4300; 4240 and 4251 kg DM/ha for HA, MA and LA, respectively) and forage height pre-grazing (15.2; 15.0 and 14.9 cm for HA, MA and LA, respectively) were similar ($P \geq 0.05$) for all grazing targets. However, there was a difference ($P < 0.05$) in forage mass and forage height post-grazing due to the difference in pasture allowance between treatments. The forage mass at the beginning of the experiment was high - on average 4264 ± 352 kg of DM/ha. The highest and lowest pasture use efficiency was obtained with treatments LA (51.2%) and HA (33.1%), respectively. The pasture managements with low PA (26.8 kg DM/cow/day) provided better grazing efficiency. Thus, it is possible to reach greater animal performance per area of dairy cows graze mixed pasture swards due increase of stocking rate. However, the impact of higher stocking rates on the persistence of pasture species needs to be investigated.

Key Words: forage mass, grazing management, stocking rate

T117 Milk production and composition of dairy cows in response to pasture allowance. A. de Moura Zanine[1], G. P. M. Rebuffo[2], G. S. Oliveira*[1], D. de Jesus Ferreira[1], R. M. Araújo Pinho[1], M. O. M. Parente[1], and H. N. Parente[1], [1]*Universidade Federal do Maranhão, Chapadinha, Maranhão, Brazil,* [2]*Universidade Federal do Mato Grosso, Cuiabá, Mato Grosso, Brazil.*

Daily pasture allowance (PA) is defined as the product of pregrazing pasture mass and offered area per animal. Determining the optimum PA is not an easy task because it depends on, and varies with, pasture and animal characteristics; however, it is very important because is directly related to farmer decisions. The objective of this study was to evaluate the effects of PA on milk yield and composition of Holstein cows during autumn. The experiment was conducted at the Mario Antonio Cassinoni Experimental Station (Universidad de La República), Paysandú-Uruguay, during autumn from April 9 to June 3, 2010. Eighteen hectares of pasture with Tall Fescue (*Festuca arundinacea*) intercropped for the second productive year with legumes white clover (*Trifolium repens*) and bird's-foot-trefoil (*Lotus corniculatus*) were utilized in this study. Total area was divided into 4 blocks of 4.5 ha, and each block was divided into 3 grazing paddocks of 1.5 ha. Thirty-six Holstein cows calving in the autumn were allocated to blocks considering: calving date, body weight, and body score. Treatments consisted of 3 pasture allowances as follows: high (HA, 38.4 kg DM/cow/day), medium (MA, 30.3 kg DM/cow/day), and low (LA, 26.8 kg DM/cow/day). Means were compared using Tukey test ($P \leq 0.05$). There was an effect of PA on the milk yield per hectare and milk yield per cow. The highest production per hectare was observed in LA (438 L/ha/day) and the lowest in HA (314 L/ha/day). On the other hand, cows increased individually milk production in HA and MA (25.6 and 26.6 L per cow/day, respectively). The content of protein and fat in milk did not differ among treatments. The yield of milk protein from cows in the LA management decreased (718 g/cow/day) compared with MA (846 g/cow/day), but did not differ from HA (820 g/cow/day). Different pasture allowances affected milk production and composition of dairy cows. The LA provided better conditions for milk yield per area, likely due to the better grazing efficiency. Thus, farmers may increase stocking rate to reach higher milk production when lactating dairy cows graze mixed pasture swards.

Key Words: grazing management, milk protein, mixed pasture

T118 Pasture dry matter intake in intensive dairy production systems: Effects of grazing and feeding management. M. N. Méndez*[1], P. Chilibroste[2], and M. Aguerre[1], [1]*Red Tecnológica Sectorial de Lechería, Montevideo, Uruguay,* [2]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, UdelaR, Paysandú, Uruguay.*

The competitiveness of export based dairy production systems (e.g., Uruguay, New Zealand) is supported by an intensive use of pasture on the diets. The aim of this work was to evaluate which factors linked to feeding and grazing management in commercial dairy farms, determine herbage DMI. Biweekly visits to commercial dairies were carried out between June 2016 and May 2017 to record individual milk production, quantity and type of supplemented on offer, access time to the grazing plots and sward characteristics like sward height, herbage mass and allowance. Herbage DMI (kgDM/cow/d) was determined according to Baudracco et al. (New Zeal. J. Agr. Res., 2010) considering herbage allowance, and by energy balance according to NRC (2001). The dairy farms were categorized in 2 groups according to the differences in DMI between the 2 estimation methods (overall mean ± 15%). Farms that had low differences between both DMI estimation methods were considered farms with high pasture harvesting efficiency (HE, 4.12 ± 1.85 kgMS/cow/d) and farms with large differences were considered farms with low pasture harvesting efficiency (LE, 7.18 ± 0.75 kgMS/cow/d). The 2 groups were compared with the MIXED procedure and considered different when $P \leq 0.10$. No differences were detected between groups in access time to grazing pasture (11.7 ± 4.8 h), sward height (32.1 ± 17.7 cm), herbage mass (2600 ± 1491 kgDM/ha) and herbage allowance (39.7 ± 29.7 kgDM/cow/d). However, HE dairy systems had high pasture and less supplement DMI (kgDM/cow/d) than LE dairy systems

(Table 1). Despite a lower individual milk production in the HE dairy systems, the margin over feeding cost (U$S/cow/d) was not different between groups.

Table 1 (Abstr. T118). Intake, milk production, and margin over feeding cost in dairy systems with high (HE) or low (LE) pasture efficiency use

Item	HE	LE	*P*-value
Herbage intake (kgDM/cow/d)[1]	8.4 ± 0.72	6.5 ± 0.84	0.10
Supplement intake (kgDM/cow/d)	9.2 ± 0.64	14.0 ± 1.10	≤0.01
Milk production (L/cow/d)	20.3 ± 1.02	25.3 ± 1.21	≤0.01
Margin over feeding cost (U$S/cow/d)	4.1 ± 0.30	3.9 ± 0.26	0.60

[1]Estimated by energy balance difference.

Key Words: commercial dairy system

T119 Effect of stocking rate on pasture production and utilization on a grazing dairy system during winter and spring. G. Ortega*[1], T. Nuñez[1], D. Custodio[1], R. Mello[1], Y. Lopez[1], and P. Chilibroste[2], [1]*Agronomy Faculty, Animal Science Department, Progreso, Canelones, Uruguay,* [2]*Agronomy Faculty, Animal Science Department, Grass Production and Utilization on Grazing Systems, Paysandú, Paysandú, Uruguay.*

A farmlet study was conducted to determine the effect of 2 stocking rates [medium (MSR) or high (HSR)] on grass production and utilization. Four farmlets (2 per treatment) on a Uruguayan pasture based dairy system were grazed either with 1.5 (MSR) or 2.0 (HSR) milking cows per hectare from June to December in 2016 and 2017. Nighty 6 cows were randomized to the farmlets based on parity (3.3 + 1.3; 2.1 +1.6), BW (500 ± 91; 520 + 87) and BCS (2.95 ± 0.87; 2.9 + 0.5) for 2016 and 2017, respectively. Every week, the sward mass of each individual plot in each farmlet was assessed through the double sample technique. Based on these observations, herbage intake per hectare was estimated as well as mean growth rate (GR, kgDM/ha/day) for each paddock and the whole farmlet. The grazing area was adjusted weekly based on the GR registered for each treatment. The data were analyzed with a mixed model, which included stocking rate, month (year), as fixed effects. The repeated measurement was week. Differences were declared significant when $P < 0.05$ by Tukey HSD test. Cows grazed a daily strip with a mean herbage allowance above ground of 15.2 vs 13.4 kg DM/cow/d for 2016 ($P < 0.05$) and 18.4 and 15.5 kg DM/d/cow ($P < 0.05$) for MSR and HSR, respectively. Cows on HSR and MSR grazed the same amount of grass (14.5 vs 14.4 kgDM/day/ha) during 2016 but did graze differently during 2017 where HSR cows ate 19.6 vs 14.6 k g DM/day/ha ($P < 0.05$) than MSR cows. Differences in GR (kgDM/ha/day) for the same year were not significant between treatments. Nevertheless, GR was greater in 2016 than in 2017 ($P < 0.05$). The amount of grass harvested in 2016 was higher for MSR (7140 kg DM/ha; 42% grazing:68% haylage) than for HSR (3045 kg DM/ha; 100% grazing). During 2017, total grass harvested was 6339 kg DM/ha for HSR (65% grazing: 35% haylage) and 6441 kg DM/ha for MSR (47% grazing: 53% haylage). Increasing stocking rate (HSR) grazed higher amount of grass per hectare, despite the less harvest opportunity per cow. However, the estrategy of harvest (grazing vs mechanical) will be affected according to stocking rate, where MSR systems were able to harvested more haylage (mechanically).

Key Words: stocking rate, grazing, haylage

T120 Silage feeding programs on intensive dairy farms. T. Bernardes*, M. Cardoso, and L. Lima, *Department of Animal Science, University of Lavras, Lavras, Minas Gerais, Brazil.*

Assessments of feeding programs currently utilized by high-producing dairy herds are important to identify issues and adequate management practices. The aim of this study was to provide silage-feeding programs adopted by intensive dairy farms across the top 6 milk producing Brazilian states. One hundred and 46 farms were surveyed from September 2017 to January 2018 for their feeding practices. Herd managers, and their respective nutritionists, were interviewed by using a common survey form. The form consisted of 21 questions, including information on the characteristics of the herd (n = 5), corn silage (n = 4; whole-plant, high-moisture, reconstituted, and earlage/snaplage), sorghum silage (n = 3; whole-plant, high-moisture, and reconstituted), haylage (n = 5; alfalfa, ryegrass and other crops), other silages (n = 1), hay (n = 1), fresh forage (n = 1), and forage/concentrate ratio (n = 1). The mean, standard deviation, maximum, and minimum of data were calculated using the PROC MEANS procedure of SAS. The number of lactation cows ranged from 26 to 2,020 and daily milk production ranged from 600 to 75,100 L/d. The forage/concentrate ratio ranged from 36/64 to 80/20. Fifty-eight percent of the farms had 2 or more forage sources in the diet. Whole-plant corn silage was fed on all farms, while whole-plant sorghum silage was fed by 6.9% of the farms. High-moisture corn, reconstituted grain corn, and earlage/snaplage were used by 24.8, 16.6, and 3.4% of the farms, respectively. Reconstituted grain sorghum was included in 7.6% of the diets. Thus, more than half of the farms (52.4%) adopted grain silages (corn or sorghum) in the diets. Haylage, hay, and fresh forage were used by 28.3, 45.5, and 31.0% of the farms. Ryegrass and bermudagrass haylage were the most used (56.1 and 39%, respectively). Bermudagrass hay was the most used (68.2%), followed by ryegrass (18.2%) and oat hay (10.6%), respectively. Bermudagrass was also the most used as fresh forage (37.8%). Overall, corn was the most widely grown crop for silage. The majority of herds opted for grain silages as sources of grain in the diet.

Key Words: grain silage, cow feeding program, silage utilization

T121 New approach to properly characterize molasses composition. A. Palmonari[1], L. Mammi[1], D. Cavallini*[1], C. J. Sniffen[2], L. Fernandes[3], P. Holder[3], and A. Formigoni[1], [1]*DIMEVET, Università di Bologna, Bologna, Italy,* [2]*Fencrest LLC, Holderness, NH,* [3]*ED&F Man Liquid Products/Westway Feeds.*

Beet and cane molasses are produced worldwide and widely used in ruminant rations to improve diet palatability and sugar content. However, DM composition is not fully described, and even variability is not considered. Objective of this study was to analyze different molasses to better characterize their composition. Sixteen cane (CM) and 16 beet molasses (BM) were sourced worldwide and analyzed. Gravimetric method was used to determine DM, Kjeldahl for CP, sugars and starch via enzymatic method, minerals by ICP, organic acids and other components in HPLC. Statistical analysis was conducted via ANOVA using the JMP-12 software. Significance was declared for $P < 0.05$. This approach was able to characterize 97.4% and 98.3% DM of CM and BM, respectively. CM showed a numerically lower dry matter content as fed (a.f.) compared with BM (76.8 ± 1.02 vs 78.3 ± 1.61%), as well as CP content (4.8 ± 1.7 vs 10.5 ± 1.1% a.f. $P < 0.05$), with a minimum value of 1% in CM to a maximum of 12% a.f. in BM. The amount of sucrose was higher in BM compared with CM (48.4 ± 1.5 vs 37.5 ± 4.8% a.f. $P < 0.05$), but with great variability even within CM (51.00 max to 33.31 min, % a.f.) and BM (52.00 max to 33.31 min, % a.f.). Glucose and fructose were detected in CM (4.06 ± 2.07 and 6.20 ± 2.17% a.f., respectively),

showing high variability. Organic acid composition differed among molasses. Lactic acid was more concentrated in CM compared with BM (4.69 ± 2.16 vs 3.48 ± 1.37% a.f.), varying from 9.77% maximum to 1.23% minimum within CM, and from 5.55% maximum to 1.37% minimum in BM. Aconitic acid was found only in CM, while glycolic acid in BM. The total sum of acids ranged from 2% to 14% a.f. Sulfates, phosphates, and chlorides had a higher concentration in CM, which showed a lower DCAD compared with BM (4.47 ± 4.97 vs 53.94 ± 33.36 mEq/100g a.f. $P < 0.05$). Within the cane group, it varied from +117.63 to −58.59 mEq/100g a.f., while in beet from +129.20 to +3.24 mEq/100g a.f. In conclusion, data obtained in this study demonstrates the significant differences in molasses composition, highlighting that a more accurate description and characterization is possible and strictly required especially if its use in animal feed is to be fully optimized.

Key Words: molasses, chemical composition, variability

T122 Effects of microbial inoculant and molasses on whole-plant soybean silage harvested in different reproductive growth stages. L. G. Ghizzi, T. A. Del Valle, E. M. de C. Zilio, A. N. Tenório, M. S. S. Dias, J. A. Marques, L. S. Gheller, T. B. de P. Silva, and F. P. Rennó*, *University of São Paulo, São Paulo, São Paulo, Brazil.*

The objective of this study was to evaluate a microbial inoculant and molasses effects on DM losses, dry matter recovered, pH and buffering capacity of whole-plant soybean silage (SS) harvested in different reproductive growth stages. Two hundred mini-silos were used in a completely randomized experiment with treatments in a 5 × 2 × 2 factorial arrangement. Treatments consisted of 5 harvest stages of soybean plant (R3, R4, R5, R6, and R7), 2 levels of molasses inclusion (0 and 40 g/kg of fresh forage), and 2 levels of microbial inoculant inclusion Silobac (Chr. Hansen Industry and Trade Ltda, Valinhos, São Paulo; 0 and 2.5 × 10^{10} cfu/g of a mixture of *Lactobacillus plantarum* and *Pediococcus pentosaceus*). Soybean plants were harvested at 5 cm from the ground, chopped with cut length of 20 mm and ensiled into polyvinyl chloride pipes with 0.5 m length and 0.1 m diameter. Bulk density was set at 650 kg of natural matter/m3. The total DM losses were obtained by the difference between DM ensiled and DM recovered when the silos were opened after 90 d from ensiled. Effluent losses were quantified by weight difference pipe, after silage removal. Gas losses was quantified by the difference in weight of the whole silo after silage and immediately before opening. Silage samples were collected for pH and buffering capacity expressed as miliequivalent of alkali required to change the pH from 4 to 6 per 100 g of dry matter. Data were analyzed using PROC MIXED of SAS 9.3 including the fixed effects of molasses, microbial inoculant and reproductive growth stages. There were 3 ways interactions for gas losses, where molasses increase ($P < 0.001$) dry matter recovered and reduced ($P < 0.001$) pH without microbial inoculant addition. The molasses decrease ($P < 0.05$) total dry matter losses regardless reproductive growth stage except in R4. Molasses addition decrease ($P < 0.03$) effluent losses in R4, R5, and R6 when associated with microbial inoculant. In general the use of molasses as a fermentative additive improved the quality of soybean silage.

Key Words: dry matter, leguminous silage, additive

T123 Growth rate and biomass accumulation in forage maize (*Zea mays*), forage millet (*Echinochloa utilis*), elephant grass (*Pennisetum purpureum*) and gamba grass (*Andropogon gayanus*). O. M. Makinwa, A. H. Ekeocha*, and A. A. Aganga, *Federal University Oye-Ekiti, Oye-Ekiti, Ekiti State, Nigeria.*

To have higher profits and sustained production of livestock it is important to understand the biomass accumulation (BMA) and growth rate (GR) of the forages the animals feed on. This work looked into the nutrient content at various growth stages and biomass accumulation of forage maize (*Zea mays*), forage millet (*Echinochloa utiliz*), elephant grass (*Pennisetum purpureum*), and gamba grass (*Andropogon gayanus*). Experiment was conducted between July and August at a location within Federal University Oye-Ekit, Ekiti State, Nigeria. Planting was done using completely randomized design (CRD) in 4-rows with 4 replicates. Plot size was 27 × 72 cm. Linear additive model was used to test for the effect of the grass specie and the effect of age of cuting on the final BMA of individual cuttings. Harvest was done biweekly starting from 2 wk after planting until wk 8 of planting when experiment was terminated. Highest growing grass in terms of plant height and sward height was *Pennisetum purpureum* throughout the period of carrying out this experiment. *Zea mays* had significantly higher values in leave width (5.44 cm at 8 wk) and number of leaves (8.67 cm at 8 wk) during the experiment when compared with other grass species $P < 0.05$. At the end of 8 wk; *Zea mays* had the highest BMA of 114%, *Echinochloa utiliz* had a BMA of 51.31%. *Andropogon gayanus* had a BMA of 45.53%, *Pennisetum purpureum* had a BMA of 44.32%. Samples from the last cuttings (wk 8) had the highest CP content (11.88% in *Andropogon gayanus*) although there was no significant differences between the CP levels of the different species statistically $P > 0.05$. Crude protein was found to increase nearly linearly as the grasses grew. Crude fiber content of the 3 grass species was observed to undulate as the grasses grew although there was no significant difference $P > 0.05$ between the species. Crude fiber, moisture content and the fat content all followed the same irregular patterns and there was no significant differences $P > 0.05$ between their percentage compositions in all the plant samples. In conclusion, GR reduces with increasing BMA.

Key Words: *Zea mays, Echinochloa utilis, Pennisetum purpureum*

T124 Effect of nitrogen dose and harvesting age on *Tithonia diversifolia* yield and quality. J. A. Elizondo-Salazar*[1] and M. G. Mora-Mora[2], [1]*Universidad de Costa Rica, Facultad de Ciencias Agroalimentarias. Estación Experimental Alfredo Volio Mata,* [2]*Ministerio de Agricultura y Ganadería.*

Tithonia, commonly known as Mexican sunflower, is a shrub belonging to the Asteraceae family. It is widely distributed throughout the humid and sub humid tropics of Central and South America, Asia and Africa. Tithonia has been shown to be an excellent forage for ruminant nutrition, however few studies have been undertaken to evaluate yiel and nutrional quality for dairy cattle feeding. The objective of this study was to evaluate biomass production and nutritional quality of *Tithonia diversifolia* harvested at 3 different ages and fertilized with 3 N doses. The experiment was carried out in the province of Guanacaste, Costa Rica. A randomized block experimental design was used in a split plot arrangement with 4 replications. N rates (0, 150, and 300 kg/ha/yr) were allocated to the main plots, and harvest times (45, 60 and 75 d of regrowth) were allocated to the subplots. DM yield was affected ($P < 0.05$) by the different variables. Plants receiving 300 kg of N and harvested at 75 d of age, yielded 12.1 tons of DM/ha/yr, while plants receiving 0 kg of N and harvested at 45 d of age produced 4.8 tons of DM/ha/yr. CP concentration was affected by treatments ($P < 0.05$) and values ranged between 10.4 and 25.7%. CP yield was affected ($P < 0.05$) by N rate and harvesting age. Plants fertilized with 300 kg of N and harvested at 75 d of age produced 1.9 tons of CP/ha/yr. NDF and lignin concentration were highest ($P < 0.05$) when plants received 0 kg of N and were harvested at 75 d of age (57.9 and 19.2%, respectively). Ash

content was also affected by treatments ($P < 0.05$) and values ranged from 9.8 to 17.5%. Results suggest that *Tithonia diversifolia* seens to be a promising forage for dairy cattle feeding in Costa Rica with best yields and nutritive quality when using N rates ranging from 150 to 300 kg/ha/yr and a harvesting age of 60 or 75 d.

Key Words: forage, nitrogen rate, dairy cattle feeding

T125 Aerobic stability and ruminal degradation of savoy grass silage (*Megathyrsus maximus*) with increasing levels of passion fruit (*Passiflora edulis*) peel. I. F. E. Guerra*, M. L. M. Villacís, L. B. M. Vivas, A. R. S. Laiño, M. J. R. Romero, E. D. T. Navarrete, L. A. G. Montiel, and M. A. M. Villacis, *Universidad Tecnica Estatal de Quevedo, Quevedo, Los Rios, Ecuador.*

The objective of the present study was to determine the aerobic stability and in situ ruminal dry matter degradation of the silage of savoy grass including increasing levels of passion fruit residue (10, 20, 30, and 40% on fresh basis) in the silage of 45-d-old savoy grass. The silage of the chopped and homogenized products was carried out in experimental microsilage (5 replications per treatment). The data for aerobic stability and ruminal degradability were analyzed with the GLM procedure using the treatment as a fixed effect, and the least squares means were compared with the Tukey test. When the effect studied was time, the linear trend was investigated by orthogonal polynomial contrasts. Additionally, the Dunnet test was carried out, using as a reference the value at 0 h, when the linear effect was significant. Statistical significance was set at $P < 0.05$. After 21 d, the microsilages were opened and the aerobic stability temperature (T) and pH for 6 d and the in situ degradation of the dry matter (DM) at 0; 3; 6; 12; 24; 48 and 72 h (h) were determined. The combination between 10 and 40% of passion fruit residue fresh weight with savoy grass did not have important effects on aerobic stability and ruminal degradation. In all the treatments, a low aerobic stability was observed with an increase in T higher than 1°C at 6 d and more than 0.5 pH units after 2 d. The ruminal degradation of the DM was higher ($P < 0.05$) in silages that included 30 and 40% of passion fruit residue, which would indicate a higher nutritional value. Savoy grass silage with passion fruit residue could be an efficient and environmentally acceptable way to dispose of food to sustain the productivity of bovine herds during the dry season. However, the high aerobic instability must be taken into account when handling silage to avoid material and nutrient losses.

Key Words: residue, passion fruit, degradability

T126 Effect of cellulase and fermentation period on the nutritive value of *Panicum maximum* (cv. Mombasa) silage. P. A. Helrigel, V. L. Banys, M. Dias, L. R. Q. Carvalho, N. P. S. Morais, and E. A. Collao-Saenz*, *Universidade Federal de Goiás-UFG, Jataí, GO, Brazil.*

Although Mombasa grass is used for silage production in the tropics, little is known about the changes caused by the addition of fibrolytic enzymes in the ensiled mass. The objective of this study was to determine the effect of cellulase addition on the chemical composition, fiber digestibility, fermentation characteristics and the storage period of *P. maximum* (cv. Mombasa) silage. The experiment was in a completely randomized, 3 × 3 factorial design with 4 replicates (CELLUCLAST doses: 0, 3, and 6% wt/wt, g cellulase/100g cellulose; fermentation periods - 30, 60 and 120 d). Mombasa grass was cut at an approximate height of 80 cm, at 20 cm from the ground level and ensilaged in 36 PVC mini-silos of 4-L capacity. Fermentation of soluble carbohydrates released by cellulose hydrolysis promoted pH and titratable acidity adequacy, demonstrating that there was a higher VFA production and lactic acid favoring the desirable forage fermentation, reducing the DM loss and improving the nutritive value of the silage. NDF was reduced linearly and TDN increased up to 13.8% (Table 1). Cell wall degradation by cellulase during ensiling decreased NDFD by 10.53%, less than the increase in TDN, indicating that the fibrolytic enzyme addition improved the nutritive value and stability of *P. maximum* silages. Mass and nutrient losses (13.8% DM, 8.8% CP) observed in the longer period may indicate that the best storage time is up to 60 d. Enzyme addition was efficient to improve the fermentative and bromatological parameters.

Table 1 (Abstr. T126). Effect of cellulase adding and fermentation period on *P. maximum* silage nutritive value

Variable and fermentation period	Cellulase dose (%)[1]			Effect[1]		
	0	3	6	L	Q	SEM
NDF						
30 d	77.3[a]	72.3[a]	72.1[a]	<0.01	0.1	0.80
60 d	77.7[a]	74.6[a]	65.3[b]			
120 d	74.1[a]	68.7[b]	67.5[b]			
TDN						
30 d	52.6[a]	56.6[a]	55.7[a]	<0.01	0.03	0.56
60 d	52.4[a]	54.5[a]	60.8[a]			
120 d	54.8[a]	58.5[a]	59.3[a]			
DM, % NM	22.8	24.6	24.8	<0.01	<0.01	0.12
ADF, % DM	46.3	42.7	43.0	<0.01	<0.01	0.66
NDFD, %	36.2	32.4	35.2	0.50	0.01	1.01
pH	4.7	3.8	3.6	<0.01	<0.01	0.04
Titratable acidity, °D	5.9	12.4	13.2	<0.01	<0.01	0.28

[a,b]Means followed by the same letter, in a column, do not differ by Tukey test ($P < 0.05$).

[1]L = linear; Q = quadratic.

Key Words: fibrolytic enzyme, TDN, titratable acidity

T127 Effects of overstocking at the feedbunk on the growth performance of replacement Holstein dairy heifers. W. K. Coblentz*[1], M. S. Akins[2], N. M. Esser[2], R. K. Ogden[1], and S. L. Gelsinger[2], [1]*US Dairy Forage Research Center, Marshfield, WI,* [2]*University of Wisconsin, Madison, WI.*

Various forms of overcrowding are common in heifer-rearing operations. Our objectives were to evaluate the effects of overstocking at the feedbunk on the voluntary intake, growth performance, feedbunk-sorting behaviors, displacements from the feedbunk, and hygiene of 128 gravid Holstein heifers (475 ± 55.3 kg) consuming an alfalfa haylage/corn silage diet diluted with processed wheat straw at an inclusion rate of 25.2% (DM basis). Data were analyzed as a randomized complete block design with 4 feedbunk stocking rates (100, 133, 160, or 200% of capacity) represented within each of 4 blocks; pens (n = 16) were considered to be the experimental unit. The statistical analyses of heifer sorting behaviors and displacements from the feedbunk were expanded to include evaluations over time, which were treated as repeated measures. Nutrient intakes were not affected by stocking rate at the feedbunk ($P \geq 0.122$). Overall, the effects of feedbunk stocking rate on growth performance were minor, with only trends for linear increases in total weight gain ($P = 0.086$) and BCS ($P = 0.066$) observed. Collectively, overstocked rates also exhibited a trend ($P = 0.078$) for a better feed:gain ratio than pens stocked at 100% of feedbunk capacity (10.3 vs. 11.0 kg/kg). Heifers sorted against large (>19-mm) particles and NDF, but exhibited preference for short (>1.18-mm, and <8-mm) and fine (<1.18-mm) feed particles, as well as CP over time. However, these responses were not affected by feedbunk-stocking rate ($P \geq 0.205$) or the interaction of stocking rate and daily sampling times ($P \geq 0.364$). During the first hour after daily feed distribution, heifer displacements from the feedbunk were greater ($P \leq 0.007$) for overstocked pens, and increased linearly ($P \leq 0.030$) with stocking rate for most evaluation periods (wk 1, 2, 4, 6, 8, 10, and 12 of the trial). Displacements exceeded 70/h in pens stocked at 200% of capacity during wk 1, 2, and 12. Heifer hygiene of legs and flanks was not affected ($P \geq 0.136$) by competition at the feedbunk. While overstocking at the feedbunk did not affect heifer performance, it should not be practiced blindly without attention to other critical components of animal welfare.

Key Words: dairy heifer, displacement, feedbunk stocking rate

T128 Estimation of starter intake in young dairy calves during the preweaning phase. V. L. Daley*[1], J. K. Drackley[2], C. M. M. Bittar[3], L. O. Tedeschi[4], S. Y. Morrison[2], P. A. LaPierre[2], and M. D. Hanigan[5], [1]*National Animal Nutrition Program (NANP), Lexington, KY,* [2]*University of Illinois, Urbana, IL,* [3]*University of São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil,* [4]*Texas A&M University, College Station, TX,* [5]*Virginia Tech, Blacksburg, VA.*

Equations to predict calf starter intake (SI) were developed and evaluated using individual animal data from studies carried out at the University of Illinois (UI: 1973 observations from 448 calves, initial BW = 42 ± 4.4 kg) and University of São Paulo (USP: 3050 observations from 527 calves, initial BW = 37 ± 5.4 kg). Each data set was randomly divided into 2 subsets (60% for development, 40% for evaluation). The mixed model for weekly repeated measures included the random effect of study. The autoregressive covariance structure (AR1) was adopted in the model. Body weight (BW, kg), daily metabolizable energy intake from the liquid diet (MEiLD, Mcal/d), average daily gain (ADG, g/d) and time relative to first offer of starter (FPstarter, wk) were used as independent variables. The mean square error of prediction (MSEP), mean bias, concordance correlation coefficient (CCC), and analysis of linear regression were used to select models. The UI calves were fed greater amounts of milk replacer than Brazilian calves (761 vs 574 g/d). Models showed a better accuracy after 3 wk of voluntary SI. The model for UI data explained about 65% of the variation in observed SI with a mean bias of –86 g/d, while the model for USP data accounted for 59% with a mean bias of –47 g/d (Table 1). Both models, however, accurately predicted the SI (Cb >0.90). The lower precision of model for USP data is likely because of differences in genetics, management, and environmental variables. These empirical models can provide guidelines for SI of young calves, but additional models might be needed to further improve the predictability of SI.

Table 1 (Abstr. T128).

	Temperate conditions		Tropical conditions	
Variable	Observed (n= 789)	US model[1]	Observed (n= 1220)	Brazilian model[2]
Starter DMI (g/d)	544	630	371	418
Mean bias (Y – X, g/d)	—	−86		
MSEP (g × g)	—	132,823		
CCC	—	0.75		
Cb	—	0.93		
r	—	0.81		
r^2	—	0.65		

[1]Starter DMI (g/d) = [(23.6928 × BW) + (−198.77 × MEiLD) + (0.06441 × ADG) + (61.9202 × FPstarter) − 430.40].

[2]Starter DMI (g/d) = [(14.0273 × BW) + (743.11 × MEiLD) + (−149.24 × $MEiLD^2$) + (0.2696 × ADG) + (−0.00007 × ADG^2) + (−32.5774 × FPstarter) + (14.1437 × FPstarter ^{2}) − 1640.72].

Key Words: dairy calves, starter intake, model

T129 Effect of type of gradual weaning program on intakes and growth of dairy calves fed a high level of milk. S. D. Parsons*[1], K. E. Leslie[2], M. A. Steele[3], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [3]*Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to investigate how 2 gradual weaning programs affect intake and growth of dairy calves during the milk-feeding (d 1–42), weaning (d 43–56), and post-weaning (d 57–70) periods. Sixty Holstein heifer calves were housed in individual pens (152 × 167 cm) and randomly assigned to 1 of 2 treatments: continuous gradual weaning program (CG) or multi-step gradual weaning program (MSG). Calves were offered 12.5L/d of milk replacer (in 2.5-L meals, 5×/d) by an automated rail milk feeder (AMF) until d 43, when weaning commenced according to their assigned treatment. Calves had access to ad libitum water from birth and starter ration of mixed concentrate (95%) and chopped (2.54 cm) wheat straw (5%) from d 5. Calves on CG program are weaned from 12.5L/d to 2.0L/d in small, equal increments until d 57. Calves on MSG program were offered 10L/d for 3 d, 8L/d for 4 d, 6L/d for 3 d, and 3L/d for 4 d until d 57. At d 57, all calves did

not receive milk and were monitored until d 70. Feed and water intakes were measured daily and milk intakes were recorded automatically by the AMF. Calf BW was measured 2 × /wk. Data were summarized by period and analyzed using repeated measures general linear mixed models. Milk intake was similar during the milk-feeding period (CG = 9.1L/d, MSG = 8.9L/d; SE = 0.21; $P = 0.63$). During weaning, milk intake varied by day depending on treatment (treatment × day = $P < 0.01$), but on average CG calves consumed more milk than MSG (6.7 vs 6.1 L/d; SE = 0.12; $P < 0.01$). Feed intake did not differ between treatments in the milk-feeding (CG = 0.063 kg/d, MSG = 0.060 kg/d; SE = 0.0047; $P = 0.66$), weaning (CG = 0.75 kg/d, MSG = 0.80 kg/d; SE = 0.056; $P = 0.54$), or post-weaning periods (CG = 2.97 kg/d, MSG = 2.91 kg/d; SE = 0.093; $P = 0.68$). Growth rates did not vary by treatment ($P = 0.8$) during the milk-feeding (1.08 ± 0.03 kg/d) and post-weaning (1.18 ± 0.06 kg/d) periods. Growth was subject to a treatment × wk interaction (SE = 0.051, $P = 0.006$) during weaning, resulting in CG calves growing at 1.08 kg/d during wk 7 and 0.59 kg/d in wk 8, versus MSG calves growing at 0.94 kg/d in wk 7 and 0.70 kg/d in wk 8. The results indicate that type of gradual weaning program may affect nutrient consumption and growth during the weaning period.

Key Words: dairy calf, weaning, growth

T130 Effect of maternal supplementation with essential fatty acids and conjugated linoleic acid on fatty acid status in neonatal calves. H. M. Hammon*[1], K. L. Uken[1], L. Vogel[1], M. Gnott[1], A. Tuchscherer[1], A. Tröscher[2], and D. Dannenberger[1], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*BASF SE, Lampertheim, Germany.*

Rations for dairy cows are mainly based on corn silage (CS) but contain no fresh grass resulting in low essential fatty acid (EFA), especially α-linolenic acid (ALA), and conjugated linoleic acid (CLA) supply. We investigated the fatty acid status of neonatal calves born from dams, which were supplemented with EFA and CLA and fed a CS based TMR with low fat and ALA content. All calves were delivered by dams abomasally supplemented either with coconut oil (76 g/d; CTRL; n = 9), linseed and safflower oil (78 + 4 g/d; EFA; n = 9), Lutalin® (10 g/d *c*9, *t*11 and *t*10, *c*12 CLA, resp.; CLA; n = 9) or a combination of EFA and CLA (EFA+CLA; n = 11) during the last 9 wk of gestation and the following lactation. During the experimental period, each calf was fed with colostrum from its own dam. Fatty acids (mg/100 mg total fatty acids) were measured in colostrum and in blood plasma of calves on d 1 (before colostrum intake) and d 5 of age. Data analysis was conducted by means of the MIXED procedure of SAS as repeated measurement ANOVA containing treatment, time (blood plasma), treatment × time, and sex as fixed effects and supplementation period and duration of gestation as covariates. Maternal supplementation with EFA and EFA+CLA increased the content of ALA in colostrum ($P < 0.01$), whereas CLA and EFA+CLA supplementation led to higher concentrations of *t*10, *c*12 CLA in colostrum ($P < 0.05$). In calf, plasma total fatty acid concentration and linoleic acid increased ($P < 0.05$) from d 1 to d 5 of age without any treatment effect. Plasma concentration of ALA increased ($P < 0.05$), but docosahexaenoic acid (DHA) decreased ($P < 0.05$) from d 1 to d 5. Concentrations of ALA, DHA, and eicosapentaenoic acid were higher ($P < 0.05$) in EFA and EFA+CLA than in CLA and CTRL on d 1 and 5 of age. Concentration of *c*9, *t*11CLA increased in all groups and on d 1 and 5 was higher ($P < 0.05$) in CLA and EFA+CLA than in EFA and CTRL. Results indicate an improved n-3 fatty acid and CLA status in neonatal calves when cows receive respective fatty acids during late gestation.

Key Words: calf, maternal fatty acid supply, essential fatty acids

T131 Effect of maternal supplementation with essential fatty acids and conjugated linoleic acid on postnatal glucose metabolism in calves. H. M. Hammon*[1], K. L. Uken[1], L. Vogel[1], M. Gnott[1], S. Görs[1], J. M. Weitzel[1], A. Tuchscherer[1], and A. Tröscher[2], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*BASF SE, Lampertheim, Germany.*

The hypothesis was tested that the maternal supply with essential fatty acids (EFA), especially α-linolenic acid (ALA), and conjugated linoleic acid (CLA) affects glucose metabolism in neonatal calves. Dairy cows were fed corn silage based TMR with low fat and ALA content for the last 3 mo of gestation. All calves were delivered by dams abomasally supplemented either with coconut oil (76 g/d; CTRL; n = 9), linseed and safflower oil (78 + 4 g/d; EFA; n = 9), Lutalin (10 g/d *c*9, *t*11 and *t*10, *c*12 CLA, resp.; CLA; n = 9) or a combination of EFA and CLA (EFA+CLA; n = 11) during the last 9 wk of gestation and lactation. Each calf was fed with colostrum from its own dam. Plasma concentrations of glucose and insulin were measured daily in calves from d 1 until d 5 of age before feeding. On d 4, calves were fed their morning meal (6% of BW) mixed with 10 mg/kg BW [$^{13}C_6$]-glucose and 0.5 g/kg BW xylose. Immediately afterward, an intravenous bolus dose of [6,6-$^{2}H_2$]-glucose (5 mg/kg BW) was applied. Blood samples were taken to measure plasma ^{13}C and ^{2}H glucose enrichments and to calculate rates of glucose appearance and fractional first pass splanchnic uptake (FPU) of glucose. Data analysis was conducted by means of the MIXED procedure of SAS containing treatment, time, treatment × time, and sex as fixed effects and supplementation period and duration of gestation as covariates. Before first colostrum intake, plasma glucose concentration was higher ($P < 0.05$) in EFA than in CTRL and CLA calves. Plasma insulin concentration on d 4 was higher 60 min after feeding in EFA than in CLA ($P < 0.05$) and 120 min after feeding in EFA+CLA than in CTRL ($P < 0.05$). Plasma xylose and [$^{13}C_6$]-glucose enrichment increased ($P < 0.05$) in all groups after feeding, the increase of [$^{13}C_6$]-glucose was faster in EFA+CLA than in CLA, and the decrease of xylose was faster ($P < 0.05$) in EFA than in CTRL. Glucose FPU did not differ among treatment groups. Results indicate minor changes of postnatal glucose metabolism with respect to maternal fatty acid supply, but point at an effect of maternal EFA on fetal glucose supply.

Key Words: calf, maternal fatty acid supply, neonatal glucose status

T132 Effects of corn processing and bypass soybean meal in calf starter on growth and digestibility in young dairy calves. T. S. Dennis*, F. X. Suarez-Mena, T. M. Hill, J. D. Quigley, W. Hu, and R. L. Schlotterbeck, *Provimi, Brookville, OH.*

This study evaluated performance and diet digestibility of pre-weaned calves fed starters with 2 types of corn processing and 2 levels of heat-treated soybean meal (SBM). The 4 starters used were whole corn (WC) with conventional SBM (CS; 25% of diet as-fed), WC with a 55:45 blend of CS and SoyPass® (LignoTech USA; SP), flaked corn (FC) with CS, and FC with SP. Starters were texturized (35% corn, 35% protein pellet, 27% whole oats, and 3% molasses) and formulated to be equal in CP (20%), starch (44%), and NDF (17%) on a DM basis. Calves were fed one milk replacer (26% CP, 18% fat on DM basis) at 0.66 kg DM for 39 d then 0.33 kg for 3 d. The trial used 96 male Holstein calves (42.7 kg BW, 3–4 d of age) in 2 separate blocks. Calves were housed in individual pens in a naturally ventilated nursery and bedded with wheat straw. Intake was measured daily, calves were weighed weekly, and hip width (HW) and body condition score (BCS) was measured every 2 wk. In the first block, fecal samples were taken from 5 calves/treatment from d 33–37 and d 54–56 to estimate diet digestibility. Fecal pH was evaluated using a digital pH meter before compositing fecal samples

by calf and period. Data were analyzed as a completely randomized design with calf as experimental unit and block as a random variable. Pre-weaning (d 0–42), HW change was greater (3.2 vs. 2.8 cm; $P < 0.05$) for calves fed WC+CS vs. other treatments. Post-weaning (d 43–56), starter intake was greater (2.08 vs. 1.95 kg/d; $P < 0.05$) and BCS change was lesser for calves fed WC vs. FC ($P < 0.05$). No other differences in performance were observed. Digestibility of NDF was less for calves fed FC vs. WC pre-weaning (33 vs. 48%) and post-weaning (46 vs. 51%; $P < 0.05$). Digestibilities of OM, CP, and fat were greater pre-weaning, whereas NDF and ADF digestibilities were greater post-weaning ($P < 0.05$). Fecal pH tended to be greater ($P < 0.10$) for calves fed FC vs. WC (7.51 vs. 7.46) but was not biologically different. In this study, increased corn processing and heat-treated SBM did not improve growth or diet digestibility in calves under 2 mo of age.

Key Words: calf starter, whole corn, steam-flaked corn

T133 Effects of corn processing and bypass soybean meal in calf starter on growth and digestibility in dairy calves from 2 to 4 mo of age. T. S. Dennis*, F. X. Suarez-Mena, T. M. Hill, J. D. Quigley, W. Hu, and R. L. Schlotterbeck, *Provimi, Brookville, OH.*

This study evaluated performance and diet digestibility of weaned calves fed starters with 2 types of corn processing and 2 levels of heat-treated soybean meal (SBM). The 4 starters used were whole corn (WC) with conventional SBM (CS; 25% of diet as-fed), WC with a 55:45 blend of CS and SoyPass® (LignoTech USA; SP), flaked corn (FC) with CS, and FC with SP. Starters were texturized (35% corn, 35% protein pellet, 27% whole oats, and 3% molasses) and formulated to be equal in CP (20%), starch (44%), and NDF (17%) on a DM basis. Calves were previously fed one milk replacer (26% CP, 18% fat on DM basis) at 0.66 kg DM for 39 d then 0.33 kg for 3 d and received the same starter treatments. Ninety-six male Holstein calves (77.6 kg initial BW) were grouped by previous starter treatment (4 calves/pen) and fed a 95% starter, 5% chopped grass hay diet (as-fed basis) for 56 d. Pen intake was measured daily and BW, hip width (HW), and body condition score (BCS) was measured every 28 d. From d 27–30, fecal samples were taken from the pen floor and composited by pen to estimate diet digestibility. Data were analyzed as a completely randomized design with pen as experimental unit and block as a random variable. No interactions of corn processing and heat-treated SBM were observed for performance or digestibility estimates. Overall, BCS change was less ($P = 0.04$) and HW change tended to be less (4.5 vs. 4.7 cm; $P = 0.07$) for calves fed FC vs. WC. Digestibility of starch was greater (97.8% vs. 95.4%; $P = 0.01$) and NDF digestibility was lesser (49.8% vs. 57.4%; $P = 0.02$) for calves fed FC vs. WC. Other digestibility measurements did not differ. In this study, increased corn processing moderately improved starch digestibility but reduced fiber digestibility substantially and did not increase growth or feed efficiency. Additionally, using bypass SBM did not improve performance or digestibility estimates. It does not appear that increasing grain processing or including more bypass protein results in better energy or protein utilization in calves under 4 mo of age but would increase feed costs.

Key Words: calf starter, whole corn, steam-flaked corn

T134 Supplementing pasteurized colostrum from primiparous cows with colostrum replacer improves colostrum quality and serum IgG levels in Holstein neonate calves. S. Vázquez-Flores*[1], A. J. Geiger[2], A. E. Olamendi-Uresti[1], D. M. Aguilar-López[1], L. E. Díaz[1], and C. L. Rodríguez[1], [1]*Department of Bio-engineering, Tecnológico de Monterrey campus, Querétaro, Mexico,* [2]*Zinpro Corporation, Eden Prairie, MN.*

The objective of this study was to assess the effect of providing additional IgG to colostrum from primiparous cows on neonatal Holstein calves. The study was conducted on 60 Holstein bull calves enrolled at birth. During their first feeding, 30 calves received pasteurized colostrum (GC) and 30 calves received pasteurized colostrum supplemented with a commercial colostrum replacer (GSC; 55 g IgG). All colostrum was collected from primiparous cows, pasteurized at 60°C for 30 min, supplemented (if GSC) and frozen until use. When suckling was the strongest, calves received colostrum from one of the treatments, warmed to 38°C. All calves were bottle fed in the first feeding, with an average intake of 2.3L within the first 2 h of life. Blood samples were taken at 24h of life in vacutainer tubes and tested for total proteins with a manual refractometer, brix, and radial immunodiffusion (RID). A Wilcoxon test was used to analyze and compare serum IgG and apparent efficiency of absorption of IgG (AEA) while colostrum brix was compared with HSU´s MCB test. Analysis of colostral IgG indicated that GC had 1455 ± 7.40 mg/dL, and GSC had 1505.5 ± 7.32 mg/dL ($P = 0.008$). Brix results for colostrum were greater for GSC than GC (9.58 ± 0.6 vs. 8.40 ± 1.1; $P < 0.05$). Calves fed GC had greater serum IgG than calves fed GSC (1661 ± 1955 vs. 2031 ± 1987 mg/dL; $P = 0.0041$). Calves fed GSC had greater AEA compared wtih calves fed GC (48.1 ± 7.1 vs. 43.9 ± 5.6%; $P < 0.001$). The study indicates that pasteurized colostrum from primiparous cows supplemented with a commercial colostrum replacer resulted in high serum IgG and AEA in neonatal Holstein calves. This approach is useful for feeding colostrum from primiparous cows without compromising passive immunity.

Key Words: primiparous cow, colostrum replacer, pasteurization

T135 Dietary effects on rumen epithelial proliferation dynamics in preweaned calves. T. T. Yohe*, C. L. M. Parsons, H. L. M. Tucker, B. D. Enger, N. R. Hardy, and K. M. Daniels, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Epidermal stem cell function is regulated by external cues from the environment and replenishing the epidermal cell population is initiated in the basale layer of its stratified epithelium. Like the epidermis, ruminal stratified epithelium responds to external stimuli, such as diet. The objectives of this study were to assess the effects of 2 different feeding programs on abundance of potential stem and progenitor cell populations and proliferation of rumen epidermal cells in preweaned calves. Neonatal Holstein bull calves (n = 12) were individually housed and randomly assigned to one of 2 diets. Diets were isoenergetic (3.87 ± 0.06 MCal of ME/d) and isonitrogenous (0.17 ± 0.003 kg/d of apparent digestible protein). Diets were: milk replacer only (MRO; n = 6), or milk replacer with starter (MRS; n = 6). Milk replacer was 22% CP, 21.5% fat (DM basis) while the textured calf starter was 21.5% CP (DM basis). Water was available ad libitum. Feed and water intake were recorded daily. Putative stem and progenitor cells were labeled via administration of a thymidine analog (5-bromo-2′deoxyuridine; BrdU; 5 mg/kg BW in sterile saline) for 5 consecutive days and allowed a 25-d washout period. Calves were euthanized on d 43 ± 1. Rumen tissue was obtained from the ventral sac and processed for fluorescent immunohistochemical analyses of BrdU (putative stem and progenitor cells) and Ki67 (cell proliferation). Data were analyzed using the MIXED procedure in SAS 9.4. Body growth did not differ between treatments, but empty reticulorumens were heavier in MRS calves (MRS: 0.67 ± 0.04 kg; MRO: 0.39 ± 0.04 kg; $P = 0.001$). The percentage of label-retaining BrdU basale cells was higher in MRO compared with MRS calves (2.0 ± 0.3% vs 0.3 ± 0.2%, respectively; $P = 0.001$). A higher percentage of basale cells

undergoing proliferation was observed in MRS than MRO calves (18.4 ± 2.6% vs 10.8 ± 2.8%, respectively; $P = 0.079$). These results indicate that presumptive stem and progenitor cells within the rumen epidermis are identifiable by their ability to retain labeled DNA long-term and change proliferative status in response to diet.

Key Words: dairy, calf nutrition, rumimant physiology

T136 Ruminal and whole-tract diet digestion in calves before, during and after weaning. S. L. Gelsinger*[1], W. K. Coblentz[2], G. I. Zanton[2], and R. K. Ogden[2], [1]*University of Wisconsin, Madison, WI,* [2]*United States Department of Agriculture Dairy Forage Research Center, Madison, WI.*

An experiment was conducted to determine ruminal and whole-tract digestibility of 2 starter feeds in calves at 5, 7, 9, 11, 13, and 15 wk of age. Ten (n = 5/diet) bull calves were cannulated at 3 wk of age. They received milk replacer and one of 2 calf starter diets through wk 7, then starter only (up to 4500 g/hd per d) through wk 15. Diets were a complete pellet (A; 42% starch, 13% NDF) and texturized (B; 31% starch, 22% NDF). Portions of each diet were dried, ground and 1.25g was inserted into concentrate in situ bags (5 cm × 10 cm, 50µm porosity). Each calf received duplicate bags of each diet for a total of 8 bags/calf (2 diets × 2 timpoints). All bags were inserted at the time of starter feeding and removed 9 or 24 h later. This process was repeated over 3d each wk. Daily starter intake and fecal output were recorded during the same 3-d periods. Each diet, refusals, and feces were subsampled, dried, ground, composited by calf by wk and analyzed for NDF and N content. Apparent digestibility coefficients, total intake and output were calculated and submitted to the mixed procedure of SAS with $P < 0.05$ considered significant. In situ ruminal DM disappearance was greatest for diet A at 9 and 24h. Ruminal DM disappearance of both diets increased with age. Disappearance of DM for diet A and B after 24-h incubation increased from 79.4 and 71.7% at 5 wk to 84.3 and 77.1% at 15 wk, respectively. In situ ruminal DM disappearance after 24h tended to be greater for both diets when placed inside the rumens of calves consuming diet B (79.0 vs 77.9%; $P = 0.10$). Total starter intake and fecal output of DM, NDF, and N increased with time and were greater for B calves. While in situ DM disappearance increased, whole-tract starter DM digestibility changed quadratically with time: increasing from 74.7% in wk 5 to 80.7% in wk 7 and then progressively declining to 74.6% in wk 15. Whole-tract digestibility of DM and N was greater ($P < 0.01$) and NDF tended to be greater ($P = 0.07$) for A calves. This is likely due to diet formulation rather than physiological differences between groups as the consumed diet did not significantly effect in situ ruminal DM disappearance.

Key Words: calf, in situ, digestibility

T137 Phenotypic evaluation of body weight at first calving. L. Han*[1], A. J. Heinrichs[1], A. DeVries[2], and C. D. Dechow[1], [1]*Department of Dairy and Animal Science, The Pennsylvania State University, University Park, PA,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objectives of this study were to investigate the association of body weight at first calving (BWFC) with first lactation milk production in Holstein heifers and examine the relationship between BWFC and the percent body weight change (WC) during the first month of lactation. We retrieved daily milk production records and daily body weight records from Afimilk. Edited data included records from 716 Pennsylvania State University and 1113 University of Florida Holstein heifers that calved from 2001 to 2016. BWFC was the mean body weight from 5 DIM to 10 DIM at first calving. Average BW from 30 to 40 DIM (BW35) was determined and WC % derived as 100 × (BW35 − BWFC)/BWFC. The 305d milk yield was analyzed with a generalized linear model that included significant ($P < 0.05$) effects of herd-year-season of calving, age at calving, and either BWFC (10 levels within farm-year), WC (10 levels within farm-year), or both BWFC and WC. WC % was analyzed with the same model to determine associations with BWFC. The average of 305d milk yield, BWFC, WL were 9497 kg, 538 kg, and −2.8%, respectively. Least-squares-means for 305d milk yield was lowest (9510kg) for the lowest BWFC group (group mean 459kg) and highest (10,219kg) for the second highest BWFC group (group mean 591kg), then numerically dropped to 10,160 kg for the highest BWFC group (group mean 628kg). Least-squares-means for 305d milk yield was highest (10,506kg) for the lowest WC group (group mean −9.76%) and lowest (9248kg) for the highest WC group (group mean 3.78%). When considered in the same model as WC, the effect of BWFC was not significant though higher BWFC was numerically associated with greater milk yield. The highest BWFC group was associated with the most WC (−3.75%), whereas the lowest group had the smallest WC (−0.90%). These results indicate that heavier heifers and those that lose the most weight within a given farm and year tend to produce more milk during the first lactation.

Key Words: body weight, calving, milk yield

T138 Performance effects of feeding Holstein calves a whole milk formulated milk replacer. A. J. Keunen*[1] and D. L. Renaud[2], [1]*Mapleview Agri Ltd., Palmerston, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Nutrient intake during the pre-weaning period has short and long-term effects on the health and performance of calves. Following birth and colostrum feeding, many calves are fed a commercial milk replacer (MR) formulated to have a significantly lower energy content than whole milk. The objective of this study was to evaluate the health and performance of male calves fed a MR with similar milk components of a Holstein cow (26% CP and 32% fat) (WMF), compared with an accelerated MR formulation (26% CP and 17% fat) (AMF). All calves enrolled (n = 240) were sourced from 35 local dairy farms and were approximately 7 d of age. Calves were randomized at arrival, fed individually for the first 28 d, and then commingled into groups of 5 consecutive calves of the same treatment group until d 49. MR was fed twice daily and refusals were monitored and recorded. On average, each calf consumed 0.58 kg of MR daily on a dry matter basis. Calf starter (18% CP) was offered throughout the trial and all intakes were recorded. Calves were observed daily for signs of respiratory disease and fecal scores were recorded individually for the first 14 d. Body weights were recorded at arrival and on d 49. A total of 10 calves (8%) died in the WMF group, whereas 2 calves (2%) died in the AMF group. No differences were observed between groups with respect to diarrhea or respiratory disease treatment. The mean average daily gain (ADG) from arrival to d 49 was 0.63 kg for the enrolled calves. A mixed linear regression model was built to evaluate the impact of the MR formulation on ADG. Calves fed the WMF had a 0.13 kg per day ($P < 0.001$) reduction in ADG when compared with the AMF group. If calves were treated for diarrhea during the trial, they had a 0.15 kg per day ($P < 0.001$) reduction in ADG compared with those that were not treated. It also found that calves that weighed >51 kg at arrival, gained 0.16 kg per day ($P = 0.01$) more than calves <44 kg at arrival. The outcome indicates that calf performance is compromised

when feeding a MR formulated with equivalent CP and fat components of milk from a Holstein cow.

Key Words: milk replacer, dairy calf, performance

T139 Prediction of metabolizable energy in calf starters. J. D. Quigley*, T. M. Hill, J. R. Knapp, W. Hu, T. S. Dennis, F. X. Suarez-Mena, and R. L. Schlotterbeck, *Provimi, Brookville, OH.*

Total-tract digestibility (TTD) data from 3 published studies were used to evaluate NRC (2001) estimates of metabolizable energy (ME) in calf starters (CS). Holstein bull calves (2–3 d of age at initiation of each study) were fed varying amounts of milk replacer (MR) with texturized or pelleted CS and water for ad libitum consumption. Calves (n = 60) were housed in individual pens for 8 wk (Trials 1, 2a, 3a). In 2 trials, calves were subsequently moved to group pens (4 calves/pen; n = 24) for an additional 8 wk (Trials 2b, 3b). Apparent TTD was measured at 3, 6 and 8 wk (Trial 1); 5 and 8 wk (Trial 2a); 11, 13 and 16 wk (Trial 2b); 5, 7 and 8 (Trial 3a); and 11 and 15 wk (Trial 3b). Feed and feces were collected from 20 calves (Trials 1, 2a, 3a) or 12 pens (Trials 2b, 3b) during each 5-d fecal collection period. Chromic oxide in MR and acid insoluble ash in CS were used as digestion markers before and after weaning, respectively. Apparent TTD of NDF, non-fiber carbohydrate (NFC), CP and fat were used to calculate ME in CS, which was subsequently adjusted for contributions of CP and fat from MR. Mixed model analyses for meta-analyses outlined by St. Pierre (2001; J. Dairy Sci. 84:741–755) were used to build prediction models. A total of 210 observations were used. Estimates of NFC and NDF TTD in CS were low early in life and increased with increasing CS nutrient intake (particularly NFC) in a logarithmic fashion. Because CS were composed mainly of NDF and NFC, calculated ME in CS was low, but increased with increasing NFC intake. Natural logarithm of daily NFC intake (lnNFCi, kg/d) accounted for 61, 12, 22, 57, and 87% of the variation in TTD of DM, CP, fat, NDF and NFC, respectively, in CS. Equation for ME (Mcal/kg DM) in CS was 0.3938 ± 0.0511 ´ lnNFCi + 2.9785 ± 0.0551 (r^2 = 0.70). Calculated ME in CS was < 75% of NRC estimated ME when calves consumed 0.2 kg/d of NFC, 98% of NRC estimated ME at 1 kg/d of NFC intake and 109% of NRC estimated ME at 2 kg/d of NFC. Ability of the calf to extract energy from CS is dynamic and depends on intake of NFC and NDF from CS. Feed management that delays CS intake may reduce TTD of NFC and NDF and calculated ME in CS.

Key Words: calf starter, energy, digestibility

T140 Weaning based on starter intake: Effects on weight gain and behavior. J. Benetton, H. W. Neave, J. H. C. Costa, M. A. G. von Keyserlingk, and D. M. Weary*, *University of British Columbia, Vancouver, BC, Canada.*

Automated feeders can be used to wean calves according to individual solid feed intakes. The aim of this study was to compare feeding behavior and performance of calves that were weaned based on individual starter intakes versus a step-down procedure based upon calf age. Dairy calves (n = 32) were randomly assigned to either step-down (Step) or individual starter intake weaning (ISW). All calves were offered 12 L/d milk until d 30 of age. At d 31, Step calves were reduced to 6 L/d of milk over 5 d and received 6 L/d milk from d 35 until d 63 when milk was reduced over 7 d until complete weaning at d 70. At d 31, ISW calves were reduced to 75% of that individual's previous 3-d milk intake average. Milk was further reduced (by 25%) when calves consumed a 3-d rolling average of 225 g/d of starter. Milk was again reduced by 25% when calves reached 675 g/d, and then completely weaned at 1300 g/d. Milk and starter intake, number of unrewarded visits to the milk feeder and ADG were calculated for 3 periods: weaning (d31–70 for Step; d31 until 1300 g/d of starter was consumed for ISW), post-wean (d70–98 for Step; d of completed weaning until d 98 for ISW), and experimental period (d0–98). Over the experimental period, no difference in performance was found between treatments, but calves in the ISW treatment consumed less milk (123 ± 15.2 L) and more starter (47 ± 8.9 kg) compared with Step calves. ISW calves tended to gain more weight during the weaning period compared with Step calves (1.1 ± 0.1 vs 0.7 ± 0.1 kg/d) but there were no treatment differences during post-wean. ISW calves consumed less milk and ate more starter during the weaning period compared with Step calves (milk: 5.0 ± 0.2 vs 2.0 ± 0.2 L/d; starter: 1.5 ± 0.1 vs 0.6 ± 0.1 kg/d, respectively). However, ISW calves had more unrewarded visits to the milk feeder during weaning compared with Step calves (12.4 ± 1.6 vs 6.4 ± 1.2 visits/d, respectively). These results suggest that calves weaned based on individual starter intake consume less milk and eat more starter but show greater signs of hunger during the weaning period compared with calves weaned on a step-down method. Further research is needed to develop appropriate weaning methods.

Key Words: precision dairy, automated milk feeder, weaning

T141 Effects of feeding different amounts of milk replacer on nutrient digestibility in Holstein calves to 2 months of age using different weaning transition strategies. R. N. Klopp*[1], T. M. Hill[2], F. X. Suarez-Mena[2], R. L. Schlotterbeck[2], and G. J. Lascano[1], [1]*Clemson University, Clemson, SC,* [2]*Nurture Research Center, Provimi, Brookville, OH.*

Growth and the digestibility of nutrients can be greatly affected by diet pre-weaning and the rate at which calves are weaned. A 2x2 factorial design (moderate (MOD) or high (HI) milk replacer (MR) feeding rates and abrupt (AB) or gradual (GR) weaning) was utilized to compare how nutrient digestibility pre-weaning is affected. Calves (n = 50) were randomly assigned to one of 4 treatments (13 for MOD-AB and MOD-GR, 12 for HI-AB and HI-GR). Data were analyzed as a completely randomized design with repeated measures when applicable by PROC MIXED in SAS. Calves assigned to MOD-AB were fed 0.66 kg (DM basis) MR for first 42 d then 0.33 kg for last 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d. All calves received the same MR (25% CP, 17% fat DM basis) and were given ad libitum access to water and a textured starter (42% starch and 20% CP). On d 26–30 and d 45–49, a fecal sample was taken from 5 calves in each treatment via the rectum to estimate apparent digestibility coefficients (dC). Apparent dC of DM (P = 0.01), OM (P = 0.01), and fat (P = 0.04) were greater for HI vs. MOD calves. Apparent dC of NDF (P < 0.01), ADF (P < 0.01), and sugar (P = 0.02) were greater for MOD vs. HI. Apparent dC of DM (90.9, 89.0 ± 0.5384; P = 0.05) was greater for AB vs. GR and the apparent dC of ADF (39.5, 32.3 ± 1.67; P = 0.01) was greater for GR vs. AB. There were no significant differences between CP or starch dC based on treatment and no interactions were observed. Starter intake was greater for MOD calves compared with HI (P < 0.01) and GR consumed more than AB (P < 0.01). These results suggest that calves receiving HI MR pre-weaning digest readily available nutrients more efficiently but MOD MR results in increased fibrous fractions dC. Accordingly, AB weaning strategy had higher dC for DM and OM but there was a depression in fiber dC.

Key Words: calves, digestibility, weaning

T142 Effects of feeding different amounts of milk replacer on nutrient digestibility in 2- to 4-month-old Holstein calves using different weaning transition strategies. R. N. Klopp*[1], T. M. Hill[2], F. X. Suarez-Mena[2], R. L. Schlotterbeck[2], and G. J. Lascano[1], [1]*Clemson University, Clemson, SC,* [2]*Nurture Research Center, Provimi, Brookville, OH.*

Growth and the digestibility of nutrients can be greatly affected by diet pre-weaning and the rate at which calves are weaned. A 2 × 2 factorial design (moderate (MOD) or high (HI) milk replacer (MR) feeding rates and abrupt (AB) or gradual (GR) weaning) was utilized to compare how nutrient digestibility post-weaning is affected by pre-weaning program. Fifty calves were randomly assigned to 1 of the 4 pre-weaning treatments (13 for MOD-AB and MOD-GR, 12 for HI-AB and HI-GR). Data were analyzed as a completely randomized design with repeated measures when applicable by PROC MIXED in SAS. Calves assigned to MOD-AB were fed 0.66 kg (DM basis) MR for first 42 d then 0.33 kg for last 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d. For the 56 d grower portion of the trial, calves were grouped into 12 pens (n = 12) based on MR program, 4–5 calves per pen (3 pens/TRT). All calves received ad libitum access to water and a textured starter (42% starch and 20% CP) blended with 5% chopped grass hay. On d 66–70, d 87–91, and d 108–112, fecal samples were taken from the pen floor with care not to sample floor materials and composited by pen to estimate apparent digestibility coefficients (dC). Apparent dC of DM ($P < 0.01$), NDF ($P = 0.01$), and ADF ($P < 0.01$) were greater for MOD vs. HI. Apparent dC of DM (82.2, 81.3 ± 0.24; $P = 0.01$) and ADF (54.1, 50.5 ± 1.06; $P = 0.02$) were greater for GR vs. AB. There were no significant differences between OM, CP, fat, starch, or sugar dC based on treatment and no interactions were observed. Starter consumption was greater for MOD calves compared with HI calves ($P < 0.01$) and GR consumed more than AB ($P < 0.01$). These results suggest that calves receiving MOD amounts of MR pre-weaning are more adapted to consuming more complex nutrients and that a GR weaning is beneficial during transition.

Key Words: calves, digestibility, weaning

T143 Effect of milk replacer feeding program on calf performance and digestion. F. X. Suarez-Mena*[1], T. S. Dennis[1], T. M. Hill[1], W. Hu[1], J. D. Quigley[1], R. L. Schlotterbeck[1], R. N. Klopp[2], G. J. Lascano[2], and L. E. Hulbert[3], [1]*Provimi, Brookville, OH,* [2]*Clemson University, Clemson, SC,* [3]*Kansas State University, Manhattan, KS.*

The objective of this study was to evaluate 4 milk replacer (MR) feeding programs on calf performance and digestion. Male Holstein calves (n = 96; 40 ± 1 kg BW; 1 to 2 d of age) were randomly assigned to 4 milk replacer programs: 0.66 kg for 35 d then 0.33 kg for 7 d fed only in the a.m. (0.6KG); 0.66 kg for 7 d, 0.82 kg for 35 d, 0.33 kg for 4 d in a.m. only, and 0.22 kg for 3 d in a.m. only (0.8KG); 0.66 kg for 7 d, 0.82 kg for 7 d, 1.1 kg for 21 d, 0.82 kg for 7 d, 0.33 kg for 4 d in a.m. only, and 0.22 kg for 3 d in a.m. only (1.1KG); ad libitum for 35 d, 0.82 kg for 7 d, 0.44 kg for 4 d in a.m. only, and 0.22 kg for 3 d in a.m. only (ADLIB). Calves were fed a common MR (25% CP, 17% fat DM basis; 14% solids) in equal meals at 0600 and 1530 h. Calves on ADLIB were given unrestricted access to MR for 2 h at 0600 and 1530 h. Calves were individually housed, and bedded with straw for the duration of the study (56 d). A textured calf starter (20% CP, 39% starch on a DM basis) and water were fed for ad libitum intake. During d 52- 56, fecal samples were collected from 5 calves/treatment to estimate digestibility using acid insoluble ash. Data were analyzed as a completely randomized design with repeated measures when appropriate and calf was the experimental unit. All pair-wise contrasts were used to separate means. Intake of MR averaged 25.3, 35.3, 40.9, and 46.5 kg DM per calf for 0.6KG, 0.8KG, 1.1KG, and ADLIB treatments, respectively. Starter intake was greatest ($P < 0.05$) for 0.6KG compared with other treatments (0.71 vs. 0.48 kg/d) and ADG differed among all 4 programs with 0.6KG <0.8KG <1.1KG < ADLIB. Intake of MR for ADLIB was highly variable and reflected in ADG. Digestibility of DM (80.2 vs. 73.7%), OM (81.3 vs. 74.7%), ADF (46.2 vs. 25.0%), NDF (59.8 vs. 39.6%), and sugar (94.4 vs. 89.9%) was greater ($P < 0.05$) for 0.6KG vs. ADLIB. Abnormal fecal days and body condition score change for 1.1KG and ADLIB were greater ($P < 0.05$) than for 0.6KG and 0.8KG. Hip width change was similar among programs ($P > 0.05$). Feeding more milk replacer increased ADG but not frame growth and delayed digestive system maturation as post-weaning diet digestibility was reduced.

Key Words: feeding rate, growth

T144 Effect of previous milk replacer feeding program on calf performance and digestion from 2 to 4 mo of age. F. X. Suarez-Mena*[1], T. S. Dennis[1], T. M. Hill[1], W. Hu[1], J. D. Quigley[1], R. L. Schlotterbeck[1], R. N. Klopp[2], G.J. Lascano[2], and L. E. Hulbert[3], [1]*Provimi, Brookville, OH,* [2]*Clemson University, Clemson, SC,* [3]*Kansas State University, Manhattan, KS.*

The objective of this study was to evaluate the carry-over effects of 4 milk replacer (MR) feeding programs on calf performance and digestion from 2 to 4 mo of age. Male Holstein calves (n = 96; 75 ± 3.7 kg BW; 57-58 d of age; received in 2 separate groups of 48 calves) were grouped by previous milk replacer program (4 calves/pen): up to 0.66 kg/d DM from MR (0.6KG), up to 0.82 kg/d DM from MR (0.8KG), up to 1.1 kg/d DM from MR (1.1KG), or for ad libitum intake (ADLIB). Total intake of MR for each program averaged 25.3, 35.3, 40.9, and 46.5 kg DM per calf for 0.6KG, 0.8KG, 1.1KG, and ADLIB, respectively. A textured calf starter (20% CP, 39% starch on a DM basis) was mixed with 5% chopped grass hay (6% CP, 64% NDF on a DM basis) and fed for ad libitum intake for the 56 d study. Hip width, BW, and body condition score were measured on d 0, 28, and 56. During d 10–14, fecal samples were taken from the pen floor with care not to sample floor materials and composited by pen for use to estimate diet digestibility using acid insoluble ash. Data were analyzed as a completely randomized design with repeated measures when appropriate and pen was the experimental unit. All pair-wise contrasts were used to separate means. Initial BW was greater ($P < 0.05$) for 1.1KG and ADLIB vs. 0.6KG and 0.8KG. Hip width change was greater ($P < 0.05$) for 0.6KG vs. others; ADG for 0.6KG was greater ($P < 0.05$) than ADG for 1.1KG and ADLIB programs. Intake, body condition score change, and final BW and hip widths did not differ ($P > 0.05$) among treatments. Digestibility of DM differed for all treatments with 0.6KG >0.8KG >1.1KG > ADLIB. Digestibility of CP was greater ($P < 0.05$) for 0.6KG vs. other treatments. Digestibility of OM, ADF, and NDF were greater ($P < 0.05$) for 0.6KG and 0.8KG vs. 1.1KG and ADLIB. Previous milk replacer program influenced performance and feed digestion in calves from to 2 to 4 mo of age. Calves previously fed more milk replacer started with a larger BW at 2 mo but this difference disappeared by 4 mo of age. Feeding more than 25.3 kg of milk replacer pre-weaning can negatively affect performance and digestion from 2 to 4 mo of age.

Key Words: digestibility, post-weaning

T145 The effects of fatty acid supplementation and provision of a dry teat on the growth of veal calves. L. L. Deikun*[1,2], G. G. Habing[1], J. D. Quigley[2], and K. L. Proudfoot[1], [1]*The Ohio State University, Columbus, OH,* [2]*Provimi, Brookville, OH.*

Veal calves face many challenges early in life which can lead to poor growth. Research is needed to determine interventions that promote growth of veal calves. The aim of this study was to determine the effects of fatty acid supplementation (NeoTec5g, Provimi) and the provision of a dry teat on calf growth. A total of 240 Holstein bull calves from 2 cohorts were randomly assigned to 4 treatments using a 2 × 2 factorial design (n = 60/treatment): control (CON), NeoTec5g (N0), NeoTec5g+Teat (NT), and Teat (T0) upon arrival to a commercial veal facility (d 0). Calves were housed in individual pens from 0 to 9 wk and then paired by treatment at wk 9. Milk replacer (MR) was fed twice daily using a proprietary step-up program. NeoTec5g was added to MR at a feeding rate of 0.5g/kg of BW/hd/day for N0 and NT groups. A pelleted calf starter mixed with straw was offered to all calves from d 1. Serum IgG was determined using radial immunodiffusion assays on d 1 to be used as a covariate; 33% of the calves had failure of passive transfer (FPT), defined as < 10 mg of IgG/mL. Body weight (BW) and body condition score (BCS) were recorded at wk 0, 5, and 10. Average daily gain (ADG) was calculated for wk 1 to 5, wk 5 to 10 and wk 1 to 10. BW and BCS were analyzed using repeated measures ANOVAs, ADG was analyzed using a generalized linear model with pair as the experimental unit. There was no effect of NeoTec5g ($P = 0.95$), or teat ($P = 0.30$) or their interaction ($P = 0.32$) on BW. There was no effect of NeoTec5g ($P = 0.11$) or teat ($P = 0.90$) or their interaction ($P = 0.19$) on BCS. There was no effect of NeoTec5g on ADG wk 1 to 5 ($P = 0.80$), wk 5 to 10 ($P = 0.94$), or wk 1 to 10 ($P = 0.98$). There was a tendency for teat to decrease ADG from wk 1 to 5 ($P = 0.08$), but not from wk 5 to 10 ($P = 0.60$) or wk 1 to 10 ($P = 0.26$). There was a tendency for the interaction of NeoTec5g and teat to decrease ADG for wk 1 to 5 ($P = 0.096$; CON: 0.44 ± 0.2 kg/d, N0: 0.46 ± 0.2 kg/d, NT: 0.40 ± 0.2 kg/d, T0:0.43 ± 0.2 kg/d), but not for wk 5 to10 ($P = 0.26$) and wk 1 to 10 ($P = 0.14$). We saw no effect of our interventions on calf growth. Other factors, such as low milk allowance and FPT, may have affected the efficacy of our interventions.

T146 Varying the ratio of Lys:Met while maintaining the ratios of Thr:Phe, Lys:Thr, Lys:His, and Lys:Val alters bovine mammary cell transcriptome profiles measured by RNAsequencing. X. Dong[1,2], Z. Zhou*[3], A. Helmbrecht[4], C. Parys[4], Z. Wang[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Sichuan Agricultural University, Ya'an, Sichuan Province, China,* [3]*Clemson University, Clemson, SC,* [4]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

The objective of this study was to determine the influence of increasing supplemental Met, based on the ideal 2.9:1 ratio of Lys to Met, on mammary cell transcriptome profiles. MAC-T cells, an immortalized bovine mammary epithelial cell line, were incubated (n = 5 replicates/treatment) for 12 h with 3 incremental doses of Met while holding Lys concentration constant to achieve the following: Lys:Met 2.9:1 (ideal AA ratio; IPAA), Lys:Met 2.5:1 (LM2.5), and Lys:Met 2.0:1 (LM2.0). The ratios of Thr:Phe (1.05:1), Lys:Thr (1.8:1), Lys:His (2.38:1), and Lys:Val (1.23:1) were the same across the 3 treatments. Extracted total RNA was sequenced using the Illumina platform, and mapped to the Bos taurus genome assembly (UMD_3.1.1). Statistical analysis was conducted using the Bioconductor edgeR package, with treatment as fixed effect. The Dynamic Impact Approach software was used to uncover the most-impacted cellular pathways. Analysis of variance at a False Discovery Rate (FDR) of 0.15 and raw *P*-value <0.01 identified 687 differentially expressed genes (DEG) in response to changes in Met level. When compared with IPAA, the bioinformatics analysis using the DEG revealed unique responses to LM2.5, including an overall downregulation of some metabolic pathways (ketone body metabolism, galactose metabolism, pentose phosphate), and upregulation of glycosphingolipid biosynthesis and fatty acid synthesis. Fewer pathways were downregulated when comparing LM2.0 with IPAA, and among the top were antigen processing and presentation, protein processing in the ER, and steroid biosynthesis. Compared with the effect caused by LM2.5, a greater number of metabolic and signaling pathways were upregulated by LM2.0 relative to IPAA including apoptosis, glycerolipid metabolism, glutathione metabolism, MAPK signaling, and PPAR signaling. Overall, preliminary evaluation indicates that increased methionine supply can elicit changes in mammary cell transcriptome profiles. The relevance of these changes to mammary cell function in vivo remains to be determined.

Key Words: milk protein, transcriptomics, bioinformatics

T147 Methionine and valine activate the mTORC1 pathway through heterodimeric amino acid taste receptor (TAS1R1/TAS1R3) and intracellular Ca^{2+} in primary bovine mammary epithelial cells. Y. Zhou[1,3], Z. Zhou*[2,1], and J. J. Loor[1], [1]*University of Illinois, Urbana-Champaign, Urbana, IL,* [2]*Clemson University, Clemson, SC,* [3]*Huazhong Agricultural University, Wuhan, Hubei, China.*

Amino acid taste 1 receptor member 1/3 (TAS1R1/TAS1R3) heterodimer contributes to the sensing of most AA via activating mTOR signaling in non-ruminants. Objectives were to determine indispensable AA signaling through TAS1R1/TAS1R3 and their roles in regulating casein mRNA abundance via mTOR signaling pathway in primary bovine mammary epithelial cells (pBMEC). pBMEC were treated with complete medium (positive control, PC), medium without EAA (−EAA) or medium supplemented with only one of the 10 EAA respectively in triplicates before harvested for gene and protein abundance analyses using qPCR and Western blot. Non-essential AA were maintained at the same level across all treatments. Small interference RNA targeting TAS1R1 were designed and transfected into pBMEC. Data were analyzed with PROC MIXED of SAS 9.4 with fixed effect of treatment and random effect of wells. Compared with -EAA, PC and individual Arg, Val, Leu, His, Phe, Met or Ile supply lead to greater ($P < 0.05$) mTOR phosphorylation. S6K1 phosphorylation was also greater ($P < 0.05$) in response to Val, Leu, Trp, Met, and Ile supply compared with −EAA. As expected, compared with −EAA, intracellular Ca^{2+} was greater ($P < 0.05$) in PC. Met or Val supply also lead to greater ($P < 0.05$) intracellular Ca^{2+}. Compared with non-transfected cells, knockdown of TAS1R1 result in reductions of 32% and 21% ($P < 0.05$) in intracellular Ca^{2+} levels. Similarly, mTOR, S6K1, and 4EBP1 phosphorylation were also reduced upon TAS1R1 knockdown in pBMEC supplemented with Val and Met. Additionally, TAS1R1 knockdown resulted in lower β-casein mRNA abundance in −EAA, Met or Val treated pBMEC compared with non-transfected cells. Overall, these findings suggest TAS1R1/TAS1R3 receptor in bovine mammary cells can sense extracellular AA and regulate mTOR signaling via modulating intracellular Ca^{2+} level. This mechanism appears particularly important for Met and Val to upregulate β-casein mRNA abundance.

Key Words: amino acid, lactation, dairy cow

T148 Increasing the availability of Thr, Ile, Val, and Leu relative to Lys while maintaining an ideal ratio of Lys:Met alters bovine mammary cell transcriptome profiles measured by RNA-sequencing. X. Dong[1,2], Z. Zhou*[3], A. Helmbrecht[4], C. Parys[4], Z. Wang[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Sichuan Agricultural University, Ya'an, Sichuan Province, China,* [3]*Clemson University, Clemson, SC,* [4]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

The objective of this experiment was to determine if varying the ratio of Lys:Thr, Lys:Ile, Lys:Val, and Lys:Leu while maintaining an ideal ratio of Lys:Met and a fixed ratio of other essential AA (IPAA) elicits changes in mammary cell transcriptome profiles. MAC-T cells were incubated for 12 h (n = 5 replicates/treatment) with IPAA (2.9:1 Lys:Met; 1.8:1 Lys:Thr; 2.38:1 Lys:His; 1.23:1 Lys:Val; 1.45:1 Lys:Ile; 0.85:1 Lys:Leu; 2.08:1 Lys:Arg) or IPAA supplemented with Thr, Ile, Val, Leu to achieve a Lys:Thr 1.3:1 (LT1.3), Lys:Ile 1.29:1 (LI1.29), Lys:Val 1.12:1 (LV1.12), or Lys:Leu 0.78:1 (LL0.78). Extracted total RNA was sequenced using the Illumina platform, and mapped to the *Bos taurus* genome assembly (UMD_3.1.1). Cellular protein was extracted for Western blot to examine phosphorylation status of mTORC1 pathway components. Statistical analysis was conducted using the Bioconductor edgeR package, with treatment as fixed effect. The Dynamic Impact Approach software was used to uncover the most-impacted cellular pathways. Compared with IPAA, ANOVA at *P* value <0.05 identified 1730, 1188, 907, and 799 differentially expressed genes (DEG) in response to LL0.78, LV1.12, LI1.29, and LT1.3, respectively. When compared with IPAA, the bioinformatics analysis revealed unique responses to treatments, including an overall upregulation of sulfur metabolism and glycosphingolipid metabolism with LL0.78, upregulation of butirosin and neomycin synthesis and retinol metabolism with LV1.12, and upregulation of ubiquinone biosynthesis with LT1.3. In contrast, LI1.29 resulted in downregulation of cytochrome P450 and glutathione metabolism among the top-impacted pathways. Combined

with the greatest phosphorylation status of AKT, mTORC1, and RPS6 in response to LV1.12, the transcriptome data underscored a potentially unique role of enhanced Val supply on mammary cell protein synthesis and cellular function. Overall, data indicate that enhancing the supply of Thr, Ile, Val, and Leu when Lys:Met is at the ideal ratio can alter mammary transcriptome profiles to various extents. The significance of these alterations to mammary cell function in vivo remain to be determined.

Key Words: transcriptome, mTOR, amino acid

T149 Milk composition of Holstein cows through two lactations. A. Pape*[1], H. M. Dann[1], D. M. Barbano[2], and R. J. Grant[1], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*Department of Food Science, Northeast Dairy Food Research Center, Cornell University, Ithaca, NY.*

The objective of this study was to measure how certain milk composition features vary with parity by using them to build logistic regression models to differentiate between lactations 1 and 2. Understanding differences in milk composition between lactations is useful for herd diagnostics and troubleshooting. The data came from 50 Holstein cows for which a complete set of milk samples from both lactations was available. Sampling for a lactation was considered complete if samples from at least 20 d (3 samples/d weighted proportionally by milk yield) distributed over at least 8 mo were available. The data set consisted of 2,783 observations. Milk samples were analyzed by mid-infrared methodology. Composition features were normalized relative to the weekly mean and standard deviation (SD) for the herd to eliminate the effect of systematic change over time. Data were stratified into 4 stages of lactation: fresh (0–21 DIM), early (22–90 DIM), mid (91–150 DIM), and late (>150 DIM). Three types of models were used: a collective model with principal components of all composition features, a collective model with selected variables, and individual models for selected variables. The selected variables were fat (4.00 ± 0.65%; mean ± SD), true protein (3.08 ± 0.31%), anhydrous lactose (4.62 ± 0.20%), de novo fatty acids (FA; 23.3 ± 2.3 g/100g FA), mixed origin FA (39.0 ± 3.2 g/100g FA), preformed FA (37.8 ± 4.8 g/100g FA), and unsaturation index (0.28 ± 0.04 double bonds/FA). Models were evaluated with 10-fold cross-validation and performance was measured with area under the ROC curve (AUC). Data processing, modeling, and evaluation were done using R. The principal components regression yielded AUC of approximately 0.88–0.97, indicating that there is strong separability between samples from lactations 1 and 2 in terms of composition. In particular, de novo FA, preformed FA, and lactose showed the strongest variation, while fat and true protein showed the weakest. Strong differences were found in separability by stage of lactation, with separability in general being strongest in the fresh and early stages, but separability in terms of lactose being strongest in late lactation.

Key Words: lactation, milk composition, milk fatty acid

T150 Method for calibrating parlor milk meters and adjusting milk weights for stall effects. D. M. Andreen*, I. J. Salfer, Y. Ying, and K. J. Harvatine, *Pennsylvania State University, University Park, PA.*

Milk yield is a fundamental observation in many experiments and is commonly determined using integrated milk meters that determine milk weight as the cow is being milked. These meters are used heavily in a harsh environment and commonly are not calibrated regularly, so mechanical or other problems may create variation in milk weight data. Additionally, direct calibration by collection of milk in a bucket is difficult and imperfect as use of the bucket may change flow rates. The objective of this work was to define a method to easily check parlor meter calibration and adjust milk weight values for variation between individual stalls in a parlor. Because most cows are milked in a different stall at each milking, Dr. L. Armentano (University of Wisconsin) originally proposed that an effect of stall could be determined that would represent the calibration of the stall. The effect of stall on milk yield was modeled using 7 d of data from approximately 200 cows using a mixed model that included the fixed effect of stall and the random effects of day, milking (AM/PM), and cow. Stalls requiring service can be identified based on the stall deviations, which can exceed 2 kg in malfunctioning stalls. A correction factor for each stall can be generated by dividing the least squared mean of the stall by the overall mean. Milk yields can then be corrected by multiplying the meter weight value by the correction factor. The method was used in multiple experiments with 250 to 1500 milk yield observations. In all data sets, stall correction of milk weights slightly improved model fit and decreased standard errors, indicating reduced variation. Second, raw meter and corrected values were compared with weight of milk collected in a bucket (n = 3 per stall). The corrected values had a 4% greater R^2 than uncorrected values. Modeling stall deviations in milk meters is a simple, convenient, and low cost method to monitor milk meter functionality and accuracy and can be used to reduce data variation and experimental error.

Key Words: milk weight, variation, stall

T151 Intramammary 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 treatments differentially modulate serum calcium and mammary immune responses. T. L. Williams*, M. B. Poindexter, M. F. Kweh, L. P. Blakely, and C. D. Nelson, *University of Florida, Gainesville, FL.*

The 1,25-dihydroxyvitamin D_3 (1,25D) metabolite increases calcium in blood and expression of inducible nitric oxide synthase (*iNOS*) and β-defensin 7 (*DEFB7*) genes in milk somatic cells of healthy cows. Immune cells in the mammary gland produce 1,25D from 25-hydroxyvitamin D_3 (25D) without affecting serum calcium; so, we hypothesized 25D treatment would alter gene expression in the mammary gland without affecting serum calcium. The objective was to determine the effect of dose and source of intramammary vitamin D treatments on gene expression and serum calcium. Lactating cows received intramammary injections with 100 or 500 mg 25D, or 10 or 50 mg 1,25D (n = 4 cows/dose) in 2 ipsilateral quarters and a placebo in the other 2 ipsilateral quarters after milking. Control cows (n = 4) received placebo treatments in 2 ipsilateral quarters. Milk samples were collected from each quarter at 0, 6, 12, 24, and 48 h relative to the start of treatments. Blood samples were collected at 0, 12, 24, and 48 h. Data were analyzed by ANOVA using mixed models. Significance was declared at $P < 0.05$. The 50 µg 1,25D treatment, but not other treatments, increased serum calcium compared with control cows. All treatments increased vitamin D 24-hydroxylase (*CYP24A1*) in milk somatic cells at 6 h in which the 50 mg 1,25D treatment had the greatest (198-fold) increase. *CYP24A1* remained elevated in 500 mg 25D quarters at 24 h but did not differ from control for other treatments at 24 h. The 50 mg 1,25D and 500 mg 25D treatments increased *iNOS* expression at 6 h. Expression of *iNOS* remained elevated for 500 mg 25D treatment at 24 h but did not differ from control for 50 mg 1,25D treatment at 24 h. The 10 mg 1,25D and 100 mg 25D treatments did not increase *iNOS* expression compared with control and *DEFB7* in treatment quarters did not differ from control. In conclusion, increasing the intramammary dose of 1,25D from 10 mg to 50 mg increased serum calcium. Intramammary 1,25D increased expression of *CYP24A1* and *iNOS* genes in milk somatic cells

to a greater extent than 25D, but duration of increase was greatest for the 500 mg 25D treatment.

Key Words: vitamin D, mammary

T152 Effects of in vivo estradiol administration on abundance and localization of yes-associated protein, an evolutionarily conserved molecule implicated in organ size regulation, in prepubertal bovine mammary tissue. P. D. Granger*[1], A. J. Geiger[1,2], C. L. M. Parsons[1], K. M. Daniels[1], R. M. Akers[1], and B. A. Corl[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*Zinpro Corporation, Eden Prairie, MN.*

Yes-associated protein (YAP) is an evolutionarily conserved protein that regulates organ size, but is not yet reported on in dairy cattle. The hypothesis was that in vivo estradiol administration increases the abundance and nuclear localization of YAP in prepubertal bovine mammary epithelial cell (MEC) and myoepithelial (MYO) cells. Twelve Holstein heifers (6 ± 2 d old) received enhanced milk replacer (EH; 28.9% CP, 26.2% fat, DM basis; 1.08 kg powder DM/d), and were offered starter (26% CP; 23.2 g DM/kg BW from 4 wk of age). Heifers received an ear implant containing either estradiol EH-E_2 (n = 6) (25.7 mg estradiol) or placebo EH (n = 6) at weaning (8 wk). Post weaning, both groups received starter (25.6% CP, 4.0% fat, 19.8% neutral detergent fiber; DM basis). Heifers were slaughtered at 10 wk. Mammary parenchyma was collected (EH-E_2, n = 6; EH, n = 4) and immunofluorescently labeled for YAP, MYO nuclei, and MEC nuclei. Images, manually identified compartments, and nuclear objects were manipulated with CellProfiler. Data were analyzed using SAS 9.4 with the fixed effect of treatment and random effect of heifer within treatment. Generally, YAP was localized to the cytoplasm of MEC and nuclei of MYO. Within MEC, estradiol treatment did not affect nuclear ($P = 0.974$), cytoplasmic ($P = 0.394$), or total intensity of YAP ($P = 0.979$). Similarly, within MYO, estradiol treatment did not affect nuclear ($P = 0.997$), cytoplasmic ($P = 0.624$), or total intensity of YAP ($P = 0.308$). In this experiment, in vivo estradiol administration did not affect the sub-cellular localization of YAP (nucleus versus cytoplasm) nor the intensity (abundance) of YAP within MEC and MYO compartments. Findings suggest: 1) sub-cellular YAP localization is compartmentalized in bovine mammary tissue and may affect growth independent of estradiol, 2) YAP signaling might work in concert with non-estradiol pathways to affect organ size, as estradiol treatment 2 wk before slaughter did not elicit change in YAP localization in MEC and MYO.

Key Words: mammary growth, heifer, organ size

T153 Regulation of lipogenic genes by AMPK in bovine mammary epithelial cells. J. Huang* and M.-A. Guesthier, *McGill University, Sainte-Anne-de-Bellevue, QC, Canada.*

The synthesis of milk components requires a sufficient supply of energy substrates. AMPK is a cellular energy gauge that controls anabolic and catabolic processes to maintain a balance between energy supply and demand. The objective of this study was to determine the role of AMPK on de novo lipid synthesis in bovine mammary epithelial cells (BMEC). Primary BMEC were isolated from lactating mammary tissue and induced to differentiate by addition of lactogenic hormones (5 µg/mL insulin, 5 µg/mL prolactin, and 5 µg/mL hydrocortisone). The phosphorylation state of AMPKα Thr172 and its downstream target TSC2 were analyzed by immunoblotting. The expression of lipogenic genes was quantified by qPCR. We first characterized activation of AMPK by pharmacological compounds in BMEC. Cells were treated with 400 µ*M* AICAR, an AMP analog, or 100 µ*M* A769662, an allosteric activator of AMPK, for up to 16 h. BMEC exposed to 400 µ*M* AICAR showed higher phosphorylation of AMPKα Thr172 at 4, 8 and 16 h compared with untreated cells (0 h). Interestingly, while treatment of BMEC with 100 µ*M* A769662 for 16 h had no effect on AMPK phosphorylation, it increased phosphorylation of its target TSC2 at by 36% ($P < 0.05$). Treatment of BMEC with 25 µ*M* STO-609, a specific inhibitor of the upstream kinase CaMKK, significantly suppressed activation of AMPK by 1 µ*M* ionomycin, suggesting it may also be implication in sensing intracellular calcium levels. To assess the physiological role of AMPK, we deprived BMEC of energy substrates for 4 h. We found that deprivation of glucose and acetate, either alone or in combination, promoted phosphorylation of AMPKα Thr172. Activation of AMPK by 100 µ*M* A769662 resulted in an increase in gene expression of SREBF1 and SCD1 by 36% ($P < 0.05$) and 41% ($P < 0.05$), respectively. In conclusion, changes in energy substrate availability and intracellular calcium levels are sensed through AMPK, which may in turn control lipid metabolism in BMEC by modifying lipogenic gene expression.

Key Words: AMPK, bovine mammary epithelial cell, lipid synthesis

T154 Stearic acid increased milk fat content in lactating dairy ewes at late lactation. R. Horstmann, G. C. De Aguiar, L. P. Batalha, and D. E. Oliveira*, *Santa Catarina State University, Lages, Santa Catarina, Brazil.*

Fat supplements are fed to lactating dairy ewes to increase the energy density of the diet, increasing milk yield and milk fat content and yield. However, studies using lipid supplements with higher content of saturated fat and its effects in lactating ewes are still not fully understood. This study evaluated the effects of a lipid supplement rich in stearic acid (C18:0, 88%) fed to Lacaune lactating ewes, on milk production and composition. Thirty-two lactating ewes (122 DIM) were used in a completely randomized design for 21d (7d of adaptation and 14d of measurements), receiving the following treatments: Control (corn silage + 1 kg of concentrate - DM basis) and C18:0 (corn silage + 1 kg of concentrate - DM + 32 g/d of C18:0). The C18:0 was mixed to the concentrate and fed individually. Milk yield was measured individually and milk samples were taken every 2d. Data for milk yield and composition were analyzed as repeated measures using the PROC MIXED of SAS, assuming the treatment as a fixed effect. The data for milk yield and composition from d0 (day zero) collected just before starting the experiment was used as a covariate in the model. Compared with Control, respectively, the C18:0 increased milk fat content by 4.4% ($P = 0.02$) and decreased lactose content by 3.8% ($P = 0.001$). The C18:0 also decreased milk yield by 8.5% ($P = 0.05$), fat and lactose yield in 8.5 and 3.5% ($P = 0.04$), respectively. Overall, our results showed that C18:0 was able to increase the milk fat content and decrease milk fat yield as a consequence of a reduced milk yield in lactating ewes at late lactation.

Key Words: milk synthesis, fat supplement, sheep milk

T155 Relationships of metabolites and hormones in follicular fluid and blood serum in transition dairy cows supplemented with a *Saccharomyces cerevisiae* fermentation product. J. A. Sauls*, K. E. Olagaray, S. E. Sivinski, B. J. Bradford, and J. S. Stevenson, *Kansas State University, Manhattan, KS.*

Free fatty acids (FFA), β-hydroxybutyrate (BHB), glucose, and steroid concentrations in follicular fluid (FF) and blood serum (BS), and luteal status were examined in cows individually fed a *Saccharomyces cerevisiae* fermentation product (SCFP; NutriTek, Diamond V, Cedar Rapids, IA) from −4 through +7 wk after calving (wk 0). Holsteins (n = 59) were assigned to control or SCFP diets (45 and 32% NDF, 14 and 19% starch pre- and postpartum, respectively). Blood was collected at −4, −2, +1, +2, +5, and +7 wk for FFA and BHB analyses. Milk yield and DMI were measured daily. Blood was collected before injections of GnRH, prostaglandin $F_{2\alpha}$, and GnRH at 32 ± 3, 39 ± 3, and 42 ± 3 DIM, respectively, to synchronize ovulation before dominant follicle recovery (DFR) by transvaginal aspiration at 49 ± 3 DIM (d 7 post-GnRH). Luteal status (high progesterone [P4] > 1 ng/mL) was determined on 32 ± 3 DIM. Blood collected before DFR and in FF after DFR was assayed for FFA, BHB, glucose, P4, androstenedione, and estradiol concentrations. After DFR, cows were inseminated at a fixed time at 70 ± 3 DIM and pregnancy was diagnosed 32 d later. Treatment did not affect milk yield, DM intake, or proportion of cows with early luteal function at 32 DIM (50 vs. 56% for SCFP vs. control) or pregnancy per AI (44 vs. 50%). Metabolites and hormonal concentrations did not differ between treatments in BS or FF at any sampling time. Concentrations of FFA in BS were greater ($P < 0.01$) at −4 and +1 wk in cows with low compared with high P4 by 32 DIM. Cows with low P4 also had greater ($P < 0.05$) BHB in BS at +2 and +5 wk compared with cows with high P4. Cows with low compared with high P4 had greater ($P < 0.05$) BHB in BS and FF at +7 wk. Cows conceiving to AI had less ($P = 0.03$) BHB in BS at +1 wk and less ($P = 0.05$) FFA in BS at +7 wk than cows that did not conceive. Concentrations of BHB (r = 0.61; $P < 0.001$) and glucose (r = 0.56; $P = 0.003$) in BS and FF were correlated positively. Differences in BHB and FFA in BS were predictive of luteal and pregnancy status, and BHB and glucose in BS reflected that in FF of the dominant follicle. No effect of SCFP was detected.

Key Words: metabolite, steroid, fertility

T156 Hyperketonemia does not affect proportional uptake of fatty acids by the mammary gland. K. A. Weld*, R. C. Oliveira, K. J. Sailer, H. T. Holdorf, S. J. Bertics, and H. M. White, *University of Wisconsin-Madison, Madison, WI.*

Hyperketonemia (HYK) has negative effects on dairy cattle production and health and may shift nutrient utilization. The objective was to determine if there was a difference in fatty acid (FA) uptake by the mammary gland of Holstein cows with and without HYK (nonHYK) and if those differences are reflected in FTIR predicted milk FA. Blood was sampled at the tail (representing arterial blood) and mammary vein after the a.m. milking twice within 25 d postpartum. A corresponding milk sample was analyzed by FTIR for components and predicted FA. Cows were diagnosed as HYK (≥1.2 m*M* BHB) or nonHYK on the day of sampling resulting in 9 HYK (1.4 m*M*+SE BHB) and 12 nonHYK (0.7 m*M*+SE BHB) sample sets. Plasma FA were determined by acid methylated and gas chromatography. The FA profile was multiplied by plasma FA concentration to approximate total concentration of each FA in the plasma. Data were analyzed using PROC MIXED (SAS 9.4) with a model containing the fixed effects of block, week (repeated measure), diagnosis, the interaction of diagnosis and week, and a random effect of cow. Results are expressed as mean ± SE. The mammary gland of ketotic cows took up a greater amount of FA than nonHYK cows (0.32 vs. 0.07 ± 0.4 m*M*; $P < 0.01$). The same was found for C16:0, C18:0, C18:1, and C18:2 (0.05 vs 0.01 ± 0.01, 0.05 vs. 0.01 ± 0.01, 0.07 vs. 0.01 ± 0.01, 0.11 vs. 0.02 ± 0.02 m*M*, for HYK and nonHYK, respectively; $P < 0.01$). However, the total FA disappearance (arterial-venous difference) relative to available concentration was not different (33.3 vs. 15.4 ± 9.6%; $P = 0.19$). This pattern was also observed for C16:0, C18:0, C18:1 and C18:2 (35.1 vs. 16.0 ± 9.9, 33.6 vs. 16.0 ± 9.6, 37.1 vs. 19.4 ± 9.5, 33.3 vs. 12.8 ± 9.9%, respectively; $P > 0.17$). Although no difference was observed in FTIR predicted milk FA output from the mammary gland, the proportion of FTIR predicted long chain FA and C18:1 relative to total milk fat was greater ($P = 0.02$) in HYK cows (31.6 vs. 27.5 ± 1.4% proportional C18:1). These data indicate that proportional uptake of FA by the mammary gland is not altered by HYK status. Cows with HYK do not have a different FTIR predicted FA profile, although an increase in relative C18:1 contribution may be detectable.

Key Words: ketosis, transition cow, milk fat

T157 The relationship between plasma and liver fatty acid profiles in transition dairy cows. K. A. Weld*, R. C. Oliveira, K. J. Sailer, H. T. Holdorf, S. J. Bertics, and H. M. White, *University of Wisconsin-Madison, Madison, WI.*

Mobilized fatty acids (FA) provide an energy source to the liver during negative energy balance but can also be stored as liver lipids. The objectives were to determine the change in FA profile across the transition period and the correlations between circulating FA profile changes, blood concentration, and liver lipid profile in Holstein cows. Blood samples were taken at −28, +1, +3, +14, and +28 (n = 36, 27, 25, 38, and 25, respectively) and liver biopsies were taken at +1, +14, and +28 (n = 27) days relative to calving (DRTC). Liver and plasma FA were determined by acid methylated and gas chromatography. To calculate delta plasma values, the proportion of each FA at −28 was subtracted from the proportion on each day postpartum. Data were analyzed with PROC MIXED and CORR (SAS 9.4). The mixed model included the fixed effect of DRTC and the random effect of cow using repeated measures and data expressed as mean ± SE. Proportionally, C16:0 and C18:1 were increased ($P < 0.01$) postpartum and were greatest at +1 (4.0 ± 0.4 and 7.5 ± 0.6%) and +3 DRTC (4.0 ± 0.4% and 7.5 ± 0.6% increase for C16:0 and C18:1). Plasma FA concentration and delta plasma concentrations at +1 DRTC were correlated ($P < 0.01$) for C16:0 (r = 0.57) and C18:1 (r = 0.74). These data support that C16:0 and C18:1 are the primary mobilized FA from adipose tissue. Relative to −28 DRTC, the proportion of C18:2 was decreased ($P < 0.01$) at +1 (−6.3 ± 1.1%) and +3 DRTC (−6.0 ± 1.1%) yet greater at +14 (2.7 ± 1.1%) and +28 DRTC (4.5 ± 1.1%) suggesting contributions beyond adipose mobilization. The proportion of C18:0 was decreased ($P < 0.01$) postpartum (−2.3 ± 0.5%) and not correlated ($P > 0.1$) to total plasma FA. The correlation between plasma and liver profiles differed by fatty acid. Plasma and liver C18:0 was not correlated ($P > 0.1$) on +1, +14 and +28 DRTC (r = 0.20, 0.10, −0.23). Correlations were greater between plasma and liver for C16:0 (r = 0.59, 0.35, 0.33), C18:1 (r = 0.79, 0.60, 0.60), and C18:2 (r = 0.5, 0.49, 0.46) at +1, +14 and +28 DRTC. Taken together, these data suggest that circulating FA during negative energy balance

reflect adipose mobilization patterns and preferential metabolic fates in hepatic tissue that should be further examined.

Key Words: liver lipid, hepatic metabolism

T158 Fatty acid profiles in plasma and liver lipids are influenced by hyperketonemia in dairy cows. K. A. Weld*, R. C. Oliveira, K. J. Sailer, H. T. Holdorf, S. J. Bertics, and H. M. White, *University of Wisconsin-Madison, Madison, WI.*

Plasma and liver fatty acid (FA) profiles in transition cows are influenced by the degree of FA mobilization from adipose tissue which is often exacerbated in cases of hyperketonemia (HYK). The objectives were to examine the liver and blood FA profiles of cows diagnosed with HYK (≥1.2 m*M* BHB) or not (nonHYK). Liver biopsies were taken at +1, +14, and +28 and blood samples at −28, +1, +3, +7, +11, +14, and +28 d relative to calving (DRTC). Liver and plasma total FA were acid methylated and analyzed using gas chromatography. Data were analyzed using PROC MIXED and PROC CORR (SAS 9.4). The model contained the fixed effects of block, HYK diagnosis, DRTC, the interaction of HYK diagnosis and DRTC, and the random effect of cow using repeated measures. PROC CORR was used to determine correlations between liver and plasma FA within DRTC and FA. Comparisons between HYK and nonHYK are expressed as mean ± SE. Plasma of cows diagnosed with HYK had an increased proportion of C18:1 (18.2 vs. 16.7 ± 0.5 g/100g FA; $P = 0.02$) and decreased proportion of C18:2 (36.2 vs. 38.7 ± 0.6 g/100g FA; $P = 0.01$). Plasma C16:0 and C18:0 were not different by HYK. Reflective of plasma differences by HYK status, liver of cows diagnosed with HYK had an increased proportion of C18:1 (27.0 vs. 25.3 ± 0.9 g/100g FA; $P < 0.04$) and decreased proportion of C18:2 (10.2 vs. 11.4 ± 0.5 g/100g FA; $P < 0.10$). Conversely, liver C16:0 was increased (29.6 vs. 27.0 ± 1.4 g/100g FA; $P < 0.09$) and C18:0 was decreased (15.5 vs. 17.9 ± 1.4 g/100g FA; $P < 0.08$) in cows with HYK. Correlations between liver and plasma FA proportions varied dependent on HYK diagnosis for C16:0 (r = 0.35, 0.44, 0.30 vs. 0.67, 0.21, 0.33 for HYK and nonHYK), C18:0 (r = 0.20, 0.41, −0.19 vs. 0.86, −0.08, −0.33) and C18:1 (r = 0.74, 0.84, 0.58 vs. 0.62 −0.25, 0.63) at 1, 14 and 28 DRTC. Generally, C18:2 was similar between HYK and nonHYK at all time points (r = 0.35, 0.44, 0.30 vs. 0.67, 0.21, 0.33). These data suggest that FA profiles during mobilization may be shifted in cows with HYK and may influence downstream FA profiles of deposited liver lipids. Potential influences on preferential liver FA oxidation or storage during metabolic challenge should be further examined.

Key Words: ketosis, transition cow

T159 Effects of dietary zinc on energetic requirements of an activated immune system following lipopolysaccharide challenge in lactating cows. E. A. Horst*[1], E. J. Mayorga[1], S. L. Portner[1], M. Al-Qaisi[1], C. S. McCarthy[1], M. A. Abeyta[1], B. M. Goetz[1], H. A. Ramirez-Ramirez[1], D. H. Kleinschmit[2], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*Zinpro Corporation, Eden Prairie, MN.*

Objectives were to evaluate effects of replacing 40 ppm of zinc from zinc sulfate (CON) with zinc amino acid complex (Zn; Availa-Zn, Zinpro Corp., Eden Prairie, MN) on production, SCC, and metabolism following an i.v. lipopolysaccharide (LPS) challenge in lactating cows. Cows were enrolled in a 2 × 2 factorial design and assigned to 1 of 4 treatments: 1) pair-fed (PF) control (PF-CON; 5 mL saline; n = 5), 2) PF Zn (PF-Zn; 5 mL saline; n = 5), 3) LPS-euglycemic clamp control (LPS-CON; 0.375 μg/kg BW LPS; n = 5), 4) LPS-euglycemic clamp Zn (LPS-Zn; 0.375 μg/kg BW; n = 5). A pre-feeding phase (42 d) was included before initiation of the experimental phase, and then cows were enrolled in 3 experimental periods. During P1 (3 d), cows received their respective dietary treatments and baseline data were obtained for covariate analysis. During P2 (2 d), a 12-h LPS-euglycemic clamp was conducted or cows were PF to their respective dietary counterparts to eliminate effects of dissimilar nutrient intake. During P3 (3 d), cows consumed feed ad libitum and received their respective dietary treatment. LPS administration decreased DMI (47%) during P2, and by experimental design, the pattern was similar in PF cohorts. DMI of LPS cows remained decreased (15%) throughout P3 relative to PF cows ($P = 0.03$). Milk yield from LPS cows decreased (54%) during P2 relative to PF cows, but treatments did not differ during P3. During P2, SCC increased (3-fold) in LPS cows, relative to PF controls ($P < 0.01$). Between 54 and 72 h post-LPS, Zn supplementation tended to decrease (70%) SCC relative to LPS-CON cows ($P = 0.10$). Insulin increased 7-fold in LPS cows at 12 h post-LPS and remained increased (4-fold) for the duration of P2 relative to PF cows ($P = 0.01$). NEFA in LPS-Zn cows remained at baseline, while in all other treatments it gradually increased ($P = 0.02$). The 12-h total glucose deficit, quantified immediately post-LPS, was 36 and 1,606 g for the PF and LPS treatments, respectively, but glucose utilization was not influenced by Zn. In summary, supplemental Zn tended to reduce milk SCC following immunoactivation, with no evidence of altering production or metabolism.

Key Words: LPS, zinc

T160 Effects of heat stress and nutritional plane on neutrophil function. E. A. Horst*, M. Al-Qaisi, E. J. Mayorga, S. K. Kvidera, M. A. Abeyta, and L. H. Baumgard, *Iowa State University, Ames, IA.*

Objectives were to evaluate the acute and chronic effects of electric heat blanket (EHB)-induced hyperthermia and feed restriction on immune cell dynamics and neutrophil function (as assessed by oxidative burst [OB] and production of myeloperoxidase [MPO]) in lactating Holstein dairy cows. Cows (n = 18) were allowed 4d to acclimate and then subjected to 2 experimental periods (P). Period 1 lasted 4 d during which cows were housed in thermoneutral conditions with ad libitum feed intake. During P2 (4 d), cows were assigned to 1 of 2 treatments: (1) artificially induced heat stress using an electric heat blanket (Thermotex Therapy Systems Ltd. Calgary, Canada) with ad libitum intake (HS; n = 10) or (2) thermoneutral conditions and pair-fed (PF; n = 8). Blood samples were obtained via jugular venipuncture on d4 of P1 and at 24 and 96h of P2. The EHB decreased DMI 32% on d1 and 52% on d4 of P2 in HS cows and, by experimental design, the pattern was similar in the PF cohorts. There were no treatment effects on circulating lymphocytes or monocytes. Circulating neutrophils from HS cows initially increased (35%) at 24 h but returned to baseline by 96 h, while they did not change in PF controls ($P = 0.08$). Band neutrophils progressively decreased in HS cows while they continuously increased in PF controls ($P = 0.01$). HS gradually decreased OB (6%, $P < 0.01$), while it initially decreased in PF controls (9%; $P < 0.01$) but returned to baseline by 96 h. Total MPO initially increased (13%; $P < 0.01$) in HS cows and then gradually returned to baseline by 96 h. Total MPO of PF cows increased (10%; $P = 0.07$) after 96 h of feed restriction. Overall stimulated MPO production increased (26%; $P < 0.01$) in HS cows, while it initially increased in PF controls (17%; $P = 0.01$) but it returned to baseline by 96 h. MPO exocytosis increased (20%) after 96h of HS while it initially increased (12%) in PF cows but it eventually decreased (14%) relative to baseline by 96 h. In summary, both feed restriction and HS discordantly influence circulating neutrophil dynamics. Whether these changes are reflective of "immune suppression" or rather immune activation are not clear.

Key Words: heat stress, neutrophil

T161 Thermoregulatory response of lactating Holsteins to an acute heat stress after a pharmacologically induced LH surge. C. R. Abbott*, A. M. Saxton, T. Walker, R. Long, L. A. Rispoli, R. R. Payton, R. O. Filho, F. G. Dantas, K. A. Campen, R. Carvalho, K. G. Pohler, N. Schrick, and L. Edwards, *University of Tennessee, Knoxville, TN.*

Objective was to examine thermoregulatory response of lactating cows to an acute heat stress occurring after an LH surge. Cows with a corpus luteum were given $PGF_{2\alpha}$, and 11 d later, CIDR and GnRH were administered. Seven day thereafter, CIDR was removed and $PGF_{2\alpha}$ was given. Cows with a dominant follicle (15.2 ± 2.6 mm) were transported to a climate-controlled room 35 h after final $PGF_{2\alpha}$. Forty hours after final $PGF_{2\alpha}$, GnRH was given to induce LH surge. Thermoneutral cows (TN; n = 17) were kept at a THI of 65.8 ± 1.5; heat-stressed cows (HS; n = 12) experienced THI increases of 2.1 units per h for 12 h (up to a final THI of 85.2 ± 0.2). Frequent rectal temperature (RT) and respiration rate (RR, breaths/min, bpm) were recorded. Blood samples were taken at last GnRH, 2, 3, and 4 h thereafter, and after HS ceased. Mixed model regression with repeated measures with various combinations of treatment, THI, previous THI, DIM, milk yield, parity, RT, and RR was used to address a priori hypotheses. Elevating THI increased ($P = 0.001$) RT and RR in HS (39.5 ± 0.6°C and 105.4 ± 22.2 bpm) versus TN (38.3 ± 0.4°C and 47.0 ± 10.5 bpm) cows. Changes in RT and RR by increasing THI were noted within 40 and 110 min, respectively (0.05 ± 0.3°C and 3.4 ± 0.1 bpm per unit rise in THI). Changes in RR lagged THI and preceded rises in RT by 30 min. Average THI 3 d before imposing HS conditions influenced RT and RR ($P < 0.0001$). Serum progesterone 2 h after final GnRH was negatively related to RT changes ($P = 0.0001$); serum estradiol was positively related ($P = 0.009$). After HS ceased, cows were returned to TN conditions; RT and RR decreased by 1.08 ± 0.4°C and 45.9 ± 9.4 bpm per 15 min. At 45 min, HS and TN cows were similar (38.9°C and 66.9 bpm vs 38.7°C and 59.6 bpm). Rapid changes in RT after an acute heat stress event emphasizes the importance of strategically cooling periovulatory cows, especially since estradiol levels may heighten heat-induced elevations in RT. *Project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24899 from USDA National Institute of Food and Agriculture.*

Key Words: heat stress, estrus, thermoregulation

T162 Sweat gland cross-sectional cut areas comparisons between slick and wild type-haired Holstein and Senepol cows in Puerto Rico. J. M. Muñiz-Cruz*[1], N. Peña-Alvarado[2], W. Torres-Ruiz[2], J. R. Almodóvar-Rivera[3], K. I. Domenech-Pérez[1], Z. E. Contreras-Correa[1], G. C. Muñiz-Colón[1], A. C. Cortés-Arocho[1], J. M. Santiago-Rodríguez[1], S. Ruiz-Ríos[1], G. A. Soriano-Varela[1], N. N. Cortés-Viruet[1], A. L. Jiménez-Arroyo[1], G. M. Jiménez-Arroyo[1], H. L. Sánchez-Rodríguez[1], [1]*Department of Animal Science, University of Puerto Rico at Mayagüez Campus, Mayagüez, Puerto Rico,* [2]*Laboratorio de Investigaciones Pesqueras, Departamento de Recursos Naturales y Ambientales, Cabo Rojo, Puerto Rico,* [3]*Department of Biology, University of Puerto Rico at Mayagüez Campus, Mayagüez, Puerto Rico.*

A preliminary study reported larger sweat glands cross-sectional cut areas (ASW) in slick-haired Holstein cows (SLICK) than in their wild-type (WT) counterparts. Being Holstein a breed developed under temperate weather conditions, the present study aimed to evaluate how such values compare with those in Senepol (SEN), a *Bos taurus* breed, slick-haired and highly adapted to the tropical weather. Hair coat types were visually chosen and genetically confirmed. Skin biopsies (n = 1 / cow; 6 mm in diameter) were obtained from 9 SLICK (2.4 ± 0.17 lactations; 569.9 ± 11.39 kg of body weight (BW)), 8 WT (3.7 ± 0.26 lactations; 645.8 ± 12.16 kg of BW) and 9 SEN cows (7.4 ± 0.47 lactations; 610.6 ± 18.28 kg of BW) behind the right shoulder after local anesthesia. Biopsies were immediately preserved in 10% formalin, embedded in paraffin, cut perpendicularly to the skin layers (7 µm thickness), and stained with hematoxylin-eosin in microscope slides. A total of 6 skin cuts per cow were randomly selected and the ASW (10–100 / cut) was evaluated using the NIS Element D Software (Nikon, Melville, NY) under 4x magnification. Data were averaged by skin cut (PROC MEANS) and compared between hair type/breed groups (PROC GLIMMIX) using SAS. Without considering the SEN cows, the ASW values were larger in the SLICK than in the WT cows (6,946.16 ± 623.10 and 4,902.67 ± 588.15 μm^2, respectively; $P = 0.0307$). However, when included in the data set, SEN cows had the largest ASW (9,651.74 ± 868.89 μm^2; $P = 0.0031$), while no differences were observed between the SLICK and WT cows. The present study corroborates a larger sweat gland size in the SLICK than in the WT cows; however, these are smaller than what is found in a well-adapted tropical breed such as SEN. Future studies should evaluate if a larger sweat gland implies greater heat dissipation via evaporation.

Key Words: slick-haired Holstein cow, sweat gland, thermoregulation

T163 microRNA involvement during the onset of ketosis and fatty liver in periparturient Holstein dairy cows. R. E. Buck-trout*[1], V. Palombo[2], M. V. Riboni[1], and J. J. Loor[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL,* [2]*Università degli Studi del Molise, Campobasso, Italy.*

To understand the possible role microRNA (miRNA) have during the onset of fatty liver, we applied an in-silico approach to identify a miRNA signature from a liver transcriptome database. The database was generated from liver tissue of cows developing ketosis and fatty liver during the first 2 wk postpartum. Liver tissue (n = 7/group) for gene expression profiling was sampled at the onset of signs of clinical ketosis (anorexia, ataxia, or abnormal behavior) (9–14 d postpartum) or d 14 postpartum for control cows. Initially, a list of miRNA families and their predicted target genes for *Bos taurus* were downloaded from the Microcosm targets website (v. 5.0). Results for the effect of ketosis were then used to predict miRNA activity from the mRNA expression profiles through 3 approaches: Wilcoxon rank test, Ranked Ratio, and Mean absolute expression. After overlapping the results of the 3 approaches, a total of 7 miRNA were predicted as possible components in the transcriptomic response associated with ketosis and fatty liver: miR-101, miR-142, miR-186, miR-200b, miR-200c, miR-218, and miR-369–3p. To identify miRNA functions, the dynamic impact approach was then used for biological pathway analysis on the compiled differentially expressed target genes of the predicted miRNA. Pathway analysis relevant to fatty liver revealed these miRNA may play a role in fatty acid metabolism (miR-186, -200b and c, -218, -369), oxidative phosphorylation (all miRNA), peroxisome (miR-186, -200b and c), gluconeogenesis (miR-186, -369), PPAR signaling pathway (miR-101, -142, -186, -200b and c), insulin signaling pathway (miR-186, -200b, -218, -369), and apoptosis (miR-142, -200b, -369). Overall, the in silico analysis suggests that specific miRNA may be involved in the etiology of fatty liver through the control of key biological pathways and genes related to this disease. Further studies should be performed to verify the expression of these miRNA along with mRNA and protein expression of the target molecules.

Key Words: miRNA, transition period, fatty liver

T164 Effect of lactation stage on transfer of n-3 fatty acids to milk fat in dairy cows. N. L. Urrutia*[1,2], R. Bomberger[1], M. Baldin[1], and K. J. Harvatine[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Instituto de Investigaciones Agropecuarias, Osorno, Region de Los Lagos, Chile.*

Omega-3 (n-3) fatty acids (FA) are essential nutrients and their metabolism has not been characterized across lactation in the cow. The objective of this study was to determine the effect of lactation stage on transfer of n-3 FA to plasma and milk following a bolus infusion. Four ruminally cannulated multiparous Holstein cows received a single abomasal bolus infusion of an enriched n-3 FA mixture (flaxseed, fish and algae oil) in early (10–20 DIM), peak (50–60 DIM), and late (>200 DIM) lactation. Treatments provided 50 g of α-linolenic acid (ALA), 7 g of eicosapentaenoic acid (EPA), and 20 g of docosahexaenoic acid (DHA), as free FA over a 30 min period. Total plasma and milk FA were analyzed over 10 d starting a day before treatments. Total transfer to milk was analyzed in a model that included random effect of cow nested in lactation stage and fixed effect of lactation stage (JMP Pro). Time course data relative to bolus was analyzed as repeated measures in SAS with cow nested in lactation stage as random effect and lactation stage, time, and their interaction as fixed effects. Lactation stage and its interaction with time was significant for total plasma ALA, EPA and DHA, and for milk ALA and DHA concentration (all $P < 0.001$). Plasma ALA peaked at 12, 6, and 4 h post bolus at 5.9, 5.3, and 4.8% of total FA in early, peak, and late lactation, respectively. Plasma EPA peaked at 12 h (all stages) at 2.2, 1.3, and 1% of total FA, while plasma DHA peaked at 6, 6, and 4 h post bolus at 1.1, 0.6, and 0.5% of total FA in early, peak, and late lactation, respectively. Milk ALA and DHA (%) peaked at 14 h in all stages, while milk EPA peaked 26 h post bolus in early lactation and 14 h in peak and late lactation. Transfer of ALA to milk reached 59% and did not differ by stage; while transfer of EPA was 90% higher in peak and late lactation compared with early lactation (34% vs. 18%, $P < 0.05$). Transfer of DHA reached 32% in peak lactation, which was 42% and 29% higher than transfer at early and late lactation, respectively ($P < 0.05$). In conclusion, enrichment of plasma and milk, and transfer efficiencies of n-3 FA differs between stages of lactation, which may indicate specific n-3 FA requirements or metabolism for each stage of lactation.

Key Words: n-3, transfer efficiency, lactation stage

T165 Level of estrogen in mammary parenchyma explants from weaned Holstein heifer calves increases growth and proliferation through transcriptional mechanisms as evaluated via RNA-sequencing. M. V. Riboni*[1], V. Palombo[2], A. J. Geiger[3], R. M. Akers[3], and J. J. Loor[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL,* [2]*Università degli Studi del Molise, Campobasso, Italy,* [3]*Virginia Polytechnic Institute and State University, Blacksburg, VA.*

To study the effect of estrogen on mammary gland development in young heifers, mammary parenchymal tissue (PAR) was collected at weaning (8 wk) from 5 Holstein heifer calves reared on an accelerated milk replacer (1.13 kg/d, 28% crude protein, 25% fat), and incubated for 6h at 3 estrogen concentrations: 0, 10, and 100 pg/mL. RNA was extracted and sequenced on the Illumina HiSeq 4000 system, generating an average of 27 million reads/sample of which 94% where uniquely mapped, with 81% of gene-assigned reads. A linear model with estrogen dose as fixed and animal as random effect was fitted. Differentially expressed genes (DEG) were declared at FDR ≤0.2 and raw p-value ≤0.01. The Dynamic Impact Approach was used for pathway analyses to determine effect on biological pathways. DEG (791) were detected only for the comparison of 100 vs 0 pg/mL, with 435 and 356 up and downregulated genes, respectively. As expected, the estrogen signaling pathway was upregulated when explants were incubated with 100 pg/mL of estrogen. Among the top 15 impacted metabolic pathways, there was an overall upregulation of fatty acid metabolism and a downregulation of amino acid biosynthesis. These were driven by an upregulation of steroid biosynthesis, and lysine and β-Alanine catabolism, that together with the upregulation of DNA replication, cell cycle, AMPK and Wnt signaling pathways (among the top 15 impacted non-metabolic pathways) suggest a higher proliferating state of the cells. At a metabolic level, proliferation was supported by upregulation of purine metabolism and mucin type O-glycan biosynthesis. Furthermore, explants incubated with 100 pg/mL of estrogen had a downregulation of immunometabolic pathways (e.g., cytokine-cytokine receptor interaction, and chemokine and adipocytokine signaling pathways). Overall, results suggest a stimulation of growth and proliferation of PAR by exposure to estrogen at early stages of life in heifer calves fed an accelerated milk replacer.

Key Words: estrogen, calf mammary parenchyma, RNA sequencing

T166 Plasma metabolomics profiling of cattle with divergent residual feed intake. A. Elolimy*[1,2], Z. Zhou[3], D. Shike[2], and J. Loor[1,2], [1]*Mammalian NutriPhysioGenomics, Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [3]*Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC.*

We used targeted and untargeted profiling approaches to determine differences in plasma biomarkers between the most and the least feed-efficient animals based on residual feed intake (RFI). One-hundred forty-nine Red Angus cattle were allocated to 3 groups according to herd origin. Animals were fed a finishing diet for 78 d to determine the RFI category for each. Within each contemporary group, the 2 most-efficient (n = 6; RFI coefficient = −2.69 +/−0.58 kg dry matter/d) and least-efficient animals (n = 6; RFI coefficient = 3.08 +/− 0.55 kg dry matter/d) were selected. Plasma samples were collected at d 78 for analyses using ELISA and a high-resolution mass spectrometry-based untargeted metabolomics. Metabolites were analyzed using Q-Exactive MS system after LC separation. Data analysis was performed using the MetaboAnalyst 4.0 program. Metabolites with 'importance in projection (VIP)' scores >1.0 and a 2-fold difference between groups were considered significantly different. The top 15 metabolites with highest VIP score were identified by molecular weight (mass error ppm <5) for comparison between groups. Biomarker analysis via ELISA revealed that the most-efficient animals had greater activity of paraoxonase (166 vs. 131 U/mL) and lower concentrations of IL-6 (138 vs. 340 pg/mL) and IL1-β (3.7 vs. 17.3 pg/mL) indicating a more pronounced inflammatory status in least-efficient animals. The untargeted metabolomics approach identified 115 distinct metabolite features between groups. Multivariate analysis (PLS-DA and OPLS-DA) demonstrated a clear discrimination between groups. Among the top 15 metabolites identified by the VIP analysis, tumonoic Acid I (VIP = 3.9) was greater in the most-efficient compared with the least-efficient animals. The molecular mass for the remaining 14 metabolites overlapped with more than one molecule in currently available databases, hence, could not be identified with certainty. Overall, the data indicate that targeted and untargeted plasma biomarker profiling could be helpful for identifying RFI-specific systemic compounds that may play a role in determining feed efficiency in cattle.

Key Words: residual feed intake (RFI), plasma, metabolomics

T167 Anti-inflammatory treatment in early lactation alters global mammary DNA methylation. C. M. Ylioja*[1], A. J. Carpenter[2], M. Garcia[1], L. K. Mamedova[1], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*University of Guelph, Ridgetown, ON, Canada.*

Anti-inflammatory treatment in early lactation has increased long-term milk yield in multiparous dairy cows, but mechanisms are unclear. Epigenetic changes alter chromatin structure and can affect gene expression patterns long-term, and tissues undergoing development and cellular differentiation are more susceptible to epigenetic changes. As metabolic changes in early lactation involve multiple tissues, we examined tissue samples from 2 different studies for changes to global methylation (GM) as a potential mechanism for long-term effects of anti-inflammatory treatment. In study 1, 16 cows of parity ≥ 3 were alternately assigned to treatment at parturition and were drenched orally with sodium salicylate (SS; 125 g/d) or water (CON) once daily for 3 d following parturition, beginning ~24 h postpartum. Mammary tissue was collected on d 1 (before treatment), d 4, and d 45 of lactation. In study 2, 17 multiparous cows were similarly enrolled at calving and administered either SS (2 g/L) or molasses carrier (CON) via the drinking water for 5 d after parturition. Samples were collected on d 5 from adipose (tailhead), liver, and muscle tissue (longissimus dorsi lumborum). DNA was isolated and GM was assessed with a 5-methylcytosine ELISA. Mixed models were used to assess GM; contrasts compared treatment effects within day. No differences were detected in mammary GM on d 1 (5.6 vs. 5.8 ± 0.7 for SS vs. CON; $P = 0.78$), but SS increased GM on d 4 compared with CON (5.8 vs 4.1 ± 0.6%; $P = 0.04$). Although treatment means on d 45 were similar to d 4, mammary GM differences were not detected (5.6 vs. 4.5 ± 0.6%; $P = 0.21$). No treatment differences were detected for GM in liver, adipose, or muscle tissues ($P \geq 0.33$). Although GM does not identify regions of DNA or downstream functions affected, an observed tendency for GM to decrease from d 1 to d 4 for CON (5.6 vs 4.0 ± 0.6%; $P = 0.07$) supports the occurrence of epigenetic changes during the onset of lactation, and the possibility that SS may elicit long-term effects via alterations to methylation patterns.

Key Words: methylation, NSAID, epigenetic

T168 Does post-absorptive propionate clearance influence insulin sensitivity in dairy heifers? A. Bedford*, L. Beckett, K. Hardin, N. W. Dias, V. R. G. Mercadante, A. D. Ealy, and R. R. White, *Virginia Tech, Blacksburg, VA.*

The objective of this study was to investigate the effect of increased propionate status on insulin sensitivity in dairy heifers. Eleven Holstein heifers, 8 to 10 mo of age and 250 to 360 kg, were assigned to 1 of 2 treatments and given a continuous IV infusion of either sodium propionate (PRO; 61.6 mmol/h; n = 5) or saline (CON; n = 6) at a matching infusion volume for 5 d. Heifers rarely have compromised insulin sensitivity; however, they were used in this study as a preliminary screening tool to help direct future work in populations generally recognized as having heightened insulin insensitivity. On d 5, all animals underwent a hyperglycemic clamp where blood glucose levels were increased to twice basal levels for measures of insulin response. Plasma SCFA and hyperglycemic clamp data were analyzed using a mixed-effect model. Plasma propionate concentrations were significantly elevated at 2, 10, 24, and 72 h post initiation of infusion in PRO animals ($P < 0.01$), with a peak at 10 h and declining concentrations thereafter. At 96 h there was no difference between propionate concentrations of PRO and CON groups ($P = 0.443$). There were no differences in daily plasma acetate or β-hydroxybutyrate concentrations seen between treatments for any time points within the experimental period ($P > 0.10$). There were no differences between initial insulin, initial glucose, or plateau glucose between PRO and CON cows ($P > 0.05$). Plateau insulin concentrations in CON animals were significantly greater than those in PRO animals (249.4 ± 25.1 vs 123.9 ± 35.8; $P = 0.018$) and there was a trend for an increased insulin sensitivity index (ISI) in PRO cows compared with CON cows ($P = 0.06$). The results of this study indicate that animals have ample capacity to clear surplus propionate. Based on the trend for changes in ISI and the significant shift in plateau insulin concentrations, additional work is needed to understand how propionate metabolism influences downstream changes in insulin signaling.

Key Words: propionate, insulin, post-absorptive

T169 Effect of glucose infusion dose on glucose tolerance test kinetics in lactating dairy cows. E. N. Smith*, M.G. Erickson, F. F. Cardoso, B. P. Biese, J. G. Laguna, J. Bydalek, and S. S. Donkin, *Purdue University, West Lafayette, IN.*

Intravenous glucose tolerance tests (IVGTT) are widely used to assess glucose metabolism and insulin sensitivity in dairy cattle. Despite frequent use, the IVGTT has not been standardized for dairy cattle, hence a wide range of glucose dose and sampling frequencies are observed in the literature. The objective of this study was to determine the effect of glucose infusion load and stage of lactation during IVGTT on plasma glucose and insulin kinetics in dairy cattle. Six lactating Holstein dairy cows, 3 primiparous and 3 multiparous, were prepared with indwelling catheters in each jugular vein and subjected to IVGTT during early- (74 ± 8.5 DIM) and mid-lactation (221 ± 8.5 DIM). Cows were assigned within parity group to receive 0.092, 0.15, or 0.3 g glucose/kg BW during an IVGTT in 2 3x3 Latin squares with 7 d periods. Blood samples were collected at −15, −10, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min relative to the glucose infusion. Milk production and feed intake were measured daily. Glucose infusion dose increased ($P < 0.05$) the area under curve (AUC) for glucose (9022, 9725, 11731 ± 222.68 mg/dL min^{-1}; 0.092, 0.15, or 0.3 g glucose/kg BW respectively) and insulin AUC (30, 40, 68 ± 5.8 ng/mL min^{-1}; 0.092, 0.15, or 0.3 g glucose/kg BW respectively). There was no effect of parity or stage of lactation on glucose AUC or insulin AUC or dose × stage of lactation effect. There was an effect ($P < 0.05$) on the ratio of glucose AUC to insulin AUC (345.5, 272.2, 192.4 ± 28.4; 0.092, 0.15, or 0.3 g glucose/kg BW respectively). As the dose of glucose increased the ratio of glucose to insulin decreased due to a greater relative increase in insulin AUC. The data indicate that glucose infusion dose in lactating dairy cows alters both glucose and insulin excursion curves but the effect of glucose dose is greater for insulin release relative to changes in peripheral glucose concentrations.

Key Words: glucose, insulin, intravenous glucose tolerance tests (IVGTT)

T170 Effect of serial corpus luteum biopsy collection during early pregnancy on corpus luteum function, size, blood flow, and gene expression. R. Wijma, E. M. Sitko*, M. M. Perez, and J. O. Giordano, *Department of Animal Science, Cornell University, Ithaca, NY.*

Our objective was to evaluate the effect serial corpus luteum (CL) biopsy collection on circulating progesterone concentrations and corpus luteum size, blood flow, and gene expression in pregnant cows. Pregnant primiparous cows on d 27 of gestation with a single noncavitary CL were assigned randomly to a biopsy (BIO, n = 8) or sham biopsy (SB, n = 8) treatment. Biopsies were collected with an automatic biopsy needle guided through transvaginal ultrasonography (US). A CL biopsy was

collected every 48 h (5 total) from d 27 to 35 in BIO cows, whereas the sham biopsy procedure was performed in SB cows. Blood samples were collected at the time of biopsy collection and 10 d after the last biopsy to measure progesterone (P4) concentration. Immediately after CL biopsy or sham biopsy and 45 d after AI, B mode and Power Doppler transrectal US were performed to evaluate luteal size, blood flow, and pregnancy viability. The mRNA abundance of genes associated with steroidogenesis (3βHSD and STAR) inflammation (TNFa), and apoptosis (TNFa, BCL2, and BAX) was determined by RT-qPCR. Genes of interest were normalized against 3 stable reference genes (GAPDH, H3F3B, and RPS9). Continuous variables were analyzed by ANOVA with repeated measures using the Mixed procedure of SAS. Embryo loss ocurred in 3 BIO (38%) and one SB cow (13%) between d 27 and 45. There was no effect ($P = 0.84$) of treatment (TRT), time (TIM; $P = 0.83$) or TRTxTIM ($P = 0.15$) on P4 concentration (ng/mL) from d 27 to 45 (BIO = 1.70 ± 0.08; SB = 1.72 ± 0.08). Total CL area tended ($P = 0.05$) to be greater for cows in SB (586.2 ± 16.4 mm^2) than BIO (530.6 ± 11.7 mm^2) but was not affected by TIM ($P = 0.87$) or TRT×TIM ($P = 0.46$). Area of CL with blood flow (BIO = 44.2 ± 2.5%; SB = 38.9 ± 2.5%) was not affected by TRT ($P = 0.66$), TIM ($P = 0.14$), or TRT×TIM ($P = 0.3$). There was no effect ($P > 0.10$) of TRT, TIM, or TR×TIM on mRNA relative abundance for any of the genes evaluated. We conclude that collecting multiple CL biopsies (up to 5) every 48 h from d 27 to 35 of pregnancy did not cause mayor alterations to CL function as determined by progesterone secretion and CL size, blood flow, and gene expression. *Supported by USDA Hatch Multistate project NYC 127813.*

Key Words: biopsy, corpus luteum, dairy cow

T171 Expression of interferon-stimulated gene 15 (*ISG15*) mRNA is partially predictive of pregnancy-associated glycoprotein (PAG) concentrations during early pregnancy in dairy cows and heifers. L. M. Mayo*, S. G. Moore, S. E. Poock, F. G. Kumro, and M. C. Lucy, *University of Missouri, Columbia, MO.*

Elongating bovine embryos synthesize and secrete interferon-tau (IFNT) beginning 14 d after AI to prevent luteolysis, necessary for pregnancy maintenance. PAGs are synthesized by placental binucleate cells and can be detected in the blood at approximately 25 d after AI. Pregnancy can be detected by measuring *ISG15* mRNA (an IFNT-stimulated molecule) in circulating lymphocytes 18 to 22 d after AI or blood PAG 25 d after AI. The objective was to measure ISG15 and PAG during early pregnancy in the same cows and heifers. The hypothesis was that the lymphocyte *ISG15* mRNA would be correlated with the circulating PAG. If true, then one explanation is that a larger embryo achieving greater ISG15 would be larger later in pregnancy and synthesize and secrete more PAG. Holstein dairy cows (n = 19 primiparous and n = 23 multiparous) and heifers (n = 18) were AI and blood was collected on d 14, 18, 20, 22, and 25. Total RNA was isolated from blood and the abundance of *ISG15* mRNA was quantified using RT-PCR. The same samples were analyzed for plasma PAG concentration using ELISA. The conception rate was 45%. As expected, *ISG15* mRNA increased ($P < 0.001$) in pregnant cows from d 14 (baseline; 0.012 ± 0.007) to d 18 (0.046 ± 0.007), 20 (0.068 ± 0.007), and 22 (0.052 ± 0.007) and was greater ($P = 0.014$) in heifers compared with cows. There was an increase ($P < 0.001$) in PAG on d 25 after AI in pregnant cows but no effect of parity. In pregnant cows, PAG concentrations on d 22 were correlated with PAG concentrations on d 25 ($r^2 = 0.58$; $P < 0.001$). PAG concentrations on d 25 were not correlated ($P > 0.10$) with maximum or mean *ISG15* mRNA on d 18 to 22 or *ISG15* mRNA on individual days. PAG concentrations on d 22 were modestly correlated with ISG15 on d 20 ($r^2 = 0.20$; $P = 0.053$) and maximum *ISG15* mRNA on d 18 to 22 ($r^2 = 0.18$; $P = 0.066$). In summary, the ISG15 signal from d 18 to 22 was not correlated with circulating PAG on d 25. On d 22, correlations with *ISG15* mRNA and PAG were modest. We failed to confirm a strong correlation between ISG15 and PAG during early pregnancy in dairy cows and heifers.

Key Words: early pregnancy, maternal recognition

T172 Embryo and cow factors affecting pregnancy rate after embryo transfer to multiple-service dairy cows. E. Estrada-Cortes*[1], W. G. Ortiz[1], R. C. Chebel[1,2], E. A. Jannaman[1], J. I. Moss[1], F. C. de Castro[3], A. M. Zolini[1], C. R. Staples[1], and P. J. Hansen[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL* [2]*Large Animal Clinical Sciences, University of Florida, Gainesville, FL,* [3]*Departamento de Medicina Veterinária, Universidade de São Paulo, Pirassununga, São Paulo, Brazil.*

Embryo transfer (ET) is as a method to improve fertility in repeat-breeder cows. The supplementation with rumen-protected choline has improved reproductive performance in dairy cows. Here we evaluated whether pregnancy success after embryo transfer in cows inseminated >2 times without becoming pregnant depends upon characteristics of the embryo or recipient. Embryos were (1) produced in vitro using conventional Holstein semen and oocytes of undetermined breed collected at an abattoir and transferred fresh (abattoir; n = 16); (2) produced in vitro using X-sorted Holstein sperm and Holstein oocytes collected at an abattoir, cultured with either vehicle (n = 56) or 1.8 m*M* choline chloride (n = 48) and transferred fresh; or (3) produced in vitro using X-sorted Holstein sperm and Holstein oocytes collected at an abattoir and transferred after freezing with ethylene glycol (n = 43). Recipients were lactating Holsteins on a commercial dairy in north Florida and had been inseminated 2–7 times. The embryo transfers, conducted during summer and early fall, were performed 8 d after the last GnRH injection of an ovulation synchronization program. Pregnancy diagnosis was at d 33 after last GnRH. Characteristics of recipients examined were disease diagnosis in the current lactation (metritis, retained placenta, mastitis and other diseases), parity, milk production, days in milk and number of previous inseminations. Pregnancy rate (PR) was analyzed by the GLIMMIX procedure of SAS. PR was lower ($P = 0.007$) for cows receiving frozen embryos (7.0%; 3/43) than for cows receiving fresh abattoir embryos (43.8%; 7/16), fresh embryos cultured with vehicle (19.6%; 11/56), and fresh embryos cultured with choline (29.2%; 14/48), but there were not significant differences between fresh embryo types. The only significant recipient factor affecting PR was diagnosis of metritis ($P = 0.059$). For all embryo types, PR was lower for cows with metritis (7.1%; 2/28) than for cows without metritis (24.4%; 33/135). In conclusion, PR of multiple-service dairy cows after ET was reduced when using frozen embryos or when recipients experienced metritis in the current lactation. Further work to evaluate effects of choline supplementation of culture medium is warranted.

Key Words: embryo transfer, lactating cow, metritis

T173 Effect of dose of cloprostenol on luteal blood flow measurements in mature and immature corpora lutea. T. Minela*, E. L. Middleton, M. R. Herman, S. N. Berthume, and J. R. Pursley, *Michigan State University, East Lansing, MI.*

Color Doppler ultrasound measurements of luteal blood flow (LBF) may serve as an accurate way to estimate corpus luteum (CL) function. Luteal volume (r = 0.69) and luteal blood flow (r = 0.71) are highly correlated with circulating concentrations of progesterone. Our aim

was to determine the effect of different doses of $PGF_{2\alpha}$ on blood flow measurements of immature and mature CL. This study utilized n = 37 11- to 12-mo-old Holstein heifers. Heifers were synchronized with 500 µg of cloprostenol on d –8; 100 µg of GnRH on d –6; and 100 µg of GnRH on d 0. Only heifers that responded to each injection were utilized. On d 4 (day of treatment) all heifers had ovaries with both a d 4 and 10 CL. On day of treatment, heifers were randomly assigned to 5 treatments: full dose of cloprostenol (500 µg; n = 8), 1/2 dose (250 µg; n = 8), 1/4 dose (125 µg; n = 8), positive control (4 treatments of 500 µg cloprostenol 24 h apart; n = 5), or a negative control (no treatment; n = 8). Color Doppler was used to assess LBF (numbers of color pixels) before treatments and at 1, 48, 96, 144, 192 h post-treatment. Negative controls had less LBF 1 h following treatment compared with all other treatments for the d 10 CL. Negative controls had greater LBF 48, 96, 144, and 192 h following treatment compared with all other treatments in d 10 CL. Positive controls and heifers that received a full dose had less LBF compared with 1/2 and 1/4 dose groups at 48, 96, 144, and 192 h following treatment in d 10 CL. There was no difference at 48, 96, 144, and 192 h following treatment in 1/4 and 1/2 dose groups in the d 10 CL. There were no differences in LBF in the d 4 CL at 1 or 48 h but the positive control had less LBF at 96 and 144 h compared with all other treatments. There was no difference in the positive control compared with the full dose at 192 h in the d 4 CL. The full dose had less LBF at 192 h compared with the 1/2, 1/4, and negative control in the d 4 CL. The differential effects of treatments indicated that color Doppler might be an accurate assessment of luteal function before and following $PGF_{2\alpha}$.

Key Words: corpus luteum, luteal blood flow, color Doppler

T174 In vitro adipogenic differentiation of subcutaneous primary bovine preadipocytes: A coculture model. C. Strieder-Barboza*, E. Thompson, K. Thelen, and G. A. Contreras, *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Reductionist studies of adipose tissue biology require reliable in vitro adipocyte culturing models. Current protocols for induction of adipogenesis in stromal vascular fraction (SVF)-derived preadipocytes require extended culturing periods (12–14d) and have low adipogenic rates. Our objective was to compare the adipogenic efficiency of a 14d-standard induction (STD14) protocol with a 7-d coculture (COC) model using primary mature adipocytes. SVF-derived preadipocytes and mature primary adipocytes were obtained by collagenase digestion from subcutaneous (flank) adipose tissue of dairy cows (n = 6). Primary mature adipocytes were retained for use in COC. SVF was expanded for 2 serial passages and seeded in 24-well plates. Confluent preadipocytes were induced to differentiate with medium containing insulin, troglitazone, 2-isobutyl-1-methylaxanthine (IBMX), dexamethasone, and acetate for 48 h, and then maintained in this medium, excluding the dexamethasone and IBMX, for 7 d in COC, and for 14 d in STD14. Cells on d 7 of induction served as control (STD7). For COC, 0.4-µm transwell inserts with 900 primary mature adipocytes/cm^2 were placed over the preadipocytes for the first 5 d of adipogenic induction. At the last day of differentiation for each protocol, adipogenic efficiency was evaluated by TAG accumulation (AdipoRed) and gene expression of adipogenesis markers (qPCR). Data were analyzed using One-way ANOVA and Tukey-multiple comparisons. Primary mature adipocytes placed in the inserts in COC were assessed for viability (Vybrant) and decreased 50% from the d 1 to 5 of induction ($P = 0.03$). Despite this, COC improved adipogenic differentiation of bovine adipocytes as demonstrated by a 3.6 and 3.2 times increase on TAG accumulation compared with STD7 ($P = 0.005$) and STD14 ($P = 0.045$), respectively. COC also increased gene expression of peroxisome proliferator-activated receptor-γ (*PPARγ*, $P = 0.0001$) and diacylglycerol O-acyltransferase-2 (*DGAT2*, $P = 0.026$) compared with STD14. While PPARγ promotes lipid uptake and adipogenesis, DGAT2 enhances TAG synthesis in adipocytes. In conclusion, COC improved adipogenesis efficiency and reduced the time required to induce in vitro differentiation of bovine preadipocytes.

Key Words: adipocytes, bovine, coculture

T175 Effects of different heating time of high, medium, and low quality colostrum on IgG absorption in dairy calves. D. J. Saldaña*[1], S. L. Gelsinger[2], C. M. Jones[1], and A. J. Heinrichs[1], [1]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [2]*Department of Dairy Science, The University of Wisconsin, Madison, WI.*

The objective of this study was to determine the effect of different heating times with 3 levels of colostrum IgG content on passive transfer from a single feeding of colostrum. Colostrum was collected from The Pennsylvania State University dairy and divided by quality (high, medium, or low) based on colostrometer measurement. Colostrum within each quality was pooled to create 3 unique batches. Each batch was further divided in thirds as follows: frozen to be fed without heat treatment, heated at 60°C for 30 min, or heated at 60°C for 60 min. Colostrum samples from each treatment were collected and tested for standard plate count, gram-negative non-coliforms, coliforms, and total IgG concentration. Serum samples were collected from 108 Holstein calves before feeding colostrum and 24 h after birth. These were analyzed for total protein, total IgG, and hematocrit. Colostrum quality (high, medium, or low), heat treatment (unheated, 60°C for 30 min or, 60°C for 60 min), and their interaction were analyzed as fixed effects, with calf sex included as a random block effect. Colostrum IgG was different between quality groups (92.5, 59.4 and 48.1 mg/mL of IgG; $P < 0.01$). Heating colostrum reduced IgG concentration compared with the control by 9% when heated for 30 min and by 12% when heated for 60 min. Colostrum heated for 60 min had a lower standard plate count than colostrum heated for 30 min or not heated (3.6, 2.0 and 1.8 log cfu/mL). Serum IgG concentration at 24 h increased as colostrum quality increased (18, 22.2 and 24.8 mg/mL; $P = 0.02$) and tended to increase as heat treatment time increased (19.7, 20.3 and 25 mg/mL of IgG; $P = 0.06$). Apparent efficiency of absorption was greater in calves that received medium quality colostrum compared with calves fed high quality colostrum (38.1 and 25%; $P < 0.01$). These results suggest there may be an upper limit to the amount of IgG absorption in a given time period and that medium or high quality colostrum yields similar blood IgG absorption given the same volume of intake.

Key Words: calf, colostrum, immunoglobulin G

T176 Factors influencing the electrical resistance of various pathways through the dairy cow. R. J. Norell*[1], J. A. Spencer[2], S. M. Zoca[2], and A. Ahmadzadeh[2], [1]*University of Idaho, Idaho Falls, ID,* [2]*University of Idaho, Moscow, ID.*

In a series of trials, we evaluated several factors that may influence electrical contact resistance of dairy cattle: manure depth, standing surface, hair coat condition, mouth area connections, and bedding in manure. Mouth to single hoof pathways were created by placing a metal bit in the mouth while the cow stood in separate plastic trays for each hoof. Treatments were applied equally between right and left hooves. In trial 1, 2 manure depths (0.3 and 2.5 cm) on metal grids were compared with 20 cows on 2 consecutive days. In Trial 2, 30 cows stood in 2.5 cm of manure with half their feet on a metal grid and half on concrete. In Trials 3 and 4, all 4 feet stood on metal grids with manure. Trial 3 treatments (n = 20) varied by contact location (neck and rump) and hair coat condition (dry, mist, and wet), whereas Trial 4, treatments varied by mouth area contact (bit in mouth, nose tongs, drinking water). Trial 5 was a laboratory study comparing the electrical resistance of manure:bedding blends with 0 to 25% (wt/wt) added bedding in 5% increments for compost, sand, and straw. In trial 1, resistance of the mouth to single hoof pathway was significantly less ($P < 0.01$) for deeper versus shallow manure. In Trial 2, the resistance was less for the metal grid standing surface than the concrete standing surface regardless of manure presence ($P < 0.01$). In Trial 3, resistance of the rump to all hooves pathway was less than the neck to all hooves pathway ($P < 0.01$), and regardless of body location, decreased ($P < 0.01$) as hair coats were either misted or wetted to the skin compared with a dry hair coat. In Trial 4, resistance for the mouth to all hooves pathway was significantly greater ($P < 0.01$) when cows drank water from a metal bucket compared with a bit in the mouth or nose tongs. In Trial 5, adding compost or sand bedding to manure has a minimal effect on resistance whereas adding straw (over 10%) significantly increased electrical resistance ($P < 0.01$). We concluded that contact resistance of varying pathways through dairy cows are influenced by manure depth, standing surface, hair coat condition, mouth area connections, and straw bedding in manure.

Key Words: electrical resistance, stray voltage, dairy cattle

T177 Partitioning the resistance of electrical pathways through the cow into component segments. R. J. Norell*[1], J. A. Spencer[2], S. M. Zoca[2], and A. Ahmadzadeh[2], [1]*University of Idaho, Idaho Falls, ID,* [2]*University of Idaho, Moscow, ID.*

The electrical resistance of dairy cattle includes internal body resistance and resistance of pathway contacts. Partitioning resistance into various segments is useful for assessing relative contributions of each component and for modeling the effects of varying contact resistance on total pathway resistance. Resistance of 6 pathways through 20 lactating cows housed in a free-stall barn were measured: mouth to all hooves (M4), mouth to front hooves, mouth to rear hooves, front to rear hooves (FTR), mouth to flank, and flank to rear hooves. Electrical contacts were applied with a horse bit in the mouth, hooves on separate metal grids with a manure covering, and a 5 × 5 cm aluminum probe applied to the flank with a wet hair coat. Body resistance (ohms) was modeled and partitioned into 4 components: head and neck (HN), front legs (FL), abdomen (A), and rear legs (RL). The median resistance for NH, FL, A, and RL were 106 ± 3, 195 ± 5, 11 ± 1, and 184 ± 4 ohms (Ω), respectively. Contact resistance of the hooves can potentially change due to wet versus dry concrete, wet versus dry manure, wet hooves, presence of bedding, and loss of hoof integrity. Based on the model, assuming individual hoof contact resistance increases by 40 Ω, then the modeled M4 and FTR pathway resistance increase by 10 and 40 Ω, respectively. Assuming lameness causes a loss in hoof integrity and lowers individual hoof resistance by 50%, then the modeled M4 and FTR resistance are estimated to decrease by 19 and 65 Ω with a lame front hoof. Additional research is needed to (a) confirm whether the model component estimates are consistent between a research facility and commercial dairy operations; and (b) refine estimates of environmental factors on contact resistance.

Key Words: electrical resistance, stray voltage

T178 Effect of virginiamycin on milk yield and composition under commercial conditions in Mexico. M. A. Gorocica*[1], G. Velasco[1], and A. Relling[2], [1]*Phibro Animal Health Corp., Teaneck, NJ,* [2]*The Ohio State University, Wooster, OH.*

The objective of the present study was to evaluate the effect of virginiamycin (VM) inclusion on milk yield and composition in Holstein cows maintained under commercial conditions in Mexico. Four pens of lactating cows (approx. 100 cows each) were blocked based on lactation number (LN), DIM, and milk yield (2.1 and 2.3 LN; 190 and 115 DIM; and, 47.3 and 48.5 L/d, for blocks 1 and 2 respectively) and assigned to 2 treatments. Control cows (CT) were fed a diet balanced to meet or exceed their nutrient requirements (NRC, 2001); Treated cows (TR) were fed the CT diet plus 500 mg/cow/d of VM. Diets were randomly assigned within each block. Diets were based on corn silage and steam-flaked corn. Feed offered and refused was weighted and analyzed daily to determine pen DMI. The study lasted for 42 d; d 1–19 for adaptation, and d 20–42 to measure milk yield and composition. Individual milk records were obtained daily. Additionally, one pooled milk sample per pen was collected for milk fat and protein determination using a MilkoScan Mars equipment (FOSS, Hillerød, Denmark). A mixed model analysis with repeated measures was conducted at the end of the trial. Pen was the experimental unit. The model included treatment as fixed effect and block as random effect. No cows were diagnosed with clinical acidosis during the course of trial and no day × treatment interactions were observed (all $P > 0.10$). However, milk yield, milk fat, and ECM were significantly greater in the TR group ($P < 0.01$; Table 1). TR cows tended to have greater DMI but milk:feed ratio was not affected by treatment ($P > 0.10$). Milk fat:protein ratio was significantly increased ($P < 0.01$) suggesting the effects on milk yield and composition may have been related to VM preventing the development of SARA and other related digestive disorders.

Table 1 (Abstr. T178). Milk yield, composition, ECM yield, and DMI during the measurement period

Item	CT	TR	SEM	Treatment effect *P*-value
Milk yield, L/d	42.7	43.3	0.25	<0.01
Milk fat, %	3.43	3.49	0.120	<0.01
Milk protein, %	3.30	3.28	0.004	<0.01
ECM[1], kg/d	42.8	43.7	0.16	<0.01
Fat:Protein	1.04	1.06	0.003	<0.01
DMI, kg/d	27.4	27.8	0.20	0.08
Milk:DMI	1.57	1.56	0.011	0.67

[1]ECM, lb = 0.327 × Milk yield, lb + 12.95 × Fat yield, lb + 7.65 × Protein yield, lb.

Key Words: virginiamycin, dairy, production

T179 Milk production, intake and ingestive behavior of Holstein cow fed total mixed ration or partial mixed ration in early lactation. M. Ceriani*, A. Jasinsky, M. Carriquiry, and D. A. Mattiauda, *Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.*

The study objective was to evaluate the effect of 2 feeding strategy on animal performance, DM intake and ingestive behavior of lactating cows. Eighteen primiparous cows (528 ± 40 kg BW; 3.2 ± 0.2 BCS; autumn calving) were assigned to one of 2 nutritional treatments from calving to 65 DIM: (G0) TMR *ad libitum* (55:45, forage:concentrate) or (G1) grazing of Lucerne (6-h grazing in 3-d strips; 20 kgDM/d) + 70% of *ad libitum* PMR intake. All cows consumed 2.0 kgDM/d of a commercial ration at each milking. Milk yield was determined daily, milk composition weekly, and BW and BCS every 14 d. Diurnal cow behavior was recorded every 15 min, during 3 consecutive day, at wk 3 and 6 of lactation. During the grazing session, the number of bites were counted during 1 min. Individual herbage intake was estimated using cow energy requirements. Data were analyzed with a mixed model including treatment, DIM, and their interaction as fixed effects, block and cow as random effects and calving data as a covariate. Solid corrected milk yield tended ($P = 0.07$) to be greater for G0 than G1 cows (27.9 vs. 25.6 ± 0.58 kg/d). Cow BW did not differ but BCS was greater ($P = 0.01$) for G0 than G1 cows (2.9 vs. 2.6 ± 0.06). Neither total DM nor energy intake differed between treatments (16.7 vs. 17.3 ± 0.46 kgDM/d for G0 and G1, respectivily). For G1 cows, the proportion of time dedicated to grazing was greater ($P < 0.05$) for wk 3 than 6 (54 vs. 33%, respectively) but bite rate was less ($P < 0.05$) in wk 3 than wk 6 (36 vs. 44 ± 1.3 bite/min, respectively). In wk 3, the proportion of time spent for feed (pasture and TMR) consumption was greater ($P < 0.01$) for G1 than G0 cows (51 vs. 27%), while in wk 6 it did not differ ($P = 0.91$) between treatments. In wk 3, G0 cows spent more time ruminating and idling at morning (16 and 9%; respectively) than G1 cows. Despite similar DM intake, G1 presented decreased solid corrected milk yield and BCS than G0 cows which would suggest greater energy expenditure in the former ones, probably explained by the increased time dedicated to food collection

Key Words: nutrition, animal behavior

T180 Relationship between feed bunk refusals and feed conversion efficiency in Argentine dairy farms. J. L. Monge*[1], F. Bargo[2], E. Giugge[3], C. Chiavassa[3], A. Barrenechea[1], G. Coschica[1], M. V. Barrenechea[1], and M. P. Turiello[4], [1]*Universidad Nacional Villa María, Villa María, Córdoba, Argentina,* [2]*Universidad de Buenos Aires, Buenos Aires, Argentina,* [3]*Grupo Chiavassa, Carlos Pellegrini, Santa Fe, Argentina,* [4]*Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina.*

Our objective was to investigate the association between feed bunk refusals (FBR) with feed conversion efficiency (FCE) in 2 commercial dairy farms with compost bedded pack barns in Argentina (Chiavassa Dairy, CD; LaCenobia Dairy, LD). For 5 mo (September 2017 to January 2018), we daily registered DM offered (DMO, kg/d), DMI (kg/d), milk yield (MY, kg/d), FCE (kg milk/kg DMI), and FBR (%DMO) in 4 pens (0 to 150 DIM; 230 and 150 cows/pen at CD and LD, respectively). Milk yield was averaged by pen and DMI was estimated by difference between feed offered and refused. TMR DM content was determined with a forced-air oven for 2 h at 135°C. At CD, cows were milked 3x/d and fed 2x/d with a TMR composed by corn silage, alfalfa silage, alfalfa hay, corn grain, soybean meal, and mineral premix that averaged 49.8% DM, 15.8% CP, 29.9% NDF, and 2.89 Mcal ME/kg DM. Milk yield, DMI, and FBR (mean ± SE) was 42.06 ± 6.01 kg/d, 23.88 ± 2.42 kg/d, and 6.90 ± 4.38%DMO, respectively. At LD, cows were milked 2x/d and fed 1x/d with a TMR composed by corn silage, alfalfa silage, wheat silage, corn grain, soybean meal, peanut meal, and mineral premix that averaged 46.0% DM, 16.3% CP, 31.9% NDF, and 2.41 Mcal ME/kg DM. Milk yield, DMI, and FBR (mean ± SE) was 26.22 ± 2.15 kg/d, 22.55 ± 2.31 kg/d, and 6.45 ± 5.93%DMO, respectively. We run Pearson correlations to evaluate association between FCE and MY, DMI, and FBR (InfoStat, 2017). Effect of FBR level (0–3%, 3–6%, and >6%) was also evaluated with a mixed linear model with FBR level and day as fixed and pen within dairy as random effects. For both dairies, FCE was positively ($P < 0.05$) correlated with MY (r = 0.50 CD; r = 0.24 LD) and FBR (r = 0.38 CD; r = 0.74 LD) and negatively ($P < 0.05$) correlated with DMI (r = - 0.33 CD, r = - 0.68 LD). FBR level affected ($P < 0.05$) DMI and FCE, but it did not MY ($P > 0.05$). With FBR >6% (vs. 3 – 6% and 0 – 3%), FCE increased (1.53 vs. 1.43 and 1.39 ± 0.29 kg milk/kg DMI) and DMI reduced (22.43 vs. 23.65 and 24.43 ± 1.14 kg/d). Our

data shows the importance of FBR management to maximize FCE in commercial dairy farms. More appropriate FCE should be estimated using energy corrected milk.

Key Words: feed conversion, milk yield, feed bunk refusals

T181 The effect of compost bedded pack or sand bedded freestall barns on milk thermoduric microorganism content. M. Borchers*[1], M. Morgan[1], and J. Bewley[2], [1]*University of Kentucky, Lexington, KY,* [2]*CowFocused Housing, Bardstown, KY.*

This study's objective was to identify milk thermoduric microorganism population differences between compost bedded pack and deep sand bedded freestalls herds. Bulk tank milk samples were collected from herds housing cows exclusively on compost bedding (n = 10) or fresh sand bedding (n = 10) in the winter and summer of 2017. Three 9 mL bulk tank milk samples were pasteurized at 63°C for 30 min, 72°C for 15 s, or 135°C for 2 s. Fifty µL of milk was spiral plated on Plate Count Agar in duplicate. Plates were incubated at 35°C and 55°C for 24 h and 4°C for 10 d. A most probable number count was performed to determine growth <10 cfu/mL. Briefly, 1 mL milk was added to 3 tubes containing 9 mL Brain Heart Infusion Broth for each farm, pasteurization, and incubation combination. Inoculated broth was plated for isolation to determine growth. Morphologically unique colonies were isolated and identified using a Vitek 2 (bioMerieux, Hazelwood, MO, USA). Plate counts served as dependent variables and housing type as independent variables in a one-way ANOVA (Proc ANOVA, SAS v9.3; Cary, NC). Plate count results are shown in Table 1. *Bacillus licheniformis* was the only isolate from compost bedded pack milk. *Bacillus coagulans*, *Bacillus licheniformis*, *Brevibacillus borstenlensis*, and *Paenibacillus polymyxa* were isolated from sand bedded freestall milk. Compost bedding herds were not significantly greater than sand bedding herds in bulk tank milk thermoduric microorganisms. Future analysis will investigate bedding and teat skin thermoduric microorganism differences in bedding.

Table 1 (Abstr. T181). Results of a one-way ANOVA comparing milk thermoduric microorganism counts from compost bedded pack (CBP)barn and sand-bedded freestall (SFS) barn herds

Temperature (°C)		Count (mean ± SD; cfu/mL)			
Pasteurization	Incubation	CBP	SFS	*F*-value	*P*-value
63	4	2.0 ± 6.4	0.0 ± 0.0	1.0	0.33
	37	14.4 ± 17.6	10.3 ± 11.4	0.4	0.55
	55	2.5 ± 4.0	1.3 ± 1.1	0.9	0.36
72	4	0.0 ± 0.0	0.0 ± 0.0	—	—
	37	3.9 ± 4.8	15.8 ± 15.9	5.2	0.04
	55	5.2 ± 9.4	1.3 ± 0.9	1.7	0.20
135	4	0.0 ± 0.0	0.0 ± 0.0	—	—
	37	56.3 ± 136.9	25.7 ± 73.5	0.4	0.54
	55	0.0 ± 0.0	0.0 ± 0.0	—	—

Key Words: thermotolerant, milk quality, pasteurization

T182 Evaluation of a topical spray-on product for body temperature control in lactating Holstein cows. C. S. Takiya*[1], B. E. Voelz[1], S. E. Schuling[2], D. E. Schimek[2], L. G. Mendonça[1], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*NutriQuest, Mason City, IA.*

Heat stress is a challenge for the US dairy industry, but few topical application strategies have been attempted to mitigate it. Sixty mid-lactation Holstein cows were used in a randomized block design experiment to evaluate impacts of a spray-on product on body temperature over 42 d. Cows were blocked by milk yield and coat color, and randomly assigned to treatments that differed in spray-on product quantity (200 or 300 mL) and application frequency (once on d 1, or twice, on d 1 and 24). The placebo treatment (300 mL water) was applied twice, providing a total of 5 treatments. Treatments were applied as an aerosol spray to both sides of the animal with a low volume, low pressure spray gun. Vaginal temperature (VT) was recorded every 5 min for 4 d beginning on d 9 and 30 using the ibutton/blank CIDR method. Infrared thermographic images (ITI) were taken from right and left side of rib cage of the cows 3 × /wk in the evening during wk 2, 4, and 6. ITI of the eye and base of udder were also collected during wk 2 and 6. Statistical models included the fixed effect of treatment and the random effect of block, with repeated measures over time. Pearson correlation coefficients were calculated to compare paired VT and maximum and mean temperatures from ITI. THI exceeded 72 for 14 h/d during the study, suggesting that heat stress likely affected cows. During d 9–13 after initial treatment, spray-on product treatments increased VT relative to control (38.72, 28.86, and 38.86 ± 0.06°C for 0, 200, and 300 mL, respectively; $P < 0.01$), but no treatment differences were observed on VT on d 30 - 34 (6–10 d after the second treatment). Treatments had no effect on mean ITI temperature of the eye, the base of the udder, or over the ribs. Eyeball and udder ITI maximum temperature were positively correlated ($P < 0.01$; R = 0.39 and 0.29, respectively), whereas the mean ITI of udder and left side of rib cage were negatively correlated ($P \leq 0.046$, R = −0.36 and −0.19) with VT. We observed no evidence that the spray-on product reduces body temperature of cows. Maximum temperatures from ITI of eye had a moderately positive relationship with VT.

Key Words: heat stress, infrared image, topical

T183 Milk fatty acid profile and gene expression related to metabolism in mammary gland from cows fed two dietary zinc sources under heat stress. T. N. Marins*[1], R. M. Orellana[1], X. Weng[1], A. P. A. Monteiro[1], J. Guo[1], J. K. Bernard[1], D. J. Tomlinson[2], J. M. DeFrain[2], and S. Tao[1], [1]*University of Georgia, Tifton, GA,* [2]*Zinpro Corporation, Eden Prairie, MN.*

Heat stress and dietary Zn source influence mammary function of lactating dairy cows but their impacts on milk fatty acid (FA) composition and mammary metabolism are largely unknown. The objective of this study was to evaluate milk FA profile and mammary gland gene expression related to energy metabolism of lactating cows fed 2 dietary Zn sources under 2 environments. Multiparous lactating Holstein cows (n = 72) were randomly assigned to 4 treatments with a 2 × 2 factorial arrangement. Treatments included 2 environments: cooled (CL) using fans and misters or non-cooled (NC), and 2 dietary Zn sources: 75 mg/kg of Zn hydroxychloride (IZ) or 35 mg/kg Zn hydroxychloride + 40 mg/kg Zn-Methionine complex (ZC). All cows were cooled for 84 d (temperature-humidity index = 73) and fed respective dietary treatments before the environmental challenge for the remaining 84 d (temperature-humidity index = 78) when NC cows were deprived of cooling. After the environmental challenge, milk samples were collect from 24 cows (6/treatment) at 3, 25, and 53 d to measure FA profile by gas chromatograph. Mammary tissue was collected from 32 cows (8/treatment) at 7 and 56 d for gene expression analysis by RT-qPCR. There were environment × Zn interactions for milk C4:0 and C8:0 ($P \leq 0.02$), with ZCNC cows having lower C4:0 and ZCCL cows having lower C8:0. Compared with NC, CL cows had greater C12:0 ($P = 0.01$),

and ZC cows tended ($P = 0.06$) to have greater C12:0 than IZ. Relative to CL, NC cows had lower de novo ($P = 0.02$) but greater preformed FA (>C16, $P = 0.05$), and tended ($P = 0.10$) to have greater polyunsaturated FA. Compared with CL, mammary tissue from NC cows had greater ($P \leq 0.08$) gene expression of phosphofructose kinase-1 and 6-phosphogluconate dehydrogenase, but did not alter gene expression of FA synthase and lipoprotein lipase. In conclusion, dietary Zn source elicits different responses on milk short chain FA profile under different environments; heat stress increases preformed but decreases de novo FA, and may alter glycolysis and pentose pathway of the mammary gland.

Key Words: heat stress, milk fatty acid, mammary metabolism

T184 Evaluating the effects of Vista Pre-T on feed efficiency in heat-stressed dairy cattle. A. E. Stone*, K. B. Graves, and S. Hardin, *Mississippi State University, Starkville, MS.*

The objective of this study, conducted between April 12 and September 1, 2017, at the Mississippi State University Bearden Dairy Research Center, was to evaluate the effect of Vista Pre-T (AB Vista, Marlborough, UK) on feed efficiency (FE) and milk components during heat stress. Cows (n = 92 Holsteins) were assigned to control (CON) and treatment (TRT) groups based on parity (1 or ≥2), DIM, and milk yield. The same ration was fed except TRT was pre-treated according to manufacturer recommendations (750 mL per ton TMR dry matter). Cows were weighed and milk fat, protein, and lactose were tested weekly. Orts were weighed daily and FE was calculated by dividing energy corrected milk by dry matter intake. Reticulorumen temperature (RT) was obtained every 5 min via bolus (Smaxtec, GmBH, Austria). Temperature humidity index (THI; Tycon Systems Inc., Bluffdale, UT) was obtained hourly. Milk yield was recorded at each milking. The MIXED procedure of SAS was used to determine the relationship between FE, milk components, milk yield, RT, SCC, and THI between groups. Treatment ($P < 0.01$), yield ($P = 0.02$), RT ($P < 0.01$), and THI ($P < 0.01$) had significant effects on FE. The TRT group was more feed efficient than CON (1.54 ± 0.02 and 1.34 ± 0.05). Dry matter intake did not differ between CON and TRT (69.07 and 68.91 kg/d; $P = 0.92$). Treatment ($P = 0.04$), RT ($P < 0.01$), and THI ($P = 0.05$) had significant effects on milk fat. The TRT group had higher fat than CON (4.41 ± 0.06 and 4.02 ± 0.14%). Parity group, DIM, milk yield, weight, RT, and treatment ($P < 0.01$ for all) had significant effects on milk protein. Milk protein was greater in the TRT group compared with CON (2.80 ± 0.01 and 2.68 ± 0.03). The CON group had lower lactose than TRT (4.70 ± 0.03 and 4.85 ± 0.01; $P < 0.01$). The TRT group had lower SCC than CON (154,170 ± 15,994 and 326,670 ± 33,326 cells/mL). Parity group ($P < 0.01$) and DIM ($P < 0.01$) also had significant effects on SCC. Treatment and CON groups did not differ in RT (39.27°C and 39.11°C, $P = 0.27$). Treatment did not have a significant effect on milk yield ($P = 0.55$), but THI did ($P = 0.02$). Vista Pre-T significantly improved FE, milk lactose, protein, fat, and SCC with no measured negative implications.

Key Words: feed efficiency, heat stress, Vista Pre T

T185 The choice of dry off procedure (abrupt/gradual) is not linked to milk production level. K. Krogh*, N. Menudier, L. Meppiel, J.-F. Collin, A. de Prado-Taranilla, C. Mansanet, G. Pagny, B. Cuminal, and N. Isaka, *Ceva Sante Animale, Libourne, France.*

Dairy cows are dried off to allow for a sufficient dry period. Previous work has been focused at interaction between dry off procedures and udder health or welfare. Drying cows off gradually implies that cows will be exposed to a series of changes before the dry off that might be questionable. This work aims to investigate the practices used (feeding management and milking routine) to dry off cows in 2 European Countries; France and Denmark and evaluate a possible association of dry off procedure with average milk production. Information on dry off management was collected at herd and animal level during a multicenter prospective cohort study including 558 dairy cows from 37 herds in France and 347cows from 21 herds in Denmark. Herds were randomly selected. All cows dried-off during the study duration were included. Information on production parameters, feeding and management practices at herd and cow level were collected. Abrupt dry-off implied no prior change in either milking frequency or feeding regimen. Gradual dry-off implied any change in these routines. Levels of milk production varied across the included farms with in mean (SD) 7,795.3 (±1,881) kg and 11,387.7(±2,394.8) kg in 305 d ECM in France and Denmark respectively. The information on how cows were dried-off at herd level (representing the overall management strategy at herd level) and at animal level (representing the actual included cows in the study) were collected. It must be highlighted how definitions of dry-off methodology were understood differently by farmers taking part in the study thus creating a large difference between the overall dry-off management at farm level and what was implemented at cow level. This study demonstrated high variability in dry off methodologies applied at herd and animal level in France and Denmark. Among farms included 31 from France and 6 from Denmark indicated to perform an abrupt dry off versus 6 respective 15 a gradual dry off. At animal level 46.1% and 32.6% of cows were dried off abruptly versus 53.9% and 67.4% were dried off gradually in France and Denmark respectively. In both countries both a gradual and an abrupt dry off method was applied at animal level irrespective of major differences in milk production at both animal and herd level.

Key Words: dry off, management

T186 Milk production before dry off in dairy cows in France and Denmark. K. Krogh*, A. de Prado-Taranilla, L. Meppiel, J.-F. Collin, C. Mansanet, G. Pagny, N. Menudier, B. Cuminal, and N. Isaka, *Ceva Sante Animale, Libourne, France.*

Dairy cows and foremost the Holstein-Friesian breed has more than doubled its milk yield over the last 40–60 years. They often average more than 30 L per day with some producing more than 60 L during peak lactation. Due to flattening of lactation curves milk production levels before dry-off (DO) have also increased. The objective of this study was to describe the levels of milk production immediately before DO in 2 European countries, France and Denmark. Milk production levels were collected at herd and animal level during a multicenter prospective cohort study including 558 dairy cows from 37 herds in France and 347cows from 21 herds in Denmark. All dairy cows dried off during the study duration were included. Additional information on production parameters at herd and cow level were collected. Information on milk production 24 h before the last milking before the dry off and the methodology was recorded. Abrupt dry-off implied no prior change in either milking frequency or feeding regimen. Gradual dry-off implied any change in these routines. Overall, the milk production when the cows were dried off gradually decreased to 13 to 14 kg the day before dry off. Among the cows dried-off abruptly, the milk production the previous day of the dry-off differed between Denmark (25.1 kg) and France (14.5 kg). Populations from the 2 countries varied in other parameters such as breed, parity and overall milk production level. These study demonstrate that the milk production cut-off to dry off cows differ among farmers in France and Denmark. While French farmers dry off cows gradually with in mean (SD) 14.4 (6.1) and abruptly 14.5 (5.3) kg milk/24 h, Danish

farmers dry cows off gradually with 13.4 (7.4) and abruptly 25.1 (10.8) kg milk/24 h. Milk production during the 24 h before the dry off were thus lower in cows included in France irrespective of the applied dry off method. A third (113 of 347 included cows) of the included Danish cows were dried off abruptly with more than 15 kg/24 h, which may be detrimental from an udder health perspective.

Key Words: dry off, management

T187 Milk replacer addition to whole milk in dairy calves: Effect on growth and starter intake. A. Bogni*[2], C. Vissio[1], N. Marchetto[3], and P. Turiello[1], [1]*Facultad de Agronomía y Veterinaria UNRC, Río Cuarto, Córdoba, Argentina,* [2]*Departamento Técnico Teknal SA, Río Cuarto, Córdoba, Argentina,* [3]*Consultor privado, Río Cuarto, Córdoba, Argentina.*

The objetive of this study was to evaluate the effect of increasing the concentration of total solids in whole milk on growth and starter intake in Holstein calves. Sixty female calves were fed 4 L of colostrum at birth, blocked by dam parity (primiparous and multiparous) and randomly assigned to 1 of 4 treatments (n = 15) in a 2 × 2 factorial design. All calves were fed whole heat-treated milk under either a conventional (C, 4 L from d 1 to 49) or a step-up/step-down system (S, 4 L from d 1 to 7, 6 L from d 8 to 14, 8 L from d 15 to 28, 6 L from d 29 to 35, 4 L from d 36 to 49), divided into 2 equal feedings daily. Both systems were also tested with 0.5 kg/d of milk replacer powder (MR) during the first 30 d or without it. Treatments were C, C+MR, S, and S+MR. Starter and water were offered daily ad libitum from d 1. All calves were fed 2 L from d 50 to 56. Calves were weighted at birth and weekly until 53–59 d of age. Starter intake was calculated based on offered and remaining feed. The ANOVA procedure was used to determine the effect of treatment on BW, ADG and starter intake, and statistical differences were considered with a *P*-value <0.05. No statistical differences in BW were detected at birth (37.3 ± 4.7 kg). MR supplementation increased by 8 kg the final BW of calves in S treatment but no differences between C+MR and S+MR were detected. MR supplementation increased ADG by 0.11 and 0.12 kg/d, in C and S treatments, respectively. Starter intake during the last week (d 50–56) was similar between treatments. In conclusion, total solid increment using MR during the first month showed a greater ADG during the entire liquid feeding period with no negative effect on starter intake. The use of C+MR is a simple method to obtain higher weight gains without the need to increase the volume of milk offered.

Key Words: dairy calf, growth, milk replacer supplementation

T188 Effects of re-hydration therapy on body temperature indices in heat-stressed lactating cows. M. Al-Qaisi*[1], E. A. Horst[1], S. K. Kvidera[1], A. J. Kramer[1], C. S. McCarthy[1], E. J. Mayorga[1], M. A. Abeyta[1], N. C. Upah[2], D. M. McKilligan[2], H. A. Ramirez-Ramirez[1], J. A. D. R. N. Appuhamy[1], L. L. Timms[1], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*TechMix LLC, Stewart, MN.*

Heat stress (HS) negatively affects physiological parameters in dairy cows. Electrolyte supplementation may improve acid-base balance, water intake, and ultimately reduce body temperature. Therefore, study objectives were to determine the effects of a product containing electrolytes, osmolyte, and energetic compounds (EOEC) as re-hydration therapy on body temperature indices in HS lactating Holstein cows. Nineteen multiparous, lactating cows were housed in individual-box stalls and randomly assigned to 1 of 2 dietary treatments: 1) a control diet (n = 9) or 2) a control diet supplemented with 113 g/cow/d of EOEC (n = 10; Bovine BlueLite Pellets; TechMix, LLC, Stewart, MN) top-dressed once daily. The trial consisted of 2 experimental periods (P). During P1 (4 d), cows were fed their respective treatments and housed in thermoneutral conditions for collection of baseline data. During P2 (4 d), HS was artificially induced by using an electric heat blanket (EHB; Thermotex Therapy Systems Ltd. Calgary, Canada). Vaginal temperature (Vt) was measured every 10 min using a data logger (iButton DS1921, Maxim Integrated, San Jose, CA). Overall, the EHB increased rectal temperature (Tr), Vt, skin temperature (Ts), respiration rate (RR), and heart rate (HR) (1.6°C, 1.5°C, 1.7°C, 2-fold, and 11 bpm, respectively; $P < 0.01$) relative to P1. There were no dietary treatment differences in Tr, Vt, RR, and HR ($P = 0.40$); however, EOEC-supplemented cows had increased Ts relative to controls (0.64°C; $P = 0.04$). Compared with P1, the EHB decreased DMI and milk yield (45 and 22%, respectively; $P < 0.01$), but no differences were observed between dietary treatments. Relative to controls, circulating glucose tended to be increased (5%; $P = 0.07$) and insulin was increased (1.95 fold; $P = 0.01$) in EOEC-fed cows. A treatment by day interaction was detected for NEFA levels where EOEC-fed cows tended to have decreased (20%) NEFA levels at the end of P2 compared with controls ($P = 0.06$). In summary, feeding EOEC altered metabolism and increased Ts but not Tr, Vt, RR, and HR. Increased Ts may suggest that EOEC increased heat dissipation, likely via increased sweating.

Key Words: electric blanket, cow, heat stress

T189 Human-edible nutrient conversion and performance of cows fed a "zero land use" diet. C. S. Takiya*, A. Bennett, M. Davidson, C. M. Ylioja, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

The sustainability of dairy industry has been challenged due to competition with humans for feed and arable land. We sought to evaluate whether cows could maintain milk yield and improve conversion of human-edible (HE) nutrients in feedstuffs into milk when fed a "zero land use" diet (ZLU) and rumen-protected amino acids (AA). Twelve multiparous cows were assigned to a 3 × 3 Latin square with 21-d periods. Cows were blocked by milk yield and were randomly assigned within block to the following treatments: (1) conventional lactation TMR (CON) with 26% co-products; (2) TMR composed of ZLU feedstuffs; and (3) ZLU with top-dressed AA [ZLU-AA; 77 g/d AjiPro-L (Ajinomoto, Chicago, IL) and 45 g/d MetaSmart (Adisseo, Antony, France)]. ZLU consisted of hay from the winter intercropping of triticale and red clover, wheat middlings, corn gluten feed, corn hominy, spent coffee grounds, whole cottonseed with lint, and molasses. Cows were milked twice/d and milk samples were analyzed on d 18–21 of each period. Maximum HE metabolizable energy (ME) and protein contents were estimated based on sugar, starch, true protein, and fat concentrations in corn grain (including in silage), corn hominy, soybean meal products, wheat middlings, and molasses. Other feedstuffs were considered unsuitable for human consumption. Statistical models accounted for the random effect of cow within square and fixed effects of treatment, period, and square. ZLU diets decreased DMI, milk and solids yields, and feed efficiency (Table 1). ZLU diets did not improve the recovery of HE ME, and worsened HE protein recovery. Avoiding the use of dedicated livestock feed does not necessarily improve recovery of HE nutrients, although assumptions about human ingredient use heavily influence these calculations.

Table 1 (Abstr. T189).

Item	Diet			SEM	*P*-value
	CON	ZLU	ZLU-AA		
DMI, kg/d	26.2[a]	22.4[b]	22.5[b]	0.63	*
Milk yield/DMI	1.21[a]	1.06[ab]	0.99[b]	0.054	*
Milk yield, kg/d	31.9[a]	22.9[b]	22.9[b]	1.48	*
Fat, kg/d	1.13[a]	0.87[b]	0.89[b]	0.049	*
Protein, kg/d	1.02[a]	0.70[b]	0.75[b]	0.043	*
Lactose, kg/d	1.53[a]	1.03[b]	1.05[b]	0.073	*
Urea N, mg/dL	13.9[ab]	14.2[a]	13.7[b]	0.14	0.03
HE ME recovery in milk, %	0.64	0.65	0.63	0.029	0.90
HE protein recovery in milk, %	0.79[a]	0.42[b]	0.41[b]	0.035	*

*$P < 0.001$.

Key Words: by-product, sustainability

T190 Comparison of six handheld glucose meters used in dairy cows. R. Lopes*, A. Valldecabres, and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.*

The objective of this study was to evaluate the suitability of 6 handheld meters for measuring glucose in dairy cows [Contour Next (CT), FreeStyle Precision Neo (FS) Nova Max Plus (NM), Aga Matrix (AM), Precision Xtra (PX), and Accu-Chek Aviva Plus (AC)]. Whole blood samples from 97 Jersey cows (71 for CT) were measured in triplicate immediately after collection using the 6 handheld glucose meters. Statistical analysis was performed using Medcalc v.18. Plasma glucose concentration by hexokinase enzymatic methodology was considered the reference methodology. Based on the intra-assay coefficient of variation (CV), precision varied across handheld glucose meters: AC (2.9%), CT (6.1%), PX (6.2%), FS (7.3%), AM (8.3) and NM (8.3%). Pearson correlation coefficients between the reference methodology and handheld glucose meters were 0.53 for AM, 0.67 for NM, 0.79 for FS, 0.82 for CT, 0.83 for PX and 0.85 for AC ($P < 0.0001$). Bland-Altman plots between each meter and the reference methodology resulted in a negative bias (FS = −4.2 mg/dL; CT = −10.7 mg/dL) or a positive bias (PX = 5.9 mg/dL; AM = 7.0 mg/dL; NM = 10.4 mg/dL; AC = 11.8 mg/dL). All handheld glucose meters fell within acceptable limits of agreement (1.96SD) at least 95% of the time (PX = 96%; AC = 96%; CT = 95%; AM = 95%; NM = 95%), except by FS (94%). Optimized thresholds were calculated by receiver operating characteristics (ROC) analysis using ≥60 mg/dL as the thresholds for hyperglycemia. Adjusted thresholds were 47.0 mg/dL for CT (Sensibility [Se] = 92.1%, Specificity [Sp] = 72.7%), 51.3 for FS mg/dL (Se = 90.4%, Sp = 57.8%), 64.5 mg/dL for PX (Se = 76.9%, Sp = 73.3%), 69.7 mg/dL for AM (Se = 50.0%, Sp = 75.6%), 68.3 mg/dL for NM (Se = 73.1%, Sp = 60.0%), and 71.3 mg/dL for AC (Se = 75.0%, Sp = 75.6%). According to area under the ROC curve (AUC), CT (0.835) had the highest accuracy, followed by AC (0.799), PX (0.790), FS (0.751), NM (0.690), and AM (0.616). CT and AC had a discriminatory power comparable to a previously validated handheld meter (PX), but precision was better for AC. For dairy cows, most meters will need be used with optimized threshold or be calibrated to improve accuracy.

Key Words: cow-side meter, glucose

T191 Forage in close-up rations: Type, inclusion rate, and dry matter adjustments. R. Lopes* and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.*

The objective of the present study was to describe the type, inclusion rate (DM %), and frequency of DM adjustments of forages incorporated into the close-up recipe (CUR). Records from a 12-mo (n = 23) or 5-mo (n = 1) consecutive period were extracted from a herd feeding management software (FeedWatch 7.0). Enrolled dairies were located in California and ranged in size from 1,100 to 6,900 cows. The data set included the following variables: date, recipe type, recipe number, ingredient type, ingredient quantity, and ingredient DM. Descriptive statistics were conducted with MedCalc (v. 18.) The median number of forages included in CUR ranged from 2 to 4 across dairies. Corn silage was used in 95% of dairies and alfalfa forages in 83% of dairies [hay (low K: 60%, unknown: 23%), haylage (8%), green chop (4%)]. Half of the dairies incorporated a combination of corn silage and alfalfa hay as the sole sources of forage in CUR. Other forages included in CUR were oat hay (25%), wheat straw hay (25%), wheat silage (12%), and sorghum silage (8%). Across dairies, forage inclusion in CUR ranged from 45 to 90%, with most dairies (70%) including at least 65% of forage. Within dairy, forage inclusion varied over time; it increased up to 2 to 38% from Q_{10} to Q_{90}. Corn silage inclusion in CUR was <40% (8%), 40 to 60% (56%), and >60% (34%). Within dairy, corn silage inclusion varied, increasing 4 to 77% from Q_{10} to Q_{90}. Alfalfa hay inclusion in CUR averaged <30% (20%), 30 to 40% (30%), 40 to 50% (40%) and > 50% (10%). On dairies with 12-mo data set (n = 23), DM adjustments for corn silage were performed 0 (17.3%), 3 to < 6 (26.0%), 6 to < 12 (30.4%) or ≥12 (26.0%) times per year. For corn silage, adjustments in DM represented a change of 1.5 to 10% in percentage units of DM. DM adjustments for alfalfa hay were observed 0 (85%) or 1 (15%) time per year; the changes made were of 0.3 to 3 percentage units. Overall, in our study forage inclusion rate varied within and across dairies. Alfalfa forages were used on most dairies but only 60% recorded alfalfa as low K. There seems to be an opportunity to increase the frequency of DM adjustments for corn silage on dairies.

Key Words: forage, DM analysis, dairy cow

T192 The effect of hygiene score on somatic cell count of cows reared in a compost bedded pack dairy barn. F. Alpay[1], C. Ünal*[1], E. Çavusoglu[1], I. M. Abdourhamane[1], M. Efil[2], D. Dinçel[1], M. Ogan[1], and S. Dikmen[1], [1]*Department of Animal Science, Uludag University, Faculty of Veterinary Medicine, Bursa, Turkey,* [2]*Department of Animal Nutrition, Uludag University, Faculty of Veterinary Medicine, Bursa, Turkey.*

Compost bedded pack barn type is an alternative housing system for dairy cows and fairly new design for Turkish dairy farmers. The objective of this study was to determine the effect of hygiene score on somatic cell scores of lactating Holstein cows reared in a compost bedded pack barn system in Turkey. A total of 8,438 records of 1,068 lactating Holstein cows were used for this study. Milk samples were collected fortnighly from the cows to determine SCC, fat, lactose, protein, nonfat solids, total solids, casein, urea, freezing point, density and acidity in milk. Cow hygiene was measured using a 4-point scoring system (very clean, clean, dirty, and very dirty) for 3 different areas of the cow's body (leg, flank, and udder) and combining these scores to generate a composite cleanliness score. Hygiene score of different body parts of each cow were recorded on the day of milk sampling. Milk composition, SCS, and milk yield data were analyzed with using PROC MIXED model of SAS. The effects of parity (1–6), stage of lactation (early, mid and late lactation),

season (spring, summer, autumn, and winter), year (2014, 2015), level of milk production (high and low) and hygiene score were determined as the fixed effects and the effect of cow within each hygiene score was included as a random factor in the model. Results showed that only leg ($P < 0.01$) and udder ($P < 0.001$) hygiene scores were significantly changed the somatic cell score. On the other hand, the effects of parity ($P < 0.001$), level of milk production ($P < 0.001$), stage of lactation ($P < 0.001$) and season ($P < 0.001$) were also significant on somatic cell score of cows reared in compost bedded pack barn. In conclusion, leg and udder hygiene scores were significantly associated with SCS and also during the summer months, high producing dairy cows had highest SCS especially when they are in early stage of lactation.

Key Words: somatic cell count, hygiene score, compost bedded barn

T193 Automated body condition scoring: Evaluation of the effects of BCS around calving on metabolic disease. C. M. Truman[1], I. L. Mullins[1], M. L. Falk[1], J. M. Bewley[2], and J. HC Costa*[1], [1]*University of Kentucky, Lexington, KY,* [2]*CowFocused Housing, Bardstown, KY.*

Body condition scoring (BCS) is a non-invasive way to measure the amount of fat present on the cow's body. Cows with low or high BCS are at a higher risk of developing metabolic disease and decreased reproductive performance. The objective of this study was to evaluate the effect of automated collected BCS around calving on subclinical and clinical disease outcomes. This study was conducted from September 2016 to September 2017 on a 3,200-cow commercial dairy in Indiana. Subclinical ketosis and milk fever status were determined for each cow from one blood sample taken ≤7 DIM. Blood was analyzed for BHB and calcium concentrations. Positive subclinical cases of ketosis and milk fever were classified at ≥1.2 mmol/L BHB and <8.6 mmol/L calcium, respectively. Clinical disease data, for metritis, milk fever, and ketosis, were collected by farm personnel and recorded electronically. Daily BCS was recorded automatically, on a 1 to 5 scale, from an automated BCS camera (DeLaval International AB, Tumba, Sweden) throughout lactation and retrieved electronically. The technology has shown to be equivalent to manual scoring ($r = 0.77$, $P < 0.001$). Using Pearson correlation procedure, BCS at calving, dry-off, and test day had no correlation with subclinical concentrations of BHB and calcium. Odds of subclinical and clinical disease cases were determined by binary logistic regression. Cows with a BCS ≥3.75 at dry-off had increased odds of having a positive subclinical ketosis case (OR = 3.62; CI: 1.33, 9.86; $P < 0.01$). For every 0.3 BCS gain from calving to 7 DIM, cows were 35% less likely to develop metritis ≤14 DIM (OR = 0.65; CI: 0.53, 0.80; $P < 0.01$). Displaced abomasum ≤150 DIM was less likely in cows at heavier condition, as BCS increased by 0.2, at 21 DIM ($P < 0.01$). Assessment of BCS using an automated system may be a useful measure to alert for the risks of metabolic diseases around calving.

Key Words: BCS, ketosis, milk fever

T194 Effects of feeding OmniGen-AF on energy metabolism, fecal cortisol metabolites, and markers of immunity in overcrowded lactating Holstein dairy cows. J. M. Huzzey*[1], D. J. McLean[2], S. A. Armstrong[2], and J. P. Jarrett[3], [1]*California Polytechnic State University, San Luis Obispo, CA,* [2]*Phibro Animal Health Corp., Teaneck, NJ,* [3]*Alpha Dairy Consulting, Visalia, CA.*

The objective was to determine if feeding lactating Holstein dairy cows with OmniGen-AF (OG; Phibro Animal Health Corp.) would have an effect on energy metabolism cortisol secretion, and immunity during overcrowding. Four groups of 15 cows, balanced by parity, DIM, and 305ME milk, were exposed to 2 stocking densities (SD) [100% = 1 lying stall/cow and 0.67 m of feed bunk (FB) space/cow; 200% = 0.5 stalls/cow and 0.34 m of FB space/cow], and one of 2 feed treatments [Control = No OG; and OG = OG fed at 56 g/cow/d]. Each group assigned to a feed treatment (2 groups/feed treatment) were exposed to both 14-d SD treatments, separated by a 10-d washout period. OG was fed 45 d before the start of the SD treatment. During the SD treatment blood and fecal samples were collected every 2 d. Total RNA from blood was analyzed for CD80 gene expression and fecal samples were used to measure fecal cortisol metabolites [11,17-dioxoandrostanes (11,17-DOA)]. At the end of each SD treatment, 8 cows from each pen were given an intravenous glucose tolerance test (GTT) and glucose and NEFA were measured. Data were analyzed using the mixed procedure of SAS. 11,17-DOA was greater during the 200% vs. 100% SD periods (3.74 ± 0.26 vs. 3.04 ± 0.26 pg/10uL; $P = 0.04$); there was no effect of feed treatment on 11,17-DOA. Overstocked cows had slower glucose clearance from circulation as evidenced by greater area under the curve (AUC) for the glucose response curves (7689 ± 806 vs. 4649 ± 709 mg/dL × 180 min; $P = 0.01$). Overcrowded cows fed OG had a less AUC than overcrowded cows not fed OG ($P = 0.05$). NEFA did not differ between treatments during the GTT ($P > 0.39$). Overcrowded cows expressed less CD80 in circulating immune cells ($P = 0.04$), indicating overcrowded cows may have reduced T-Cell signaling capacity; there was no effect of feed treatment on CD80. These results provide evidence that overcrowding has negative consequences for immunity and energy metabolism, changes that may influence by increased cortisol secretion. OG may mitigate some of these physiological changes during overcrowding.

Key Words: overcrowding, OmniGen-AF, health

T195 Does the training of nulliparous cows to use a robotic milking system influence their milk yield and milking frequency? M. Peiter*[1], M. H. O. Pasetti[2], J. A. Salfer[3], and M. I. Endres[1], [1]*University of Minnesota, St. Paul, MN,* [2]*University of São Paulo-ESALQ, Piracicaba, SP, Brazil,* [3]*University of Minnesota Extension, St. Cloud, MN.*

Robotic milking systems (RMS) have attracted growing interest from dairy producers in the last few years in the United States, but the amount of research data available is limited. It has been observed that it takes some time for cows to adjust to the RMS, especially primiparous cows. Therefore, the objective of this study was to compare daily milk yield and milking frequency (number of milkings/d) between primiparous cows introduced to the RMS without any previous exposure (2016) and cows trained to access the RMS before calving for the first time (May–Nov 2017). The training was performed in a case study dairy farm 2×/d for approximately 15 d pre-partum by physically bringing cows to the RMS milking station, where they were offered 1 kg of a concentrate pellet/visit. Eight stages of lactation were evaluated: 1–7, 8–14, 15–21, 22–30, 31–60, 61–90, 91–150, and 151–210 DIM. Data were analyzed using PROC MIXED (SAS 9.4); stage of lactation and year as fixed effects and cow as random effect. Daily milk yield (n = 31,828 cow-d) was greater for trained cows at all the stages of lactation; a stage × yr interaction was detected ($P < 0.001$). Milk yield (not trained and trained cows, respectively) was 16.6 and 19.3 kg (1–7 DIM); 23.0 and 28.6 kg (8–14 DIM); 27.7 and 32.3 kg (15–21 DIM); 30.2 and 35.7 kg (22–30 DIM); 33.4 and 38.3 kg (31–60 DIM); 35.4 and 38.3 kg (61–90 DIM); 34.7 and 37.2 kg (91–150 DIM); 32.7 and 36.4 kg (151–210 DIM). Milking frequency followed the same pattern and was greater at all stages of lactation for trained heifers; a stage*yr interaction was detected ($P < 0.001$). Number of milkings/d (not trained

and trained cows, respectively) was 1.82 and 2.12 (1–7 DIM); 1.88 and 2.62 (8–14 DIM); 2.05 and 2.94 (15–21 DIM); 2.16 and 3.27 (22–30 DIM); 2.24 and 3.44 (31–60 DIM); 2.45 and 3.12 (61–90 DIM); 2.51 and 3.15 (91–150 DIM); 2.46 and 3.01 (151–210 DIM). Training nulliparous cows to the RMS before calving appeared to affect milk yield and milking frequency. However, more research is yet needed to further investigate this management practice on RMS farms.

Key Words: robotic milking, milk yield, milking frequency

T196 A comparison of milk yield and milking frequency of primiparous versus multiparous cows in robotic milking systems. M. Peiter*[1], M. H. O. Pasetti[2], J. A. Salfer[3], and M. I. Endres[1], [1]*University of Minnesota, St. Paul, MN,* [2]*University of São Paulo-ESALQ, Piracicaba, SP, Brazil,* [3]*University of Minnesota Extension, St. Cloud, MN.*

The objective of this study was to compare daily milk yield and milking frequency (number of milkings/d) of primiparous (LACT 1) and multiparous (LACT 2+) cows in a robotic milking system (RMS) at various stages of lactation. Data were collected daily for 2016 and from May–Nov 2017 from a case study farm that implemented training of nulliparous animals to the RMS in Jan 2017. Training was performed 2×/d for 15 d prepartum by physically bringing them to the RMS milking station. Ten stages (S) of lactation were evaluated: 1–7 (S1), 8–14 (S2), 15–21 (S3), 22–30 (S4), 31–60 (S5), 61–90 (S6), 91–150 (S7), 151–210 (S8), 211–270 (S9), and 271–330 (S10) DIM (S1, S3, S5, S10 are reported). Data were analyzed within year using PROC MIXED (SAS 9.4), with stage of lactation and parity as fixed effects and cow as random. Daily milk yield in 2016 (n = 82,466 cow-d) was greater for LACT 2+ cows for most lactation stages but not late lactation; an S × LACT interaction was detected ($P < 0.001$). Milk yield (LACT 1 and 2+, respectively) was 18.7 and 28.1 kg (S1); 29.8 and 46.1 kg (S3); 35.5 and 52.2 kg (S5); 28.5 and 27.7 kg (S10). Daily milk yield in 2017 (n = 69,178 cow-d) followed a similar pattern with an S × LACT interaction detected ($P < 0.001$). Milk yield (LACT 1 and 2+, respectively) was 19.3 and 29.6 kg (S1); 32.4 and 47.6 kg (S3); 38.2 and 53.8 kg (S5); 29.7 and 29.5 kg (S10). Milking frequency in 2016 was greater for LACT 2+ cows most of lactation but not late lactation; an S × LACT interaction was detected ($P < 0.001$). Number of milkings/d (LACT 1 and *2+,* respectively) was 1.94 and 2.47 (S1); 2.18 and 3.37 (S3); 2.37 and 3.05 (S5); 2.36 and 2.15 (S10). In 2017, LACT 1 cows had a higher milking frequency than LACT 2+ cows starting at S5, whereas in 2016 this only occurred at S9. Number of milkings/d (LACT 1 and 2+, respectively) was 2.13 and 2.71 (S1); 2.98 and 3.61 (S3); 3.45 and 3.44 (S5); 2.45 and 2.39 (S10). It appears that this change in management may result in improved primiparous cow performance in RMS. However, further research with a more direct comparison is warranted.

Key Words: robotic milking, milk yield, milking frequency

T197 Claw measures of Jersey cows: An anatomy study. L. T. Passos*[1,2], V. Fischer[2], J. Adaska[1,3], and N. S. Del-Río[1], [1]*Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA,* [2]*Federal University of Rio Grande do Sul,Postgraduate Animal Science Program, Porto Alegre, RS, Brazil,* [3]*California Animal Health & Food Safety Lab, Tulare, CA.*

Hoof trimming is a common practice on dairy operations to treat and prevent lameness. However, more information on claw anatomy of Jersey (JE) cows is needed before trimming guidelines can be developed for this dairy breed. The objective of this study was to describe anatomical structures of cadaver claws from JE cows. Rear claws (n = 39) from 8 primiparous and 12 multiparous JE cows with 5 to 277 DIM were collected from 3 commercial dairies in California. The following measurements were performed in intact claws: sole width (SW), heel height (HH), dorsal wall length (DWL), sole thickness (ST) and dorsal wall angle (DWA). Measurements were obtained using a precision goniometer (±0.3°) and caliper (±0.01 mm). Each toe was divided sagittaly using a band saw to measure ST at the apical margin of distal phalanx. Descriptive statistics were performed with the Proc MEANS and UNIVARIATE of SAS (version 9.4). Results (means, range) are shown in Table 1. The lateral toe was 7% wider than the medial toe in primiparous cows whereas in multiparous cows lateral toe was 1.4% longer and 13.5% wider than the medial toe. Based on industry-wide recommendations the DWL should never be trimmed below 75 mm to ensure ST of at least 7 mm and the DWA should be within 45° to 52°. In our study, primiparous cows had toes with DWL <75 mm (93.8%), ST <7 mm (46.8%) and DWA within 45° to 52° (59.3%). Multiparous cows had toes with DWL <75 mm (32.7%), ST <7 mm (39%) and DWA within 45° to 52° (58.6%).There are thin soles in JE. More studies are needed before trimming guidelines are developed for JE cows.

Key Words: Jersey, claw anatomy, hoof trimming

T198 Effects of a conventional diet or total mixed ration diet offered to Korean female cattle on blood metabolites. B. Kim*[1], M. Kim[1], S. A. Fenila[1], G. Son[1], B. Park[2], and J. Shin[1], [1]*Kangwon National Univ., Chuncheon, Kangwondo, South Korea,* [2]*Nonghyup Feed Research Institute, Seoul, South Korea.*

This study was aimed to compare the change of blood metabolites of Korean female cattle offered either a conventional diet (CON) or a total mixed ration (TMR) diet for 6 mo in prepartum. Twenty-two Korean female cattle (about 27 mo old) were randomly assigned to one of 2 diets and 2 sample *t*-tests were conducted; CON, concentrate (3kg/hd/day) + rice straw (unlimited) or TMR, (6.6kg/hd/day). The TMR offered per cow per day was composed of concentrate, maize silage, grass, molasses, and rice straw. The DM, CP and TDN intakes were not significantly affected by any of diet groups, but the tendency for higher DM, CP and TDN intakes were observed in the TMR group. The nonesterified fatty acids (NEFA) concentrations in blood were higher for the CON group than the TMR group (566.45 vs. 235.09 uEq/L; $P < 0.05$). However, the

Table 1 (Abstr. T197). Descriptive statistics of measurements from 39 JE claws, mean (range)

Claw	Parity	SW (mm)	DWL (mm)	HH (mm)	ST (mm)	DWA (°)
Lateral	1	46 (40–52)	69 (61–77)	26 (19–33)	6.8 (3.1–10.4)	50 (41–59)
	>1	48 (44–55)	73 (67–80)	27 (20–35)	7.8 (4.8–11.3)	48 (41–58)
Medial	1	43 (37–51)	69 (62–80)	23 (42–58)	6.8 (4.8–9.6)	50 (42–58)
	>1	43 (39–50)	72 (67–77)	22 (15–30)	7.5 (3.6–11)	48 (39–57)

cattle offered TMR had higher blood urea nitrogen (BUN) concentrations (21.69 vs. 10.85 mg/dL; $P < 0.05$), blood glucose levels (62.73 vs. 40.55 mg/dL; $P < 0.05$) and blood aspartate aminotrasferase levels (78. 73 vs. 57.18 u/L; $P < 0.05$) than did the CON group. The creatinine concentrations in blood were similar between 2 diet groups. In this study, Korean female cattle consuming TMR had low levels of blood NEFA, but high concentrations of blood glucose and BUN. This result indicates that feeding TMR is important for Korean female cattle in terms of increasing reproductive efficiency,which has long been recognized as the most aspect of Korean female cattle.

Key Words: total mixed ration, blood urea nitrogen, nonesterified fatty acids

T199 Characterization of the rumen microbiome resilience throughout lactation and its association with gross feed efficiency in Holstein dairy cows. Z. Zhou[1], P. M. Peixoto[1], M. S. Gomes[1], E. R. Bonsaglia[1], I. F. Canisso[1], J. L. Stewart[1], F. C. Cardoso[2], and F. S. Lima*[1], [1]*Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL,* [2]*Department of Animal Sciences, Urbana, IL.*

The objectives of this study were to characterize the rumen microbiome throughout lactation and its association with gross feed efficiency in a cohort of Holstein cows. Rumen samples were collected using an oro-esophageal-ruminal sampling device (Flora Rumen Scoop; Profs-Product, Guelph, Canada) at d 7, 21, 50, 90, and 130 d postpartum in a cohort of 18 primiparous Holstein cows. Animals were housed in a barn with tiestalls fed the same diet during the entire study. Rumen samples 16S rRNA genes were sequenced using the MiSeq platform. The Shannon richness and diversity index did not change ($P = 0.90$) over time. The discriminant analysis did not show differences over time. The predominant phyla throughout lactation were *Bacteroidetes*, *Firmicutes*, *Tenericutes*, *Proteobacteria*, and *Spirochaetes* with mean relative abundance (MRA) of 43.0%, 40.9%, 3.4%, 3.4%, and 2.8%, respectively. The only change over time for phyla was an increase ($P < 0.01$) of the MRA at d 90 for the phylum *Tenericutes* that returned to normal at d 130 postpartum. The predominant genera were *Prevotella*, *Butyrivibrio*, *Treponema*, *Shuttleworthia*, *Bulleida*, *Ruminococcus*, RFN20, *Clostridium*, *Coprococcus*, and *Fibrobacter* with MRA of 25.7%, 3.8%, 2.7%, 2.3%, 2.3%, 2.2%, 2.2%, 1.8%, 1.6%, and 1.5%, respectively. The MRA for RFN20 was greater at d 50, 90 and 130 than in d 7 and 21 postpartum. *Treponema* tended to be increased at d 90 postpartum when compared with the other time points evaluated. Gross feed efficiency (GFE), calculated as energy corrected milk over dry matter intake, was categorized as Low <1.32 or High >1.32. Discriminant analysis revealed differences between Low and High GFE. Cows with high GFE had greater MRA for *Bulleida* ($P = 0.02$) and a tendency for greater MRA for *Butyrivibrio* than Low GFE. Also, *Treponema* MRA was lower in High GFE ($P < 0.01$) than Low GFE cows. The current data suggest that rumen microbiome is resilient throughout the lactation and cows categorized as Low GFE and HIGH GFE host a different rumen microbial population.

Key Words: rumen microbiome, gross feed efficiency, resilience

T200 Effects of recombinant bovine somatotropin supplementation on periparturient dairy cows. M. S. F. Zoni[1], L. F. Moroz[2], A. F. Sica[3], R. L. Araujo[3], R. C. Chebel[4], and R. de Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Frank'Anna Farm, Carambeí, PR, Brazil,* [3]*Colorado Farm, Araras, SP, Brazil,* [4]*University of Florida, Gainesville, FL.*

Recombinant bovine somatotropin (rbST) could be an important tool to improve immune status and to optimize health in transition dairy cows. Therefore, the objective of this study was to investigate the effects of rbST on productive and metabolic parameters of periparturient dairy cows. The trial was conducted in 2 commercial herds in Paraná and São Paulo States, Southern Brazil, from September 2016 to August 2017. A total of 692 heifers and cows were blocked by lactation order and body condition score 28 d before calving. The Treatment group 1 was supplemented weekly with 0.6 mL (125 mg) of bovine somatotropin (Boostin®, Merck) only in the prepartum period, the Treatment group 2 was supplemented weekly with 0.6 mL (125 mg) of rbST both in the prepartum and postpartum periods until the 21th day after calving, while the Control group received 0.6 mL of saline until the 21th day after calving. Three blood samples were collected on d 0, 6, and 13 postpartum. Data were analyzed using the MIXED procedure of SAS with a model containing the effects of farm, calving order, time, treatment, and treatment*time interaction as fixed effects and cow within treatment as a random effect. Average milk yield in the first 100 d of lactation did not differ (40.66 kg/d in the Control, 40.66 kg/d in rbST prepartum, and 39.98 kg/d in rbST pre- and postpartum; SEM = 1.6 kg/d, $P = 0.61$) among experimental groups. Analysis of metabolites on serum blood in the day of calving (NEFA, Ca, albumin, cholesterol, triglycerides, GGT and AST) did not demonstrate ($P > 0.10$) any beneficial effects of rbST supplementation, however the analysis of β-hydroxybutyrate (BHB) on serum blood in the 6th and 13th day after calving showed an increase ($P < 0.05$) in fat mobilization in the groups with rbST administration. These results suggest an increase in the incidence of clinical and subclinical ketosis, especially in the Treatment group 2 (rbST supplementation pre- and postpartum). In summary, rbST supplementation in the prepartum and immediate postpartum periods did not show positive results in this trial.

Key Words: health, performance, transition period

T201 Microorganisms isolated from subclinical intramammary infections present in dairy cattle from the southeast United States. K. Enger*[2], C. Petersson-Wolfe[2], R. A. Almeida[1], D. T. Nolan[3], P. D. Krawczel[1], J. Bewley[6], A. E. Stone[4], S. H. Ward[5], S. P. Oliver[1], and G. M. Pighetti[1], [1]*University of Tennessee, Knoxville, TN,* [2]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [3]*University of Kentucky, Lexington, KY,* [4]*Mississippi State University, Starkville, MS,* [5]*North Carolina State University, Raleigh, NC,* [6]*Cow-Focused Housing, Bardstown, KY.*

The predominant microorganisms that cause subclinical and clinical intramammary infections (IMI) can vary with environmental conditions, personnel, and management practices. However, limited information exists regarding the types of organisms isolated from subclinical IMI of dairy cattle in the southeast US. To address this limitation, members of the Southeast Quality Milk Initiative identified 30 herds in KY (n = 9), MS (n = 3), TN (n = 9), and VA (n = 9) that were participating in Dairy Herd Improvement programs and willing to participate. The top 10–20 cows with the highest SCC according to the most recent DHI test were selected for sampling at each farm visit (2–3 times per year, Jan 2016 to Jan 2018). Quarter milk samples were collected aseptically and stored at −20C until cultured. Culture and organism identification were performed as outlined by NMC. Overall, 1,847 cows and 5,710 quarters were sampled. Of these, 647 (11.3%) were considered contaminated with either bacillus or 3+ colonies of different morphology and 2411 (42.2%) exhibited no growth. After removing these samples, 2,652 quarter samples contained 1 (95%) or 2 (5%) bacterial isolates. The dominant organisms isolated included coagulase-negative staphylococci (CNS; 38.9%), *Staphylococcus aureus* (15.6%), *Streptococcus dysgalactiae*

(7.6%), Esculin positive streptococci including *Streptococcus uberis* (7.3%) and *Escherichia coli* (5.3%). The following organisms each accounted for 1 to 3.5% of the isolates: *Staphylococcus hyicus*, *Serratia*, *Corynebacterium*, *Klebsiella*, *Enterococcus*, yeast, and *Aerococcus* species. CNS and *S. aureus* were the 2 most isolated organisms on 16 of 30 farms. Closer examination of a subset of CNS isolates, revealed 41 of 61 (67%) were *Staphylococcus chromogenes*. This suggests that *S. chromogenes* may be a more frequent cause of subclinical IMI with high SCC than *S. aureus*. Staphylococcal species, including *S. aureus*, represent the predominant isolates from high SCC cows in the southeast and strategies to prevent or minimize new infections with these organisms should be a part of mastitis control programs in the southeast US.

Key Words: mastitis, dairy cattle, microorganisms

T202 Microorganisms isolated from subclinical intramammary infections present in cattle managed on organic dairy farms in the southeast United States. G. M. Pighetti*[1], V. L. Couture[1], H. R. Bailey[1], A. Rius[1], P. D. Krawczel[1], and S. R. Smith[2], [1]*University of Tennessee, Knoxville, TN,* [2]*University of Kentucky, Lexington, KY.*

The predominant microorganisms that cause subclinical and clinical intramammary infections (IMI) can vary with environmental conditions, personnel, and management practices. However, limited information exists regarding the types of organisms isolated from subclinical IMI of dairy cattle in the southeast United States. To address this limitation, 5 certified organic herds in KY and TN that were participating in Dairy Herd Improvement programs and willing to participate were enrolled in the study. The top 10–20 cows in the herd with the highest SCC according to the most recent DHI test were selected for sampling at each farm visit (4–6 times per year, Mar 2017 to Nov 2017). Quarter milk samples were collected aseptically and stored at −20C until cultured. Culture and organism identification were performed as outlined by NMC. Overall, 168 cows and 1,230 quarters were sampled. Of these, 27 (2.1%) were considered contaminated with either bacillus or 3+ colonies of different morphology and 685 (55.7%) exhibited no growth. After removing these samples, 518 quarter samples contained 1 (92%) or 2 (8%) bacterial isolates. The dominant organisms isolated included coagulase-negative staphylococci (CNS; 26.2%), *Staphylococcus aureus* (17.0%), *Staphylococcus hyicus* (15.4%), *Corynebacterium bovis* (12.1%), and *Streptococcus uberis (10.2%)*. Closer examination of a subset of CNS isolates, revealed 67 of 106 (63%) were *Staphylococcus chromogenes*. Corynebacterium species other than *C. bovis* and *Streptococcus dysgalactiae* accounted for 2.5 and 3.5% of the isolates, respectively. The predominant species on each farm varied considerably, with CNS and *S. aureus* most frequently isolated on 2 herds, CNS and *S. hyicus* on a third, *C. bovis* and *S. uberis* on a fourth, and CNS, *C. bovis* and *S. aureus* on a fifth. This suggests local environments and management practices significantly influence dominant types of organisms associated with IMI. Strategies to prevent or minimize new infections with these organisms should be a part of mastitis control programs on organic dairy farms in the southeast US.

Key Words: mastitis, organic dairy, microorganisms

T203 Active placental *Proteobacteria* in healthy dairy cows is strongly associated with dairy calf birth weight. C. E. Owens*, H. G. Huffard, H. H. Hanling, K. M. Daniels, K. F. Knowlton, and R. R. Cockrum, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

In humans, low placental microbiome variation is associated with low birth weight and *Proteobacteria* and *Tenericutes* are associated with pre-term births. As current breeding strategies incorporate selection for decreased gestation length, the relationship observed in humans might also exist in dairy cows. Previous research suggests decreased pre-natal fetal growth is associated with mature animal lameness and decreased milk production. Composition of the placental microbiome and its impact on calf prenatal performance is not known. Therefore, we hypothesized that active bacteria within bovine placenta are associated with calf weight. The objectives of this study were to (1) quantify the presence of *Proteobacteria* and *Tenericutes* in the placenta, and (2) determine the relationships of these bacterial phyla and classes with calf birth weight and gestation length. Placental cotyledons without exposure to vaginal fluid were collected (n = 4) from multiparous Holstein cows. Total RNA was extracted and qRT-PCR was performed using 5 sets of primers: *Eubacteria*, *Tenericutes, β-proteobacteria*, *ε-proteobacteria,* and δ- and γ-proteobacteria. Pearson correlations were performed comparing bacterial group relative abundance with calf birth weight (kg) and gestation length using the rcorr package in R. Across all cotyledons tested, β-proteobacteria (56.82 ± 35.84%) was most abundant, followed by *Tenericutes* (3.18 ± 4.82%) and δ- and γ-proteobacteria (0.98 ± 0.97%). ε-proteobacteria was not detected. Both β-proteobacteria and δ- and γ-proteobacteria were negatively correlated with birth weight ($R^2 = -0.96$ and -0.97, respectively). There was no correlation between bacterial phyla and gestation length. Similar to results in humans, we observed that *Proteobacteria* are associated with neonatal weight. *Proteobacteria* might directly divert placental growth factors intended for the calf, resulting in a lower birth weight, or placental microbiota might indirectly influence fetal growth through yet unknown means.

Key Words: placenta, microbiome, reproduction

T204 Effect of feeding rumen-protected methionine pre- and postpartum on reproductive performance of lactating dairy cows. M. L. Stangaferro*[1], M. Z. Toledo[2], M. M. Perez[1], C. A. Gamarra[2], P. LJ Monteiro[2], A. B. Prata[2], D. Luchini[3], M. E. Van Amburgh[1], R. D. Shaver[2], M. C. Wiltbank[2], and J. O. Giordano[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Wisconsin-Madison, Madison, WI,* [3]*Adisseo USA Inc., Alpharetta, GA.*

Objectives were to evaluate effects of feeding rumen-protected methionine (RPM; Smartamine M) from 3 wk prepartum until 150 DIM (67 d of pregnancy) on first service pregnancy per AI (P/AI) and pregnancy loss. In addition, cyclicity, concentrations of progesterone (P4) and pregnancy specific protein-B (PSPB) in pregnant cows were evaluated. Multiparous Holstein cows at Cornell University (CU; n = 235) and the University of Wisconsin (UW; n = 233) were randomly assigned to a control (CON) or RPM group. Diets were isoenergetic with similar lysine amounts but RPM included Smartamine M pre- (PreP) and postpartum (PostP; CU: Met as 2.09 vs. 2.68% and UW: 2.10 vs. 2.59 as percent of metabolizable protein). Cows were allocated to pens (12 pens at CU and 6 pens at UW with 16 cows per pen PostP), and diets were fed to pens. Cows received first timed-AI (TAI) at 80 ± 3 DIM after the Double-Ovsynch protocol (GnRH-7d-PGF-3d-GnRH-7d-GnRH-7d-PGF-24h-PG-32h-GnRH-16h-TAI). Pregnancy was evaluated at d 25 (by PSPB), 32, and 67 (by transrectal ultrasonography) after TAI. Cyclicity was based on plasma P4 concentrations (≥1 ng/mL) at 35 and 49 ± 3 DIM. Quantitative and binary outcomes were analyzed by ANOVA with repeated measurements and logistic regression, respectively. Treatment (TRT) and location were fixed effects and PEN(TRT) was included as random effect. Pen was the experimental unit and cow was the observational unit. There were no differences ($P > 0.10$) in P/AI (Table 1), cyclicity (CON = 84.0%; RPM = 80.2%), P4 (CON = 6.8 ± 0.3; RPM = 6.6 ± 0.3 ng/mL d 8-d 29), or PSPB concentration (CON = 1.59 ± 0.06; RPM = 1.65 ± 0.06 ng/mL; d 22–67) for pregnant cows, with a numerical reduction in pregnancy loss (38% lower). Thus, RPM fed pre- and postpartum had no significant effect on measured reproductive values up to d 67. The numerical reduction in pregnancy loss in cows fed RPM warrants further investigation.

Table 1 (Abstr. T204). Reproductive outcomes

Item	CON	RPM	*P*-value
P/AI at 1st TAI, % (n)	55.7 (212)	56.1 (214)	0.48
d 25 (based on PSPB)	48.6 (212)	49.1 (214)	0.46
d 32	43.1 (211)	45.8 (214)	0.28
d 67			
Pregnancy loss	12.7 (118)	12.5 (120)	0.49
d 25 to d 32	22.2 (117)	18.3 (120)	0.23
d 25 to d 67	10.8 (102)	6.7 (105)	0.16
d 32 to d 67	55.7 (212)	56.1 (214)	0.48

Key Words: methionine, reproduction, dairy cow

T205 Estrous expression improves the success of embryo collection and transfer. T. A. Burnett*[1], A. M. L. Madureira[1], T. G. Guida[2], J. L. M. Vasconcelos[2], and R. L. A. Cerri[1], [1]*University of British Columbia, Vancouver, BC, Canada,* [2]*São Paulo State University, Botucatu, São Paulo, Brazil.*

The aim of this study was to determine the association of estrous expression and successful embryo transfer (ET) and collection. In Experiment 1, Holstein heifers (10.5 to 14.5 mo) were superovulated (n = 69 from 51 animals) for the collection of embryos using a protocol based on FSH and progesterone. On the day of estrus, the total number of follicles present on both ovaries were counted via ultrasonography. On d 7 post-AI embryos were collected, counted and assessed for viability. In Experiment 2, Holstein cows were synchronized using a protocol based on estradiol and progesterone and 7 d post-estrus cows with a CL were implanted with a fertilized embryo (n = 1147 from 657 cows). Embryos were transferred as either in vivo (fresh or frozen) or IVF (frozen only). Pregnancy was confirmed at 31 ± 3 d post-estrus. Estrous expression was monitored through a leg (Exp 1) or collar (Exp 2)-mounted activity monitor. Estrous expression was quantified using 2 parameters: 1) peak activity (maximum activity) and 2) duration (total time above threshold); both parameters were categorized using the median. Data were analyzed by ANOVA using the MIXED and GLIMMIX procedures of SAS. In Exp 1, number of follicles present at AI was not affected by estrous expression. Heifers with higher peak activity had a higher number of total embryos (10.2 ± 1.2 vs 6.0 ± 1.3 embryos; $P = 0.01$) and a higher percent of those embryos were viable (53.1 ± 5.0 vs 23.4 ± 5.1%; $P <$

0.001). Longer duration estrus episodes had a higher percentage of viable embryos (51.2 ± 5.2 vs 25.3 ± 5.3%; $P < 0.01$); but, duration did not affect total embryos. In Exp 2, 89.1% of cows expressed estrus before ET. Animals expressing estrus before ET had substantially higher P/ET than those that did not (35.8 ± 1.6 vs 5.9 ± 4.9%; $P < 0.001$). Of the animals that expressed estrus, cows with higher estrous expression had higher ET success than those with low estrous expression (41.5 ± 2.3 vs 30.6 ± 2.2%; $P < 0.001$). No interaction was found between estrous expression and embryo transfer method, but IVF was found to have lower P/ET than both fresh and frozen (30 ± 1.9 vs 38.7 ± 3.6 vs 39.0 ± 3.1%; $P = 0.02$). In conclusion, estrous expression is important for both periods before and after ET as seen by more viable embryos and higher P/ET for animals with greater estrous expression.

Key Words: embryo transfer, estrus, activity monitor

T206 Validation of an in-house bovine serum enzyme immune assay for progesterone measurement. A. Nadalin[1], A. Madureira*[1], T. Burnett[1], J. Bauer[1], K. Pohler[2], and R. Cerri[1], [1]*University of British Columbia, Vancouver, BC, Canada,* [2]*University of Tennessee, Knoxville, TN.*

The purpose of this study was to develop and validate an in-house competitive enzyme immune assay (hEIA) to measure the amount of progesterone (P4) in the serum of dairy cattle. Blood samples were collected from randomly selected multiparous Holstein cows at predetermined times of their estrous cycle (d −5 to 12), and serum separated for analysis. The in-house assay was then compared with a commercial EIA kit (cEIA; Ovucheck Plasma TRM546, Biovet Inc., QC, Canada) and a RIA method validated by Pohler et al. (2016). In short, the protocol used a double-antibody (secondary antibody, Goat antimouse; and a primary antibody, monoclonal progesterone) and matching enzyme conjugate label (Progesterone-3-HRP). 96 well microtiter plates were coated with the secondary antibody, incubated then later blocked using the primary antibody. Progesterone standards ranged from 0.1 to 20 ng/mL. Progesterone was extracted from samples using petroleum ether. Quality controls were created from serum stripped of steroids using a charcoal procedure. Microplate reader wavelength was set at 450nm and P4 calculated using a Log-Logit regression line. Both low (0.45 ng/mL) and high (6.43 ng/mL) concentration internal controls were run on 6 plates to calculate intraplate and interplate %CVs. The intraplate CV was 7.68% and 5.14%, and the interplate was 12.5 and 11.0%, respectively for the low and high samples. The prepared quality controls (2.5 ng/mL) were tested on the same 6 plates and resulted in a 6.56 and 3.61%CV for intra and interplate variability. When compared with the other two validated assays, the correlations among the 3 assays were statistically significant ($P < 0.01$) and yielded the following correlations: h-EIA and RIA (r = 0.95; slope = 1.29); c-EIA and RIA (r = 0.91; slope = 1.51); hEIA and cEIA (r = 0.96; slope = 1.10). When agreement tests were performed to detect samples below and above 1 ng/mL, the kappa values were: 1.00 for h-EIA and RIA; 0.78 for c-EIA and RIA; and 0.78 for hEIA and cEIA. In conclusion, the in-house EIA method is a reliable assay to accurately measure concentrations of P4 in bovine serum that is suitable for commercial and research purposes.

Key Words: enzyme immune assay, progesterone, bovine

T207 Effects of polymorphisms in *GHR, IGFI* and *TNFA* genes on fertility in lactating dairy cows. W. R. Butler*[1], A. Schneider[2], P. A. S. Silveira[2], D. H. Townson[3], P. C. W. Tsang[3], R. A. Dailey[4], T. L. Ott[5], and J. L. Pate[5], [1]*Cornell University, Ithaca, NY,* [2]*Universidade Federal de Pelotas, Pelotas, RS, Brazil,* [3]*University of New Hampshire, Durham, NH,* [4]*West Virginia University, Morgantown, WV,* [5]*Pennsylvania State University, University Park, PA.*

The growth hormone receptor (*GHR*), insulin-like growth factor-I (*IGFI*) and tumor necrosis factor-α (*TNFA*) genes have important effects on reproduction in cows. This study investigated SNP in each gene associated with differences in fertility among cows. Data on inseminations and days to pregnancy until 210 d in milk (DIM) were collected for cows (479 total) at 4 research stations (NY, NH, PA, WV). DNA from blood of each cow was genotyped for SNPs identified in prior studies by PCR-restriction fragment length (PCR-RFLP) methods. Statistical models (SAS GLM and Glimmix procedures) tested the effects of genotypes and linear contrasts among alleles with station and parity (1 or ≥2) as covariates. Results for effects of genotypes on conception rate and DIM to pregnancy are presented in Table 1. Effects of *GHR* and *IGFI* SNP were not significant. The SNP in *TNFA* had significant effects on fertility; BB cows being most disadvantaged. Neither *IGFI* nor *TNFA SNPs* had a linear effect on days to pregnancy. However, combining the effects of SNPs in *TNFA + IGFI*, cows carrying more favorable alleles (AA for each gene) had fewer DIM to pregnancy than cows having BB genotype for both (AAAA = 112 ± 8, BBBB = 126 ± 10; Linear effect $P = 0.019$). How the effects of these SNPs are manifested by differences in fertility cannot be ascertained from this study. However, as examples, the *TNFA* gene regulates expression of the inflammatory cytokine *TNFA* in tissues and increased plasma *TNFA* levels are known to affect liver function and ovarian follicle development in early lactation. Also, the effects of the *GHR-IGFI* system in promoting ovarian follicular and luteal function via plasma IGFI levels are well known.

Table 1 (Abstr. T207). Conception rate to first AI and days in milk to pregnancy

	Genotype			
Gene	AA	AB	BB	*P*-value
GHR				
n	189	60	210	
CR 1st AI (%)	0.38 ± 0.04	0.37 ± 0.07	0.41 ± 0.04	0.737
Days to pregnancy	106 ± 3	102 ± 6	97 ± 3	0.177
IGFI				
n	141	235	74	
CR 1st AI (%)	0.42 ± 0.05	0.42 ± 0.04	0.28 ± 0.06	0.116
Days to pregnancy	102 ± 4	105 ± 3	111 ± 6	0.358
TNFA				
n	49	225	179	
CR 1st AI (%)	0.41 ± 0.07	0.44 ± 0.04	0.33 ± 0.04	0.098
Days to pregnancy	104 ± 6	98 ± 4	113 ± 4	0.007
Contrast: AA + AB vs. BB				0.015

Key Words: dairy cow, fertility, genomics

T208 One injection of high-concentration prostaglandin $F_{2\alpha}$ is as effective as two injections of conventional prostaglandin $F_{2\alpha}$ in causing luteolysis for dairy cows subjected to a 5-d CIDR-Cosynch protocol. J. A. Spencer*[1], K. Carnahan[1], W. J. Price[2], B. Shafii[2], and A. Ahmadzadeh[1], [1]*Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*Statistical Program, University of Idaho, Moscow, ID.*

The objective of this study was to examine the effects of 1 or 2 doses of prostaglandin $F_{2\alpha}$ (PG, dinoprost tromethamine), or one dose of high-concentration formula PG (HighCon) on progesterone (P_4) concentration profiles, and luteolysis ($P_4 < 0.5$ ng/mL) in lactating dairy cows subjected to a 5-d CIDR-Cosynch protocol. On d 0, 67 cows received GnRH, and a CIDR was inserted. On d 5, CIDR was removed and cows were assigned randomly to receive one dose (5 mL) PG (1PG; 5mg/mL; n = 24), 2 doses (5 mL/dose) PG 12 h apart (2PG; 5mg/mL; n = 22), or one dose (2 mL) HighCon (12.5mg/mL; n = 21). After CIDR removal (d 5), blood samples were collected every 12 h from d 5 to 8 to measure P_4. On d 8, all cows received a second GnRH and were inseminated. Ovaries were examined by ultrasonography on d 0, 5, and 8. A repeated measures analysis assuming a lognormal distribution was used to assess treatment differences in P_4 concentrations. The model included terms for treatment, the repeated factor (time), and time by treatment interaction. Cow within treatment was the random effect within the model and d 5 P_4 concentration was used as a covariate. All cows (n = 67) used for analysis had elevated P_4 concentrations and corpora lutea before treatment (d 5). Blood P_4 concentrations decreased over time in all treatments ($P < 0.01$). There was an effect of treatment by time interaction on P_4 ($P = 0.05$). By 24 h after treatments, P_4 concentrations were lower, and remained at a lower level ($P < 0.01$) throughout the experiment for 2PG and HighCon compared with 1PG. There was no difference in P_4 concentrations between 2PG and HighCon at 72 h, and the P_4 averages were 1.02, 0.05, and 0.22 ng/mL for 1PG, 2PG and HighCon, respectively. Proportion of cows that went through luteolysis ($P_4 < 0.5$ ng/mL) were greater in 2PG and HighCon compared with 1PG (100, 90.4, and 54.1%, respectively). The results indicated that 1PG was not as effective as 2PG and HighCon in causing luteolysis, and that one injection of HighCon was as effective as 2PG in decreasing blood P_4 and causing luteolysis by the time of AI.

Key Words: prostaglandin $F_{2\alpha}$ dose, luteolysis, dairy cow

T209 The phenotype of caruncle macrophages is associated with retained placenta in dairy cows. R. K. Nelli*[1], J. De Koster[1], J. N. Roberts[1], J. de Souza[2], A. L. Lock[2], W. Raphael[3], and A. G. Contreras[1], [1]*Large Animal Clinical Sciences, Michigan State University, East Lansing, MI,* [2]*Animal Science, Michigan State University, East Lansing, MI,* [3]*Waverly Animal Hospital, Lansing, MI.*

Uterine diseases affect 25% of dairy cows in the United States and often develop from retention of the placenta. Expulsion of the placenta is a highly regulated inflammatory process. In healthy ruminants, a pro-inflammatory and chemoattractive response facilitates parturition and the expulsion of fetal membranes. Mechanisms by which dysregulation of uterine immune responses impair this process, particularly the role of caruncle macrophage phenotype in the expulsion process, are poorly understood. Our study compared uterine caruncle macrophage phenotypes in multiparous dairy cows on d 1 of lactation with either retained (RET, n = 5) or normal expulsion (NOR, n = 5) of placenta. Monocyte/macrophage cells were isolated from the stromal vascular cell digest of uterine caruncles by magnetic activated cell sorting using an anti-bovine-CD172a antibody. Further, transcriptional and protein expression studies were performed on uterine caruncles. A linear statistical model was used to detect differences between NOR and RET cows. Compared with NOR, RET samples had lower $CD14^+/CD16^+$ (pro-inflammatory phenotype) expression ($P = 0.003$) in caruncle monocyte/macrophage population. Compared with NOR, RET enhanced the expression of M2 associated genes CD206, CLEC7A, RNASE6 ($P < 0.07$) following transcriptional analyses. In addition, RET decreased STAT3 activation compared with NOR ($P = 0.06$), possibly indicating impaired proteolytic activity at the caruncle/cotyledon junction. Our studies demonstrate that macrophage populations in the caruncle of cows with RET placenta have a strong M2 macrophage polarization or an insufficient M1 macrophage stimuli. This may lead to reduced chemotactic capacity to recruit immune cells into the caruncle thus impairing the inflammatory, phagocytic, and proteolytic processes necessary for placental expulsion. Elucidating the role of macrophage phenotype during placental expulsion has the potential to develop preventative strategies for uterine disease in post-partum dairy cows.

Key Words: M1/M2-macrophages, retained placenta, uterus immunity

T210 Effect of time to resumption of ovarian cyclicity post-partum on fertility and survival of Holstein cows. P. Pinedo*[1], J. Santos[2], G. Schuenemann[3], R. Bicalho[4], R. Chebel[2], K. Galvao[2], R. Gilbert[4,9], S. Rodriguez-Zas[5], G. Rosa[6], C. Seabury[7], J. Fetrow[8], and W. Thatcher[2], [1]*Colorado State University, Fort Collins, CO,* [2]*University of Florida, Gainesville, FL,* [3]*The Ohio State University, Columbus, OH,* [4]*Cornell University, Ithaca, NY,* [5]*University of Illinois, Urbana-Champaign, IL,* [6]*University of Wisconsin, Madison, WI,* [7]*Texas A&M University, College Station, TX,* [8]*University of Minnesota, Saint Paul, MN,* [9]*Ross University, Basseterre, St. Kitts and Nevis, West Indies.*

Early resumption of ovarian cyclicity (ROC) after calving is associated with reproductive outcomes. Remarkably, 20 to 40% of Holstein cows remain anovulatory by 50 DIM. Present objective is to evaluate the association among time to ROC, fertility, and survival in a multi-State Holstein population. A secondary objective is to analyze potential risk factors for delayed cyclicity. A total of 11,733 cows calving in 16 herds in 4 regions: Northeast (4), Midwest (6), Southeast (1), and Southwest (5) were enrolled at parturition and monitored weekly for reproductive events, disease occurrence, and survival. The ROC was assessed via transrectal ultrasonography at 40 ± 3 and 54 ± 3 DIM, and cows with a corpus luteum at either examination were considered to have ROC. Pregnancy was diagnosed by ultrasonography on d 32 ± 3 after AI and reconfirmed at d 60 ± 3. Diseases included retained fetal membranes, metritis (foul-smell, watery, brownish vaginal discharge at 7 ± 3 DIM), subclinical ketosis (serum BHBA > 1.0 mmol/L at 7 ± 3 DIM), clinical endometritis (mucopurulent to fetid vaginal discharge at 28 ± 3 DIM), lameness (score > 3 at 35 ± 3 DIM), mastitis (farm records), and left displaced abomasum. Multivariate logistic regression and ANOVA were used for testing potential associations between ROC and multiple explanatory and outcome variables, with farm as a random effect in all models. Early ROC was associated with parity (AOR [95% CI] = 0.79 [0.72–0.87] for primiparous cows), calf sex (0.89 [0.81–0.97] for males and twins), BCS at calving (0.71 [0.64- 0.79] for cows with BCS < 2.75), BCS at 40 DIM (0.62 [0.56- 0.68] for cows with BCS < 2.75), subclinical ketosis (0.78 [0.70–0.87] for affected cows), lameness (0.65 [0.57–0.75] for lame cows), and clinical endometritis (0.64 [0.58–0.70] for affected cows). Early ROC resulted in reduced days open (132 vs. 150 d; $P < 0.001$) and higher odds of pregnancy at first (1.92 [1.74–2.12]) and second (1.42 [1.26–1.60]) AI. Early ROC also was associated with lower odds of being sold after 55 DIM (0.79 [0.71–0.89]). However, the probability of pregnancy loss at first and second AI and the probability of dying after 55 DIM were not associated with early ovulation. Metrics for fertility and survival are identified.

Key Words: ovarian cyclicity, fertility, survival

T211 Feeding an amino acid formulated milk replacer. B. Yan*[1], L. Ting[1], K. Hultquist[3], J. Wu[2,1], and D. Casper[3], [1]*Gansu Agricultural University, Lanzhou, Gansu, China,* [2]*Gansu Academy Agricultural Sciences, Lanzhou, Gansu, China,* [3]*Furst-McNess Company, Freeport, IL.*

The milk-fed calf has a requirement for AA instead of CP; however, most milk replacers (MR) are formulated to a specific CP concentration. Previous studies have demonstrated that feeding a modified accerelated milk replacer MR (24:20; CP:fat) improved calf growth performance. The objective was to determine if a MR formulated using synthetic AA to achieve the same AA concentrations as a 24/20 MR, but allowing for reduced CP concentrations would enhance calf growth performance compared with more standard MR formulations. Eighty 3- to 5-d-old Holstein bull calves in 2 lots (40 each) were received within the same week and blocked by BW and randomly assigned to 1 of 4 MR treatments of: 20:20 (20), 22:20 (22), 24:20 (24) and a 22:20 with amino acid concentrations of the 24:20 (AA). Each experimental MR containing decoquinate was fed at 0.28 kg/calf/d at 2 ×/d for 14 d via bucket and then increased to 0.43 kg/calf/ d at 2 ×/d until 35 d. Feedings were reduced to 1 time/d with a 0.43 kg/calf/d feeding rate to facilitate weaning at 42 d. Calves were housed in bedded Calf-Tel hutches with ad libitum access to water and 22% CP pelleted calf starter (CS). All data were statistically analyzed using a randomized complete block design (RCBD) with block considered random and treatments considered fixed effects. Initial BW was similar across all treatments (42.4 kg), while 56-d BW for calves fed AA MR were greater compared with calves fed 24 MR with calves fed 20 and 22 MR being intermediate and similar (78.7, 78.8, 76.5, and 81.8 kg for 20, 22, 24, and AA, respectively). Dry matter intake of CS was greater for calves fed AA MR (0.74, 0.78, 0.65, and 0.81 kg/d) compared with 20 and 24 MR, but similar to calves fed 22 MR, while calves fed the 24 MR had lowest CS DMI. Improved BW gains can be achieved by feeding an AA fortified MR having a lower CP concentration.

Key Words: calf, milk replacer, amino acids

T212 Production performance and nitrogen utilization in dairy cows fed low or high crude protein diets containing corn dried distillers grains with solubles and supplemented with Lactivate or ProLak. A. V. Stevens*[1], A. H. Laarman[1], P. Rezamand[1], K. Karges[2], and G. Chibisa[1], [1]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*H.J. Baker & Bro. LLC, Shelton, CT.*

Feeding corn dried distillers grains with solubles (DDGS) in low CP diets improves nitrogen (N) utilization. However, it reduces metabolizable AA supply, especially Lys, and compromises milk production. Our objective was to determine the effects of feeding by-pass protein/AA supplements in low or high CP diets containing DDGS on milk production and N utilization. Six multiparous Holstein cows (619.3 ± 49.8 kg BW; 26.8 ± 6.2 DIM) were subjected to a split-plot, 3 × 3 Latin square design with 21 d periods. The whole-plot factor was dietary CP content; low (14.6%; LP) or high (16.6%; HP), and the subplot was by-pass protein/AA supplement (top-dressed); Control (CON), Lactivate (LAC; 0.11 kg/cow/d) or ProLak (PRO; 0.45 kg/cow/d). All diets contained 10% corn DDGS. Feed intake and milk yield were recorded daily. Milk samples were collected from d 19 to 21. Spot urine (d 19 to 21) and blood (d 21, 3 h post-feeding) samples were collected to measure urine urea-N (UUN) and BUN, respectively. Data were analyzed using PROC MIXED in SAS. There was no CP level x supplement interaction ($P > 0.05$) for all data. Dry matter intake (26.2 vs. 26.9 ± 1.68 kg/d), milk (41.3 vs. 44.6 ± 4.69 kg/d) and milk component yield did not differ ($P \geq 0.38$) in cows fed the LP than HP diet. Although N intake did not differ (0.615 vs. 0.718 ± 0.0422 kg/d; $P = 0.16$), BUN (8.3 vs. 13.0 ± 0.78 mg/dL), MUN (11.9 vs. 14.5 ± 0.60 mg/dL) and UUN excretion (40.0 vs. 54.9 ± 4.03 g/d) were lower for cows fed the LP than HP diet. However, there was no supplement effect ($P \leq 0.82$) on milk (42.0, 43.5 and 43.2 ± 3.55 kg/d) and milk protein yield (1.19, 1.22 and 1.23 ± 0.092 kg/d for CON, LAC and PRO, respectively). Overall, feeding corn DDGS in LP than HP diets improved N utilization. Although not statistically significant, the numerical changes in milk and milk protein yield in cows fed the LP than HP diet; supplemented with LAC and PRO vs. CON, are important from an economic standpoint when evaluating income over feed costs.

Key Words: lactation performance, metabolizable AA supply, nitrogen utilization

T213 Impact of converting weaned dairy calves from a component-fed to a total mixed ration on growth and nutrient digestibility. L. K. Mitchell* and A. J. Heinrichs, *The Pennsylvania State University, University Park, PA.*

The objective was to determine effects of converting calves from a component-fed ration to a TMR at 8, 10, or 12 wk of age on intake, growth, and nutrient digestibility. Holstein calves (n = 40) were randomly assigned to 1 of 4 groups (no TMR, TMR conversion at 8, 10 or 12 wk; T0, T8, T10, and T12 respectively). Calves were weaned at 6 wk of age, housed individually, and studied from 7 to 14 wk of age. Rations, consisting of a 20% crude protein texturized starter and grass hay, were offered ad libitum as separate components or as a TMR with 85% starter and 15% grass hay on a DM basis. Intake and BW were measured weekly. Component intake for TMR was calculated from the proportion of each in the TMR. Fecal grab samples were collected and composited every 9 h over 3 d at 9, 11, and 13 wk of age from the same 4 calves per group. Feed and feces were evaluated for DM, NDF, ADF, and ADL (internal flow marker) to estimate digestibility. The statistical model included group (fixed), calf (random), and age (repeated). The slice option was used to determine effects of diet and age on digestibility of nutrients. Initial BW and hip width were used as covariates to evaluate DM intake and growth. Intake did not vary between groups ($P > 0.1$). However, at all ages calves fed TMR ate more hay and less starter than component-fed calves ($P < 0.05$). Average daily gain did not vary between groups ($P > 0.1$), but empty BW gain tended to be lower for calves put on TMR earlier (T0 = 1.08, T8 = 0.94, T10 = 1.04, and T12 = 1.06 kg/d $P = 0.07$). Hip growth was less for calves fed TMR earlier (T0 = 0.90, T8 = 0.80, T10 = 0.85, and T12 = 0.88 cm/wk $P < 0.05$). Digestibility of DM was greater at 9 wk for T8 vs. others ($P < 0.05$). Fiber digestibility was greater for calves on TMR at 9, 11, and 13 wk of age ($P < 0.01$ at all ages). We conclude that increased fiber digestibility allowed weaned calves on a TMR to consume less concentrate without affecting weight gain compared with component-fed calves during this study.

Key Words: feeding strategy, weaned calf, total mixed ration

T214 Concentrations of the flavonoids baicalin, baicalein, and wogonin in milk from cows supplemented or not with *Scutellaria baicalensis* extract during early lactation. K. E. Olagaray*[1], S. E. Sivinski[1], H. Liu[1], F. Robert[2], E. Dupuis[2], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*CCPA Group, Janze, France.*

The purpose of this investigation was to determine milk concentrations of 3 flavonoids found in *Scutellaria baicalensis*, and to determine whether supplementation of an extract of this plant changes concentrations of these flavonoids in milk. Multiparous Holstein cows (n = 122) were used in a randomized block design to determine responses to dietary *S. baicalensis* extract (SBE). Treatments were control or administration of SBE (100 g/d of test material). Milk samples were collected on d 3 and once during d 5–12 of lactation. A subset of randomly selected milk samples (5 control and 10 SBE) were used to determine concentrations of baicalin, baicalein, and wogonin. Serial dilutions of standards (100, 50, 25, 10, 5, 2, and 1 µg/mL) were prepared in methanol to generate standard curves. Skim milk samples were subjected to methanol extraction and analyzed by reverse-phase HPLC. Injections of baicalin, baicalein, and wogonin standards showed retention times of 15.4, 21.7, and 28.5 min, respectively. Flavonoids were not reliably detectable below 2 µg/mL, and none of the flavonoids were found at this concentration in milk. The maximum observed concentrations for baicalin, baicalein, and wogonin were 1.69, 0.19, and 0.76 µg/mL, respectively, and only a single sample generated a baicalein peak. Baicalin, baicalein, and wogonin concentrations were adjusted by their respective recoveries (83.3, 48.4, and 27.7% in skim milk) resulting in maximum estimated concentrations of 2.03, 0.39, and 2.74 µg/mL, respectively. Although concentrations were below reliable detection limits, peak area data were analyzed with repeated measures in a mixed model. Peak areas for baicalin and wogonin did not differ by treatment ($P = 0.82$ and 0.91, respectively) or day of lactation (both $P > 0.15$). In conclusion, the targeted flavonoids were not readily detectable in milk by this HPLC method, and dietary supplementation of 100 g/d of test material containing SBE showed no evidence of increasing flavonoid concentrations in milk.

Key Words: polyphenol, HPLC, bioactive nutrient

T215 Injectable trace minerals (selenium, copper, zinc, and manganese) neither hinder nor improve performance during an aflatoxin challenge in lactating multiparous Holstein cows. R. T. Pate* and F. C. Cardoso, *Department of Animal Sciences, University of Illinois, Urbana, IL.*

Aflatoxin (AF) mitigation strategies have been studied to alleviate the AF effects on dairy cattle performance. However, limited research is available on the effects of trace mineral supplementation during an AF challenge. The objective of this study was to determine the performance effects of 2 subcutaneous supplementations of 15 mg/mL Cu, 5 mg/mL Se, 60 mg/mL Zn, and 10 mg/mL Mn (Multimin 90, Multimin North America, Fort Collins, CO) given at 1 mL/90.7 kg of average body weight (BW) in response to an AF challenge. Fifty-eight Holstein cows [BW (mean ± SD) = 734 ± 60kg; DIM = 191 ± 93] were assigned to 1 of 3 treatments in a randomized complete block design. The experimental period (63 d) was divided into an adaptation phase (d 1 to 56) and a measurement phase (d 57 to 63). From d 57 to 59 cows received an AF challenge, which consisted of 100 µg of AFB1/kg of dietary dry matter intake (DMI) administered orally via balling gun. Treatments were: saline injection and no AF challenge (NEG), saline injection and AF challenge (POS), and trace mineral injection and AF challenge (MM). Injections were performed subcutaneously on d 1 and 29 at 1 mL/90.7 kg of average BW. Milk was sampled 3 times daily from d 56 to 63. Two treatment orthogonal contrasts, CONT1 (NEG vs. POS) and CONT2 (POS vs. MM), were made. Cows in NEG had lower ($P < 0.001$) AF excretion in milk (0.0 µg/d) and higher ($P = 0.02$) 3.5% fat-corrected milk (32.1 kg/d) compared with cows in POS (16.7 µg/d and 28.6 kg/d, respectively). Protein yield tended to be higher ($P = 0.10$) for cows in CON (1.13 kg/d) compared with cows in POS (1.07 kg/d). Feed efficiencies (3.5% FCM/DMI, ECM/DMI, and Milk/DMI) were higher ($P = 0.02$) for cows in NEG (1.42, 1.46, and 1.45, respectively) than cows in POS (1.16, 1.18, and 1.22, respectively). Cows in POS had higher ($P = 0.03$) milk urea nitrogen (14.3 mg/dL) than cows in MM (13.3 mg/dL). In conclusion, subcutaneous supplementation of trace minerals neither hindered nor improved performance response when an AF challenge was present.

Key Words: aflatoxin, trace mineral, feed efficiency

T216 A comparison of mathematical approaches for determining the rate of starch digestion across grains and particle sizes. M. N. T. Shipandeni*[1,2] and E. Raffrenato[1], [1]*Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa,* [2]*Department of Animal Science, University of Namibia, Windhoek, Namibia.*

Starch digestibility is often estimated in vitro at 7 h and the value is then used to calculate a rate of digestion using a previously published mathematical formula. The objective of this study was to compare mathematical approaches for determining the rate of starch digestion using data from various grains and particle sizes. Three cereals (corn, sorghum, barley) were ground through a 2-mm sieve using a Wiley mill and subsequently sieved to obtain the following sizes: <250, 250–500, 500–1180 and 1180–2000 µm. All fractions, including the unsieved one, were analyzed for starch and fermented in vitro using rumen fluid to quantify starch digestibility (Sd). Residual starch of the fermented samples were obtained at 0, 3, 6, 7, 9, 12 and 24 h. Rates of starch digestion (kd) were calculated with either a first-order decay model using all time points except 7-h (nlin-kd), or a mathematical formula using only the 7-h Sd (7h-kd). The nlin-kd's were used as reference values (observed) and compared with the 7h-kd values. Digestibility values, pooled, by grain or by size, were pairwise-compared and prediction accuracy was tested and compared using correlations and the mean square prediction error (MSPE). Fermentation run (n = 3) was considered as random effect. Predicted and observed kd were highly correlated across all grains and sizes (r = 0.94 to 0.99). Across grains, corn resulted in the highest correlation (r = 0.98) while sorghum and barley resulted in r = 0.77 and 0.87, respectively. While observed kd regressed on predicted kd resulted in intercepts not different than zero ($P > 0.44$), slopes were always larger or smaller than 1 ($P < 0.01$) resulting in the 7-h kd under- or over-estimating, respectively, the reference nlin-kd. The formula resulted in underestimated values especially for the unsieved samples, with differences up to 0.04 h^{-1}. This suggests the need of using a nonlinear estimation, using multiple time points, or the development of alternative estimations, especially when quantifying rates of starch digestion for high producing cows.

Key Words: sorghum, barley, corn

T217 Effects of lysophospholipids on nitrogen utilization, nutrient digestibility, and production in dairy cows. C. Lee*[1], D. L. Morris[1], S.-H. Kim[1], J. E. Copelin[1], P. A. Dieter[1], and I. Kwon[2], [1]*Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH,* [2]*Easy Bio Inc., Seoul, South Korea.*

An experiment was conducted to examine effects of supplemental lysophospholipids (LPL) in dairy cows. Eight cannulated lactating Holstein cows were used in a repeated 4 × 4 Latin square design. Dietary treatments were CON, a dairy ration (55% forage and 45% concentrate on a DM basis); MON, the control diet supplemented with monensin (16 mg/kg in dietary DM; Elanco Animal Health; positive control); L-LPL, the control diet supplemented with low LPL (0.05% in dietary DM; Lipidol Ultra, Easy Bio Inc.); H-LPL, the control diet supplemented with high LPL (0.075%). Experimental periods were 21 d with 14-d diet adaptation and 7-d sample collection. Daily intake and milk yield were measured and milk samples were collected weekly. Spot urine and fecal samples (8 samples per cow) were collected for 3 d to determine nutrient digestibility and dietary N utilization. All data were analyzed using the MIXED procedure of SAS where the model included group and cow within group as random and treatments, time, and their interaction as fixed. Preplanned contrasts were made to determine linear effect of LPL and effect of LPL vs. MON. Supplementation of LPL to the diet did not alter DMI but linearly increased ($P < 0.01$) milk yield, resulting in linear increases ($P < 0.01$) in feed efficiency (milk yield ÷ DMI) and milk protein and fat yields. However, total-tract digestibility of DM and OM tended to be lower (linear $P \leq 0.07$) for LPL compared with CON. In addition, linear decreases ($P \leq 0.02$) in urinary N excretion and urinary N excretion as % of total intake N were observed. Comparing to MON, supplementation of LPL increased ($P \leq 0.10$) milk fat content and yield and estimated microbial N production in spite of decreases ($P \leq 0.04$) in apparent DM and OM digestibility. In conclusion, LPL is a potential feed additive that can increase milk yield and components and dietary N utilization. However, the mechanisms for the positive effects clearly differ between LPL and monensin according to milk fat and microbial N production. Further studies are needed to examine the roles of LPL in dairy cows.

Key Words: lysophospholipid, production, nitrogen utilization

T218 The effect of supplementation type on quality and processability parameters of milk from grazing dairy cows in late lactation. Z. C. McKay*[1], M. O'Sullivan[2], M. B. Lynch[1], F. J. Mulligan[1], R. Mahon[2], and K. M. Pierce[1], [1]*Lyons Research Farm, Lyons Estate, University College Dublin, Celbridge, Co. Kildare, Ireland,* [2]*School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

Irish dairy production is primarily produced from a spring calving grazing system with the use of concentrate supplementation in the spring and autumn. Problems with milk quality are more pronounced in such a seasonal system where the majority of the herd enter into late lactation at the same time. Supplementation may offer a means of improving milk quality and composition. Oat supplementation has shown improvements in milk yield and composition in early lactation, while supplementation with vitamin E (VE) may improve cell structure and milk quality. Therefore, the objective of this research was to investigate the effect of concentrate type and VE on milk quality and processability parameters in late lactation grazing dairy cows. Forty 8 Holsten Friesian dairy cows were blocked on days in milk (+185DIM) and balanced for parity, pre-experimental milk yield and milk composition, predicted 305day milk yield and BCS. Cows were randomly assigned to 1 of 4 dietary treatments in a randomized complete block design (n = 12). The dietary treatments (T) were grass only (T1); grass + 2.6kg DM barley based concentrate +350IU VE/kg (T2): grass + 2.6kg DM oat based concentrate +350IU VE/kg (T3) and grass + 2.6kg DM oat + 1050IU VE/kg based concentrate (T4). The diets were fed for a 14day acclimatization period and then for a further 49days. Milk samples from each treatment were pooled into 3 subsamples (n = 3). Cows offered T3 had a lower milk pH (6.60) than T1 (6.62, $P = 0.02$). Rennet coagulation time was reduced in milk from cows offered T4 (2.49 min, $P = 0.004$), and increased in milk from cows offered T2 (5.36 min, $P = 0.007$) compared with cows offered T1 (4.16 min). Cows offered T3 and T4 had increased ethanol stability (67.29%, $P = 0.003$; and 67.33% $P = 0.002$ respectively) in comparison to T1 (65.54%). In conclusion, concentrate supplementation with oats or high VE had a positive effect on milk quality and processability. Supplementing dairy cows with oats improved milk pH. Offering a high level of VE to dairy cows reduced rennet coagulation time and increased ethanol stability of milk.

Key Words: late lactation, milk processability

T219 The effect of two new formulas of dietary buffer with a high buffering capacity contained Na or K on performance and metabolism of dairy cows. S. M. Nasrollahi*[1], A. Zali[1], and W. Z. Yang[1], [1]*University of Tehran, Tehran, Iran,* [2]*Lethbridge, Lethbridge, AB, Canada.*

The study was conducted to evaluate the effect of adding 2 new formulas of dietary buffer on feed intake, nutrient digestibility, rumen pH, blood metabolites, and milk production of dairy cows. Nine multiparous cows (BW = 594 ± 46 kg; DIM = 120 ± 28 and milk yield = 46.6 ± 3.4 kg/d) were randomly assigned to a triplicate 3 × 3 Latin square. Treatments were varying source and proportion of dietary buffer (DM basis): (1) 1.12% sodium bicarbonate (SB), (2) 0.87% high buffering capacity formula contained Na (HBNa), and (3) 0.74% high buffering capacity formula contained K (HBK). Each experimental period consisted of 14 d of adaptation and 7 d of sampling and cows were fed with high concentrate diets (62% of DM). Buffering capacity was 102, 150, and 137% of a pure $NaHCO_3$ for SB, HBNa and HBK, respectively. The amount of Na and K were 27 and 0, 31 and 0, and 25 and 6%, respectively, for SB, HBNa, and HBK. Data were analyzed using Mixed Procedures of SAS with a model consisting of fixed effects of treatment, square, and period, and random effect of cow within square. Intake of DM tended ($P < 0.06$) to be lower with HBK (20.6 kg/d) than SB (21.0 kg/d) and HBNa (21.2 kg/d). However, no treatment effects were observed on rumen pH (averaged 5.88) and DM digestibility in the total digestive tract (averaged 79.4%). Yields of actual milk (38.1 kg/d) and 3.5% FCM (31.6 kg/d) were not affected by treatments, whereas yields of SCM ($P < 0.07$) and milk fat ($P < 0.10$) tended to be greater with HBK than SB and HBNa. Contents of milk fat, protein and lactose were greater ($P < 0.05$) with cows fed HBK than with cows fed SB or HBNa, as a result, milk efficiency (milk yield/DMI) improved with HBK (1.86) compared with SB (1.80). Blood concentration of Ca was higher ($P < 0.01$) with HBK compared with SB and HBNa (10.4 vs. 9.7 and 9.9 mg/dL, respectively). Blood glucose concentration also tended ($P < 0.08$) to be greater with HBK (64.7) versus HBNa (58.8) and SB (58.4 mg/dL). These results indicated that under the current experimental condition, supplementation of dairy cow diet with a high buffering capacity buffer contained 6% K decreased DMI, improved milk composition and milk efficiency of dairy cows.

Key Words: dietary buffer, feed efficiency, dairy cow

T220 Upgrading of yellow wine lees through solid-state fermentation with *Candida utilis* and *Bacillus subtilis*. K. Y. Yao*, H. F. Wang, and J. X. Liu, *Institute of Dairy Institute, Zhejiang University, Hangzhou, China.*

Yellow wine lees, the main co-products in yellow wine industry, consist of high content of crude protein but are not well utilized as animal feed. Solid-state fermentation was employed in the current study to upgrade the nutritional value of yellow wine lees as protein feed for ruminant animals. A single factor study was carried out to select the proper strain combination, and another single factor and a 3-level-3-factor orthogonal design were then conducted to evaluate the effects of the water content, incubation temperature and inoculum ratio on quality of product and find out the optimal condition for solid-state fermentation. Chemical composition and in vitro digestibility were determined to assess the nutritional value of the unfermented and fermented products. Each experiment was duplicated 3 times. The data were analyzed using PROC GLM of SAS. A strain combination of *Candida* and *Bacillus* was chosen for solid-state fermentation, and the optimal fermentation condition was water to total solid medium at 53:100 (v/w), 30°C of temperature, and ratio of *Candida* to *Bacillus* at 2:1. Contents (%, DM basis) of CP (30.2) and peptides (23.6) of fermented products were 14.5 ($P < 0.01$) and 40.9% ($P < 0.01$) higher than those of unfermented material, respectively. Content of AA of the fermented products increased by 25.7% ($P < 0.01$) compared with the unfermented, and contents of essential AA (such as Lys and Met) were increased greatly ($P < 0.01$). The microbial treatment decreased the ammonia-nitrogen concentration ($P = 0.09$) and increased the synthesis of microbial protein ($P = 0.02$) with higher crude protein digestibility ($P < 0.01$). The acetate content tended to decrease by the pretreatment ($P = 0.08$). These results drew the conclusion that the microbial pretreatment obtained from this study greatly increased the nutritional value of yellow wine lees, making these materials more suitable as feeds for animals including dairy cows.

Key Words: in vitro rumen fermentation, solid-state fermentation, yellow wine lees

T221 Effect of grain- or by-product-based concentrate fed with early or late harvested first cut grass silage on dairy cow performance. D. Pang*[1], T. Yan[2], E. Trevisi[3], and S. Krizsan[1], [1]*Swedish University of Agricultural Sciences, Umeå, Sweden,* [2]*Agri-Food and Biosciences Institute, Hillsborough, United Kingdom,* [3]*Catholic University of the Sacred Heart, Piacenza, Italy.*

The objective of this study was to compare the effects of a grain-based conventional concentrate (GC) with a concentrate based on agro-industrial by-products (BC), each fed with 2 different grass silages harvested at early (ES) or late (LS) maturity stage, on dairy performance and efficiency of N and energy utilization when fed to lactating dairy cows. Twenty lactating Nordic Red cows were assigned to a replicated 4 × 4 Latin square design with 4 periods of 21 d. Dietary treatments were in a 2 × 2 factorial arrangement. The silages were harvested with 2-week interval from the same primary growth grass ley. The ES was higher in CP (173 vs. 108 g/kg DM) and lower in NDF (443 vs. 607 g/kg DM) than LS. The GC was made from grain and soybean meal, while the BC contained sugar beet pulp, wheat bran, canola meal, distillers dried grain, palm kernel expeller and molasses. The GC contained more starch (444 vs. 51 g/kg DM) and less NDF (162 vs. 351 g/kg DM) than BC. The diets were fed ad libitum as total mixed rations and were formulated from 661 g/kg of silage, 326 g/kg of concentrate and 13 g/kg minerals on DM basis. Experimental data were subjected to ANOVA using the GLM of SAS by applying a model correcting for the effect of block, period, cow within block and dietary treatments. The effects of silage maturity, concentrate type and their interaction were evaluated. The milk yield and composition were unaffected by concentrate type, except that milk protein decreased 0.7 g/kg from cows fed BC. These results were paralleled by decreased CP digestibility and increased NDF digestibility for cows fed BC. Cows fed ES on average consumed more dry matter (23 vs. 21 kg/d) and yielded more milk (28.7 vs. 25.2 kg/d) than cows fed diets with LS. The edible feed conversion ratios showed greater improvements with ES than LS when replacing GC with BC. Feeding diets with LS reduced digestibility and energy utilization efficiency, but improved N efficiency. In conclusion, a conventional concentrate can be replaced by agro-industrial by-products without compromising production in lactating dairy cows, and the silage maturity had a stronger effect on dairy performance than the concentrate type.

Key Words: by-products, dairy cow, energy utilization

T222 Regression analysis of the relationship between milk *de novo* synthesized fatty acids, *trans*-10 C18:1, and milk fat percent using treatment means from the literature. C. I. Matamoros*, R. Klopp, AR Clarke, and K. J. Harvatine, *The Pennsylvania State University, University Park, PA.*

Biohydrogenation-induced milk fat depression (BH-MFD) is caused by bioactive conjugated linoleic acid (CLA) isomers produced during altered rumen biohydrogenation. These CLA isomers appear in low concentration in milk during BH-MFD, but *trans*-10 C18:1 also increases during BH-MFD and provides a biomarker of alternate ruminal fermentation and the production of bioactive fatty acids (FA) that inhibit milk fat synthesis. The concentration of de novo synthesized FA (<16 carbons) are also decreased during BH-MFD. The objective of this study was to characterize the relationship between milk fat de novo FA and *trans*-10 C18:1 and milk fat concentration allowing prediction of BH-MFD based on milk FA profile. A database was constructed from peer-reviewed papers (n = 928 treatments) and treatment means of non-published papers from Penn State (n = 149 treatments). The inclusion criteria were that the papers reported milk yield, milk fat concentration, and milk FA profile in post-peak Holstein cows. Regression analysis was conducted using JMP Pro 13.0. The linear, quadratic, and a single and 2 pool exponential fit were tested. The 2-pool exponential model allowed modeling of a responsive and non-responsive portion of milk fat as BH-MFD results in a maximal 50% reduction in milk fat. Additionally, a comparison between methylation methods (2-step acid, 1-step acid, and sodium methoxide methylation) was also tested because they differ in the literature. The concentration of even chain saturated FA between 6 and 14 carbons and C14:1 best fit to milk fat percent using a linear model (MSE = 0.3; RMSE = 0.548; $R^2 = 0.168$). Milk *trans*-10 18:1 best fit to milk fat percent using a 2-pool exponential model (MSE = 0.149; RMSE = 0.385]. The exponential fit did not differ between data from papers using acid and base methylation procedures or between the literature and only data from our laboratory. In conclusion, BH-MFD was best predicted by milk *trans*-10 C18:1 using a 2-pool exponential model allowing prediction of expected milk fat concentration from a milk fatty acid analysis.

Key Words: *trans*-10 C18:1, milk fat, milk fat depression

T223 Impact of various forages and live yeast culture on weaned dairy calf growth and nutrient digestibility. L. K. Mitchell* and A. J. Heinrichs, *The Pennsylvania State University, University Park, PA.*

The objective was to determine effects of various forages and live yeast culture on weaned calf growth and nutrient digestibility. Holstein calves (n = 45) were randomly assigned to 2 × 3 factorial treatments: live yeast culture (Y; Yea-Sacc, Alltech Inc.) or no yeast (NY) and alfalfa haylage (AH), corn silage (CS), or grass hay (GH). Calves were weaned at 6

wk of age, housed individually, and studied from 7 to 16 wk of age. Rations, consisting of texturized grower (Y or NY) and assigned forage, were offered as separate components until 9 wk of age. After 9 wk, feed was offered as a total mixed ration (TMR). Grower intake was capped at 2.25 kg/d, while forage was offered ad libitum. The TMR contained equal forage neutral detergent fiber (NDF; 8 ± 0.5%) on a dry matter (DM) basis. Calves were limit fed TMR to restrict grower intake, and additional forage was offered after 8 h if the entire TMR allotment was consumed. Total fecal collection (12 calves) was conducted for 4 d at 11 and 15 wk of age. Feces were weighed, sampled daily, and composited. Feeds and feces were evaluated for DM, NDF, acid detergent fiber, and starch to estimate digestibility. The statistical model included yeast, forage, and their interaction as fixed effects and calf as a random effect. Age was included as a repeated effect for nutrient digestibility. Initial body weight (BW) was used as a covariate to evaluate intake and growth. Intake (DM as % of BW) was least for calves consuming GH vs. other forages ($P < 0.05$). Calves on CS had the greatest average daily gain and empty BW gain, while calves on GH had the least. There was a tendency for a yeast x forage interaction effect on feed efficiency ($P = 0.1$) with calves on YCS being the most feed efficient. Digestibility of DM decreased with age (wk 11, 73.46 vs. wk 15, 71.22%; $P = 0.05$). Fiber digestibility was least for calves on CS ($P < 0.05$). Starch digestibility was least for calves on AH (CS = 93.94, GH = 93.14, AH = 88.29%; $P = 0.05$). In this trial, weaned calves fed CS gained weight faster than calves on AH or GH, and Y provision tended to increase feed efficiency in calves consuming CS.

Key Words: forage, weaned calf, yeast

T224 Evaluation of batch culture incubation methods, NDF degradation, and bacterial FA detection. Y. Roman-Garcia*[1], C. Lee[1,2], B. Denton[1], and J. Firkins[1], [1]*The Ohio State University, Columbus, OH,* [2]*Ohio Agricultural Research and Development Center, Wooster, OH.*

To improve accuracy and precision of NDF disappearance in vitro and bacterial fatty acid (FA) quantification by GLC-mass spectroscopy, ground alfalfa hay was weighed directly (DIR) into a 50-mL tube or was weighed into an ANKOM (Macedon, NY) filter bag (BAG), sealed, and placed in tubes. Using a mixed model with random block (replication), 24-h alfalfa NDF disappearance in vitro was greater (31.0 vs. 23.7%; $P = 0.03$) vs. BAG when NDF was analyzed with filter bags; NDF analysis of DIR with filter bags was not different vs. the filter paper procedure (30.1% vs 31.4%, $P = 0.78$). At 24 h, BAG pH was higher vs. DIR (6.86 vs. 6.42; $P < 0.01$), suggesting incomplete mixing with inocula. With 50:50 alfalfa:corn, NDF degradation by filter bag was lower than with filter paper (17.0% vs. 41.2%; $P < 0.01$). To prevent artifact FA peaks with GLC, 3 methods were assessed to extract particulate-associated bacteria (PAB) for FA analysis: (1) lowering the pH to 2.0 using acid (AC); (2) cold shocking (CS) with 2 ice-cold saline washes; and (3) storing at 4°C for 24 h (4C). All 3 methods were factorialized and compared with no extraction (NO; i.e., fluid-phase bacteria). With NO, 74% of the peaks from 2 standards of bacterial FA were recovered, but CS decreased ($P < 0.01$) recovery to 54%. Combining 4C and AC had the highest recovery (85%; $P = 0.44$ vs. NO), whereas combining CS and 4C had the lowest (34%; $P = 0.04$ vs. CS). A Poisson regression was used to examine the recovery of 22 peaks not present in the 2 standards. Compared with AC, 20 unknown peaks remained with NO and decreased ($P < 0.01$) to 13 with CS. Either 2 low temperature steps (LT) or a single high temperature step (HT) for methylation were tested after incubation with ^{13}C-labeled Ile, Leu, and Val. The recovery of known standard peaks was 83% ($P = 0.38$ between methods). With HT, we detected 19 unknown peaks, which LT increased ($P = 0.04$) to 21. Ten unknown peaks were enriched with ^{13}C for both LT and HT. Feed should be placed directly in the tube during incubation, and filter papers ensured accurate NDF degradation for mixed feed. Neither methylation nor PAB extraction methods examined herein introduced artifact FA peaks.

Key Words: NDF, method

T225 Crosslinking of protein capsules containing fish oil reduces their disintegration rate in ruminal contents but allows rapid fatty acid release in intestinal proteases. T. C. Jenkins*[1], K. Murphy[2], S. J. Saunier[1], G. J. Lascano[1], and N. M. Long[1], [1]*Clemson University, Clemson, SC,* [2]*Virtus Nutrition LLC, Corcoran, CA.*

Delaying the contact of fatty acids with ruminal microorganisms was tested by treating protein capsules containing fish oil with flavoring agents and heat. Ruminal breakdown was determined by sealing a single treated capsule in a nylon bag and suspending it in ruminal contents (Daisy incubator) for 0, 6, 12, 24, and 48 h (n = 14). After each period, 4 bags were randomly selected and dried for 24 h at 105°C for determination of percentage DM, and the remaining bags were opened and capsules removed. Four of the removed capsules at each rumen exposure time were analyzed for fatty acids by GC, 3 were suspended in pepsin/HCl for 2 h in a disintegration tester at 40°C, and the remaining 3 were suspended for 2 h in a buffer solution containing pancreatin at 40°C. The completely randomized design included the main effect of incubation time with 2 orthogonal contrasts. Untreated capsules totally disintegrated with release of all fish oil in <15 min suspension in rumen buffer at 40°C. Treated porcine # 0 hard gelatin capsules suspended in ruminal contents had similar ($P = 0.82$) DM remaining at 0 (99.0%) vs 6, 12, and 24 h (average 98.2%), but lower ($P = 0.006$) DM remaining at 48 h (88.2%). Similarly, the percentage of EPA in recovered capsules was the same for 0 versus 6, 12, and 24 h (18.3%) but lower ($P < 0.01$) at 48 h (17.1%). Capsules removed from ruminal contents and then exposed to pepsin/HCl had similar DM remaining for 6 (97.4%) vs 12 and 24 h (97.1%), but lower ($P = 0.06$) DM remaining for 48 h (62.1%). Capsules removed from ruminal contents and then mixed in a pancreatin solution had similar DM remaining for all rumen exposure times (average 1.95%). Results were similar for treated small and large soft gel capsules containing fish oil. The treatment protocol developed in this study successfully slowed disintegration time of gelatin capsules in ruminal contents and minimized changes in fatty acid profile of fish oil contents. Extensive disintegration occurred when treated capsules were exposed to pancreatic proteases.

Key Words: fish oil, rumen, protection

T226 Non-linear essential amino acid use efficiency equations for milk amino acid synthesis. R. R. White*[1], H. Lapierre[2], J. L. Firkins[3], and L. E. Moraes[3], [1]*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA,* [2]*Agriculture and AgriFood Canada, Quebec, Canada,* [3]*The Ohio State University, Columbus, OH.*

The objectives of this work were to use a Bayesian approach to fit efficiency curves relating individual essential AA export (AAExp) to duodenal digestible AA (DigAA) flow, and to evaluate the responsiveness of these curves to digestible energy (DE) intake and days in milk (DIM). A literature data set of 333 treatment means (87 studies) was collected. This data set included measures of diet composition, feed intake, duodenal or omasal protein and EAA flows, and milk protein output. Where diet composition data were not reported in the paper,

they were calculated from feed library composition values. The AAExp was estimated as the sum of milk AA, scurf AA, and metabolic fecal AA output. The DigAA flow was estimated as the sum of AA from intestinally digestible microbial and rumen undegraded protein supplies minus endogenous urinary protein. Nonlinear least squares regression was used to develop informative prior parameter estimates, which were then used in a Bayesian hierarchical derivation of the relationship between individual essential AAExp and DigAA. Regressions were weighted based on the inverse of the SE for milk protein yield reported in each study. This procedure was done independently for each AA and a logistic equation form was used in all cases. Within-study parameter estimates for the asymptote, amplitude, and steepness of this logistic curve were then regressed on predicted dietary DE intake and reported DIM using simple linear regression. The DE intake influenced ($P < 0.05$) His, Ile, Leu, Lys, Met, Phe, Thr, and Val curve asymptote. Curve amplitude was influenced ($P < 0.05$) by DE intake for all EAA and was also influenced ($P < 0.05$) by DIM for Ile, Lys, Met, Phe, Thr, and Val. The curve steepness was affected ($P < 0.05$) by DIM only for Arg, and by DE only ($P < 0.05$) for Val. Both DE and DIM influenced ($P < 0.05$) the curve steepness for His, Ile, Leu, Lys, and Thr. Results suggest EAA use efficiency should be modeled as a nonlinear function dependent, in some cases, on DE and stage of lactation.

Key Words: essential amino acid, efficiency, milk protein synthesis

T227 Estimation of total fatty acid content and composition of feedstuffs for dairy cattle. V. L. Daley*[1], L. E. Armentano[2], P. J. Kononoff[3], J. M. Prestegaard[4], and M. D. Hanigan[4], [1]*National Animal Nutrition Program (NANP), University of Kentucky, Lexington, KY,* [2]*University of Wisconsin, Madison, WI,* [3]*University of Nebraska-Lincoln, Lincoln, NE,* [4]*Virginia Tech, Blacksburg, VA.*

Predictions of total fatty acid (FA) and individual FA content of feeds are necessary for formulating diets based on essential FA requirements of dairy cows. Equations to predict total FA content from crude fat (EE) for different classes of feeds were developed using data from commercial laboratories. The FA composition of total FA for each feed was taken from the CNCPS library. Feed ingredients were grouped into 8 classes. All models were linear and fitted without an intercept. The data for forages were weighted using the inverse of the standard error. Regression coefficients for prediction of total FA from EE, and the mean FA composition are shown in Table 1. Estimates of FA content and composition of feed ingredients are useful to formulate diets for dairy cows when animal feed analysis laboratories are not available.

Key Words: feed, fatty acid composition, model

T228 Effects of crude protein level and rumen degradable:undegradable protein ratios on nitrogen balance and milk production in dairy cows. O. I. Santana*[1], A. Peña-Ramos[1], and K. M. De la Cruz-Espino[2,1], [1]*INIFAP, Pabellon, Aguascalientes, Mexico,* [2]*Universidad Autonoma de Aguascalientes, Aguascalientes, Mexico.*

In Mexico, dairies overfeed CP without improvements on milk production. Our objective was to assess the effects of 2 levels of CP and 2 ratios of rumen degradable:undegradable protein (RDP:RUP, respectively) on nitrogen (N) balance and milk production of dairy cows. Eight multiparous Holstein cows (640 ± 35 kg of BW and 124 ± 14 d in milk) were placed in a replicated 4 × 4 Latin square with 2 × 2 factorial arrangement of treatments to evaluate 4 diets: Low CP and high CP (LP and HP; 14.9 and 17.1% of dry matter, respectively) with RDP:RUP CP ratios of either 68:32 or 62:38. Levels of CP and RDP:RUP ratios were manipulated with canola meal and alfalfa hay. All diets contained 29.5% neutral detergent fiber, 25% starch, and 1.55 Mcal of NEl/kg. Cows were fed once and milked 3 times per day. On d 18, 19, and 20 of each 21-d period, milk samples, urine and fecal spot samples were taken at 6 h before and 6 h after feeding. Data were analyzed using proc Mixed of SAS. Dry matter intake (DMI) was greater for cows fed HP than LP diets (26.0 vs. 25.3 kg/d) and for cows fed 68:32 than 62:38 (25.7 vs. 25.1 kg/d) (both $P < 0.05$). Treatments did not influence milk yield (MY, 39.4 ± 1.8 kg/d), milk component (3.23 ± 0.02, 3.46 ± 0.05, and 5.0 ± 0.01% of protein,

Table 1 (Abstr. T227). Fatty acid (FA) and FA composition (% of total FA) in different classes of feeds

Item	Animal protein	Plant protein	Energy sources	Grain forage crops	By-products feeds	Forages[1]	Oil seeds	Oil
Feed ingredients, n	14	28	35	15	43	57	11	11
Observations, n	6579	18263	181611	1142016	49068	405624	5426	NR[3]
Regression coefficients[2]								
Slope	0.7310	0.8476	0.7990	0.6100	0.8665	0.5678	0.9286	0.8831
SE	0.0374	0.0235	0.0238	0.0393	0.0178	0.0166	0.0268	0.0021
FA composition								
C12:0	0.24	4.70	0.05	0.61	0.76	6.11	0.18	0.08
C14:0	4.86	0.58	1.15	0.74	0.94	0.68	0.42	0.84
C16:0	27.22	15.26	16.74	19.01	20.29	19.53	17.26	7.83
C16:1	6.61	0.59	0.19	0.45	0.28	1.87	0.42	1.14
C18:0	12.31	3.63	2.15	2.38	3.51	2.93	3.60	3.51
C18:1 *cis*-9	25.52	20.18	20.28	16.32	17.12	2.60	22.90	43.03
C18:1 *trans*	1.56	0.55	NR[3]	0.01	1.88	0.42	0.04	2.17
C18:2 *cis*-9,*cis*-12	6.71	48.83	54.65	42.29	49.10	19.14	50.36	24.54
C18:3 *cis*-9,*cis*-12,*cis*-15	0.57	9.44	3.53	13.56	6.44	40.55	3.97	10.45
Others	13.85	1.23	0.81	4.68	2.07	6.55	0.96	6.42

[1]Forage grasses and legumes.

[2]Regression coefficients to estimate FA in feeds: FA (% of DM) = crude fat (% of DM) × slope.

[3]Not reported.

fat, and lactose, respectively), MY by component (1.2 ± 0.1, 1.3 ± 0.1, and 1.9 ± 0.2 kg/d of protein, fat, and lactose, respectively), and feed efficiency (MY/DMI; 1.5 ± 0.12). Cows fed HP had greater N intake (NI) than LP diets (688 vs. 602 g/d, $P < 0.05$), but no differences were detected for RDP:RUP ratios. Urinary-N, urinary urea-N, and milk urea-N were greater for cows fed HP than LP (227 vs. 148 g/d, 108 vs. 72 g/d, and 22.6 vs. 13.0 mg/dL respectively, all $P < 0.05$), but no effects of RDP:RUP ratios were detected. No differences were observed for fecal-N and milk-N (226 ± 8.4 and 195 ± 15.3 g/d, respectively), but N efficiency (milk-N/NI) was greater in cows fed LP than HP diets (34.3 vs. 28.8%, $P < 0.05$). In conclusion, LP diets did not penalize MY, but increased NE, and reduced N losses. However, RDP:RUP ratios had no influence on MY and N balance.

Key Words: efficiency, urea

T229 Evaluation of a limit feeding strategy with canola or soybean meals on dairy cow performance. S. A. E. Moore*[1] and K. F. Kalscheur[2], [1]*University of Wisconsin, Madison, WI,* [2]*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Shrinking margins in the dairy industry dictate a need for increasing efficiencies of feed conversion to milk and limiting feed waste on the farm. The aim was to determine if performance and efficiency were similar when feeding 2 protein sources (canola meal, CM; soybean meal, SBM) at 2 feeding levels (limit-fed, LF; ad libitum-fed, AL). Fifty-two multiparous Holstein cows (mean ± SD; 98 ± 38 DIM; 2.73 ± 1.03 parity) received a common diet during a 4-wk covariate (COV) period. Animals were sorted by residual feed intake (RFI = DMI_{Act} – DMI_{Pred}), grouped into quartiles, and then blocked to standardize MY, milk components, BW, DMI and DIM. Each cow was randomized to receive one of 4 treatments. An average of COV wk 3–4 ad libitum intake was reduced by 5% in LF diets. The amount offered was evaluated weekly and reduced in pairs if a LF cow had orts ≥0.907 kg as-fed. Average final restriction of the LF group was 92.8% of COV wk 3–4 ad libitum intake. Diets were formulated to contain 42, 18, and 40% DM of corn silage, alfalfa haylage, and concentrate mix, respectively. The COV diet was formulated to contain equal CP from SBM and CM. Statistical analyses were performed as repeated measures using the MIXED procedure of SAS. Dry matter intake (DMI) was greater for AL vs LF cows (mean ± SEM; 29.0 vs 27.7 ± 0.22 kg/d; $P < 0.02$) during wk 1–4. DMI was greater for CM-fed cows in wk 1 by 0.65 ± 0.22 kg/d ($P < 0.04$) and for SBM-fed cows in wk 4 by 0.71 ± 0.22 kg/d ($P < 0.03$). Protein source did not have an effect on MY. Cows fed AL had greater MY, in wk 3–4 (52.4 vs 50.4 ± 0.55 kg/d; $P < 0.02$). Milk fat concentration was less for LF compared with AL cows in wk 3–5 (3.63 vs 3.87 ± 0.07%; $P < 0.05$). MUN was lower in CM-fed cows (10.9 vs 11.6 ± 0.24; $P < 0.01$). Feed efficiency (FE = energy corrected milk/DMI) was greater in the LF treatment during wk 1 (1.99 vs. 1.92 ± 0.02; $P < 0.02$) but not different in other weeks. While the LF method was effective at equalizing DMI in SBM- and CM-fed animals, MY decreased and FE did not commensurately compensate. Continued investigation into limit feeding strategies is needed to evaluate the effect on digestion and efficiency.

Key Words: canola meal, efficiency, soybean meal

T230 Case study: Comparison of sorghum versus corn distillers grains and its effect in dairy production. L. M. Baker*[1], B. W. Jones[1,2], W. B. Smith[1], and K. C. McCuistion[3,4], [1]*Department of Animal Science and Veterinary Technology, Tarleton State University, Stephenville, TX,* [2]*Texas A&M AgriLife Research, Stephenville, TX,* [3]*Texas A&M University-Kingsville, Kingsville, TX,* [4]*United Sorghum Checkoff Program, Lubbock, TX.*

With the expansion of the US ethanol industry, the amount of grain byproducts on the market has increased. Sorghum is the second most common grain used in ethanol production, yet few studies have evaluated lactating dairy cow performance when fed distillers grains with solubles (DGS) from grain sources other than corn. Studies evaluating sorghum-based DGS in finishing cattle have reported variable results. Inconsistent findings may be the result of differences in the quality and quantity of solubles added back to the DGS, technology differences used at the ethanol plants, physical form, and DGS inclusion rates. The objective of this study, conducted on a commercial dairy farm in Wisconsin, was to evaluate the effects of sorghum dried DGS (SDGS) compared with corn dried DGS (CDGS) when fed at 8% of dietary DM. Multiparous ($\mu = 3$) Holstein cows (n = 362; 132 ± 2.8 DIM) were allocated to 1 of 3 pens based on DIM and milk yield. For comparison, a negative control pen was fed a diet that contained 3.6% corn dried distillers grains with solubles (CON). All diets were formulated to be isonitrogenous and isocaloric. Milk samples were collected and analyzed by DHIA on d 14 and 28. The MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) was used to evaluate fixed effect of diet. No effect of dietary treatment existed for daily milk yield ($P = 0.59$) or percent milk protein ($P = 0.69$) from CON (52.9 ± 0.84 kg/d and 3.00 ± 0.008%, respectively), SDGS (52.2 ± 0.83 kg/d and 3.00 ± 0.008%, respectively), or CDGS (52.7 ± 0.84 kg/d and 3.01 ± 0.008%, respectively). Percent milk fat was greater ($P < 0.05$) from CDGS (3.63 ± 0.023%) compared with CON (3.49 ± 0.023%) or SDGS (3.55 ± 0.023%). An effect of dietary treatment existed on DM intake ($P < 0.01$), where intake was less ($P < 0.05$) for cows consuming CON (27.8 ± 0.19 kg/d) compared with SDGS (28.9 ± 0.19 kg/d) and CDGS (29.0 ± 0.19 kg/d) which were not different ($P > 0.05$). Results indicate that inclusion of SDGS in the diet of lactating dairy cows should not negatively afect overall animal performance when compared with CDGS.

Key Words: milk fat, milk yield, sorghum distillers grains

T231 Effects of branched-chain amino acid supplementation in lactating dairy cows: A meta-analysis. A. J. Kramer*, H. A. Ramirez-Ramirez, and J. A. D. R.N. Appuhamy, *Iowa State University, Ames, IA.*

Besides Lys and Met, current ration-balancing programs suggest that the supply of branched-chain amino acids (BCAA; Ile, Leu, and Val) may also be limiting in dairy cows. Several studies have investigated the effects of BCAA supplementation, but results have been inconsistent. A meta-analysis was conducted to quantitatively summarize the effects of BCAA supplementation, independent of Met and Lys supplementation, in lactating dairy cows. Twenty-five observations of EAA supplementations (% of MP); milk yield (MlkY, kg/d); and milk protein (MlkP, %), fat (MlkF, %), and lactose (MlkL, %) were retrieved from 14 controlled studies with post-ruminal or jugular infusions of essential AA (EAA), all including BCAA, Met, and Lys. The effects of AA infusions on MlkY, MlkP, MlkF, and MlkL were first quantified via the mean difference (MD, MD = infusion – control), using random-effect models. Relationships between MD and changes in individual BCAA flows (% of MP) were then quantified using mixed-effect models. All analyses were conducted with a multilevel meta-analytic approach accounting for heterogeneity of infusion treatments nested within studies using the metafor package in R. Infusing EAA was related to mean increases in MlkY and MlkP of 1.15 kg/d and 0.14%, respectively ($P < 0.001$). One unit increase in Met (% of MP) supplementation was associated with 1.11 kg/d greater MlkY ($P = 0.047$). None of the BCAA supplementations had a significant

impact on the MD of MlkY, independent of Met. Net energy of lactation (NEL) had a positive effect on MlkP increment ($P = 0.016$) whereas Met or Lys supplementations had no effect on it. When adjusted for NEL, MlkP increments tended to be positively associated with ratios of Leu ($P = 0.068$) or Val ($P = 0.073$) supplementations to Met supplementation. EAA infusions were associated with reductions in MlkL and MlkF [0.07% ($P < 0.001$) and 0.09% ($P = 0.091$), respectively]. Increasing Met supplementation further amplified the MlkF reductions ($P < 0.001$). Individual BCAA may positively affect milk protein concentration when supplementations are adjusted relative to Met.

Key Words: leucine, methionine, milk protein

T232 Establishment of an ileal cannulation technique in preweaning Holstein calves: Effects on growth, health, feed intake and characterization of ileal digesta sampling. I. Ansia*[1], S. Y. Morrison[1], H.-H. Stein[1], C. Brøkner[2], and J. K. Drackley[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL,* [2]*Hamlet Protein A/S, Horsens, Denmark.*

Our aim was to evaluate the effectiveness of inserting a cannula into the distal ileum for use in future digestibility studies in preweaning calves. A T-cannula was placed in the terminal ileum ca. 5 cm anterior to the ileocecal junction in 2 calves at 15 d of age, and 2 paired non-cannulated calves were used as controls. All calves were fed 2X daily a commercial milk replacer (MR) at a rate of 2% (DM) of BW adjusted weekly. Starter was offered ad libitum from wk 6. Body measurements were recorded weekly; intake and health scores were recorded daily. Samples of digesta were collected weekly during 12 h continuously (after a.m. feeding) during 2 consecutive days. Data collected during the 4 wk after post-surgical recovery (starting d 30) were compared using the MIXED procedure in SAS. Initial BW (wk 4; 47.1 ± 2.9 kg; $P = 0.74$), mean BW (81.6 ± 10.3 kg; $P = 0.16$), ADG (894.8 ± 440.8 g/d; $P = 0.20$), MR (1.41 ± 0.27 kg/d; $P = 0.13$) and water intake (0.8 ± 0.6 kg/d; $P = 0.89$), and body dimensions were not affected by cannulation. However, starter intake (0.07 vs 0.21 kg/d; $P = 0.06$), final BW (wk 8; 89.2 vs 94.6 kg; $P = 0.05$) and gain:feed (0.57 vs 0.65; $P = 0.03$) were lower in cannulated calves. There were no effects on health (fecal, nasal, respiratory, and ear scores), rectal temperature (38.3 ± 0.4°C; $P = 0.58$), or in the odds of incurring diarrhea ($P = 0.57$) or being medicated ($P = 0.35$). Flow of digesta samples (46.4 ± 0.04 g/h) tended to increase linearly after feeding, while there was a quadratic effect of time ($P < 0.01$) in digesta pH (Table 1). There were no differences across weeks or among calves in fresh weight (0.72 ± 0.13 kg/w/calf), DM (12.4 ± 1.2%) or pH (7.1 ± 0.6) of digesta. Despite the expected negative effect on BW and gain:feed, this technique permitted sampling of representative ileal digesta while allowing satisfactory growth and health.

Key Words: calves, cannulation

T233 Ankom F57 filter bags limit in vitro undigested NDF and gas production for some materials. N. Schlau*[1], D. R. Mertens[2], K. Taysom[1], and D. Taysom[1], [1]*Dairyland Laboratories Inc, Arcadia, WI,* [2]*Mertens Innovation and Research LLC, Belleville, WI.*

The objective was to determine the effects of the Ankom F57 filter bags (F) on in vitro undigested NDF (uNDF) and gas production (IVGP). Six grass (GR), alfalfa (AL), and corn silage (CS) materials were selected for each type. Standard deviation of aNDFom indicated diversity: 58.4 ± 20.7, 40.0 ± 7.45, and 40.7 ± 12.94% DM, respectively, for GR, AL and CS; starch for CS was 28.4 ± 16.4% DM. Samples were ground to 1mm and 0.5g was weighed as a loose powder (L) into 250 mL fermentation bottles or into F bags, which were weighed to prevent floating in bottles. Blended ruminal fluid from 3 steers fed a high-NDF diet (30% DM) was used in the Goering and Van Soest (1970) in vitro method to measure IVGP using the Ankom RF system. For each material, 3 IVGP bottles were removed to measure uNDF at 12h (uNDF12h) and 2 were used for uNDF120h. For L, residues were extracted in ND using Gooch crucibles. For F, the bags were extracted using an Ankom Fiber Extractor and fermentation liquid was extracted in ND using Gooch crucibles to determine washout. There were 6 IVGP runs, but 2 runs were truncated due to computer malfunctions. Results were analyzed using MiniTab software with material within type as a random effect to account for type and IVGP run being confounded. Statistical models included type, material(type), treatment (L or F), and type × treatment interaction. Gas produced by blanks were evaluated at 12h and peak IVGP and F was larger than L for both ($P < 0.005$) and runs differed ($P < 0.001$). Differences in IVGP were evaluated at 3, 4.5, 6, 9, 12, 18, 24, 36, 48, 72 and 96h. By 3h, IVGP of F was less than L ($P < 0.05$). Maximum $F < L$ difference in IVGP was largest at 24h ($P < 0.001$). There was a trend for IVGP of CS to be greater than AL or GR ($P < 0.10$) at 18h. Precision of repeated measurements for uNDF12h were 1.05 and 0.88 for F and L, respectively, and for uNDF120h were 1.26 and 0.36, respectively. The uNDF12h of F was greater than L ($P < 0.01$) for CS (37.0 > 30.0% DM) and AL (38.9 > 34.4% DM), but not for GR. For uNDF120h, F was greater than L ($P < 0.05$) only for CS (15.2 > 12.9% of DM). Results indicate that F57 bags inhibit IVGP and increase uNDF for some materials.

Key Words: Ankom RF, forages

T234 Low-density lipoprotein ceramide accrual develops with steatosis, hyperlipidemia, and insulin antagonism during the transition from gestation to lactation. A. N. Davis*[1,2], J. E. Rico[1,2], W. A. Myers[1,2], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV.*

In non-ruminants, low-density lipoprotein (LDL) C16:0 and C24:0 ceramide mediates insulin resistance. In dairy cattle, we have established that ceramide accumulates in plasma, liver, and skeletal muscle during the peripartum. Moreover, the induction of hyperlipidemia and steatosis promotes ceramide synthesis in cows. Liver-derived ceramide

Table 1 (Abstr. T232). Characterization of digesta sampling

	Hour after feeding												*P*-value	
Item	1	2	3	4	5	6	7	8	9	10	11	12	Linear	Quad.
Flow, g/h	26.4	36.6	42.0	36.0	43.3	43.4	42.7	47.1	49.0	70.6	71.9	32.2	0.08	0.5
pH	7.7	7.2	7.4	7.4	7.4	7.2	6.7	6.6	6.4	6.8	7.2	7.7	0.09	<0.001

may contribute to impaired insulin action during the peripartum. Therefore, our objective was to characterize changes in lipoprotein ceramide levels in periparturient dairy cows. Twenty-five pregnant, multiparous lean (BCS 3.0 ± 0.2; n = 14) or overconditioned (OVER; BCS 3.9 ± 0.3; n = 11) Holstein cows were enrolled 28 d before expected parturition. Blood was sampled at d −10, 0, and 12, relative to calving. Liver biopsies and the hyperinsulinemic-euglycemic clamp were performed 2 wk before and after parturition. Serum lipoproteins were isolated using liquid chromatography. Sphingolipids were quantified using mass spectrometry. Data were analyzed using a mixed model with repeated measures. Very low density lipoprotein (VLDL) total ceramide levels declined postpartum ($P < 0.01$), whereas LDL total ceramide increased postpartum ($P < 0.01$). C24:0 ceramide represented the most abundant ceramide in VLDL and LDL. OVER had lower VLDL C16:0 ceramide ($P < 0.01$), relative to lean. LDL C16:0 and C24:0 ceramide increased by d 10 ($P < 0.01$), relative to d −12. Prepartum LDL C24:0 ceramide levels were greater in OVER ($P < 0.05$), relative to lean. The ratios of LDL C24:0 ceramide to sphingomyelin were greater in OVER ($P < 0.05$), suggesting the potential involvement of sphingomyelin hydrolysis. The levels of C24:0 ceramide were positively correlated with serum fatty acid levels ($r = 0.41$; $P < 0.01$). Prepartum LDL total and C24:0 ceramide levels were positively correlated with liver lipid content ($r = \sim0.50$; $P < 0.05$). Lastly, postpartum C16:0 LDL ceramide levels were inversely related to systemic insulin action ($r = -0.44$; clamp index; $P = 0.05$). We conclude that LDL ceramide accrual develops with steatosis, hyperlipidemia, and insulin antagonism during the peripartum.

Key Words: ceramide, insulin sensitivity, peripartum

T235 Replacing cereal grains starch with non-forage fiber in diets of dairy cows: A meta-analysis. J. I. Sanchez-Duarte*[1,2] and K. F. Kalscheur[3], [1]*Dairy Science Department, South Dakota State University, Brookings, SD,* [2]*INIFAP, Matamoros, Cohuila, Mexico,* [3]*USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI.*

A meta-analysis was conducted to evaluate the effect of reducing cereal grains starch with non-forage fiber sources (NFFS) on the productive response of lactating dairy cows. Thirty-nine studies were selected that used NFFS to replace a portion of starch from cereal grains. Studies reported cow performance, rumen fermentation, or total-tract nutrient digestibility. Data were analyzed through stepwise regression analysis by the mixed effect models of R using the study as a random effect. The variance explained by the models was evaluated calculating marginal $R^2_{(m)}$ and conditional $R^2_{(c)}$. As dietary starch intake (kg/d) increased, DMI ($Y = -0.08x^2+1.11x+19.15$) and milk fat yield ($Y = -0.005x^2+0.05x+1.07$) responded quadratically; milk yield ($Y = 0.34x+31.87$), milk protein concentration ($Y = 0.02x+3.01$), and milk lactose yield ($Y = 0.02x+1.46$) increased linearly; and milk fat concentration decreased linearly ($Y = -0.05x+3.94$). DMI was explained by the quadratic effect of DM digestibility ($P = 0.04$) as starch intake increased. Increases of milk yield and milk protein concentration was explained by the linear increase of rumen VFA concentrations (propionate, acetate to propionate ratio, isobutyrate, isovalerate, and valerate; $P < 0.05$) and CP digestibility ($P = 0.01$) in response to the increased starch intake. Decreased milk fat concentration is an effect of the reduction in the rumen acetate concentration ($P < 0.001$) and NDF digestibility ($P < 0.001$). Values of $R^2_{(m)}$ and $R^2_{(c)}$ indicated a better goodness-of-fit for significant models. Residuals and Q-Q plots of the models were symmetrical and their errors were normally distributed. Additionally, intake of DM, CP and NDF, as well as DIM contributed to the variation of the models. It is important to highlight that diets formulated with NFFS (25.9 ± 11.5% on a DM basis) had lower DMI, milk yield, and milk protein concentration than cows fed diets high in cereal grains (27.5 ± 11.5% as DM basis). Cows fed diets higher in NFFS may result in greater milk fat percentage. Therefore, all those factors should be taken into account when NFFS are used to reduce starch from cereal grains in lactating dairy cow diets.

Key Words: meta-analysis, starch, milk

T236 A new system of calcium and phosphorus requirements for lactating dairy cows. S. R. Soares, H. M. Silva, A. B. Donadia, R. N. Torres, D. C. Moura, K. C. Alessi, L. Menegazzo, M. L. Marques, I. P. Martins, M. R. C. Oliveira, and A. S. Oliveira*, *Dairy Cattle Research Lab, Universidade Federal de Mato Grosso-Sinop, Sinop, Mato Grosso, Brazil.*

A new system of Ca and P requirements for lactating dairy cows was developed from meta-analysis of 53 peer-reviewed papers, with 72 minerals balance trials and 276 treatments means. The data sets represented a wide range of dietary (258 to 1000 g forage/kg DM; 121 to 258 g CP/kg DM; 260 to 496 g NDF/kg DM; 4.0 to 22.5 g Ca/kg DM; and 1.5 to 6.7 g P/kg DM) and animal characteristics (316 to 754 kg BW; 4.5 to 52.8 kg milk/d; and 8.7 to 29.0 kg DMI/d) of 3 genetic groups (66.8% Holstein; 17.7% Jersey; and other breeds 14.6%). Endogenous fecal excretion (EFE) and endogenous urinary excretion (EUE) were estimated as intercept of the linear mixed equation between mineral intake and excretion, using study as random effect. We proposed the use of the metabolizable coefficient (MC) concept to calculate dietary requirement, that represents the proportion of mineral intake that is not excreted in feces and urine, discounting endogenous excretions. Genetic group affected ($P < 0.05$) Ca and P concentration in milk (mean ± 95% confidence interval): Holstein = 1.17 ± 0.07 g Ca/kg and 0.89 ± 0.02 g P/kg; Jersey = 1.38 ± 0.10 g Ca/kg and 1.00 ± 0.04 g P/kg; other breeds = 1.08 ± 0.07 g Ca/kg and 0.87 ± 0.05 g P/kg. Genetic group did not affect ($P > 0.05$) fecal or urinary Ca and P excretions per kg DMI. Therefore, we fitted overall equations: Ca fecal (g/kg DMI) = 1.83 (±0.59; $P < 0.01$) + 0.032 (±0.032; $P < 0.01$) × Ca intake (g/d), $R^2 = 0.66$, n = 97; Ca urinary (g/kg DMI) = 0.13 (±0.03; $P < 0.01$) + 0.0001 (±0.009; $P = 0.68$) × Ca intake (g/d), $R^2 = 0.33$, n = 81; P fecal (g/kg DM) = 0.93 (±0.21; $P < 0.01$) + 0.016 (±0.003; $P < 0.01$) × P intake (g/d), $R^2 = 0.47$, n = 211; P urinary (g/kg DM) = 0.01 (±0.007; $P < 0.01$) + 0.0003 (±0.000; $P < 0.01$) × P intake (g/d), $R^2 = 0.10$, n = 126. Endogenous fecal excretions of Ca and P are estimated in 1.83 ± 0.59 g/kg DMI and 0.93 ± 0.21 g/kg DMI, and EUE in 0.13 ± 0.03 g/kg DMI and 0.01 ± 0.007 g/kg DMI. Genetic group did not affect P-MC, but Holstein had lower ($P < 0.05$) Ca-MC than Jersey (0.74 ± 0.03 versus 0.82 ± 0.01). Our new system reduces Ca and P dietary recommendations to non-pregnant dairy cows (10 to 50 kg milk/d) in 29% and 15% compared with the National Research Council dairy model (2001).

Key Words: modeling, maintenance, nutrition system

T237 Effects of partial replacement of corn silage with whole-plant soybean silage on performance of dairy cows. L. G. Ghizzi, T. A. Del Valle, J. A. Marques, E. M. de C. Zilio, L. S. Gheller, T. B. de P. Silva, L. Y. Sakamoto, A. N. Tenório, M. S. da S. Dias, J. M. Souza, G. G. Silva, N. T. Scognamiglio, and F. P. Rennó*, *University of São Paulo, São Paulo, São Paulo, Brazil.*

The objective of the study was to evaluate the effects of increase levels of whole-plant soybean silage (SS) replacing corn silage in diet on DMI, milk yield and composition, food efficiency and FCM of dairy cows. Twenty-four Holstein cows were used in a replicated 4 × 4 Latin square design experiment, with 21-d periods (last 7 d for sampling). Treatment

Table 1 (Abstr. T237).

Item	Treatments				SEM	*P*-value	
	SS-0	SS-16	SS-33	SS-50		Linear	Quadratic
DMI, kg/d	23.6	23.6	23.1	22.9	0.351	0.020	0.505
Milk yield, kg/d	33.0	32.3	31.2	31.6	0.533	<0.001	0.115
Feed efficiency (milk yield/DMI)	1.40	1.37	1.37	1.38	0.023	0.476	0.162
FCM, kg/d	32.3	31.1	30.5	30.3	0.506	0.002	0.236
Fat, kg/d	1.10	1.05	1.04	1.03	0.191	0.012	0.222
Protein, kg/d	1.08	1.05	1.02	1.02	0.017	<0.001	0.136
Lactose, kg/d	1.62	1.57	1.52	1.53	0.025	<0.001	0.140
Fat, %	3.38	3.29	3.37	3.30	0.048	0.382	0.737
Protein, %	3.29	3.27	3.26	3.25	0.010	0.003	0.893
Lactose, %	4.93	4.90	4.90	4.86	0.015	<0.001	0.728

sequences consisted of a control diet (SS-0; with no SS), and SS replacing corn silage at rates of 16.7% (SS-16), 33.3% (SS-33), or 50.0% (SS-50) on diet DM basis. Feed intake and milk yield were recorded daily. Cows were milked twice daily and milk samples were collected in 6 consecutive milking. Milk samples were analyzed for fat, true protein and lactose using a ultrasonic milk analyzer (Lactoscan, MCC, Milktronic Company, Nova Zagora, Bulgaria). Data were analyzed using the MIXED procedure of SAS 9.3. Whole-plant soybean silage linearly decreased DMI, milk yield, FCM, milk solids yield, as well as protein and lactose content (Table 1). The replacement of corn silage by whole-plant soybean silage decrease de milk yield and composition, despite did not alter the fat content and food efficiency of cows.

Key Words: leguminous silage, milk yield

T238 Predicting the concentration and yield of milk fatty acids from diet nutrient composition in dairy cows. J. de Souza*[1], N. St-Pierre[2], and A. L. Lock[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Perdue Agribusiness, Salisbury, MD.*

We determined milk fatty acid (FA) concentration and yield responses in relation to diet nutrient composition in dairy cows. Our analysis used individual observations (n = 1202) from 212 lactating Holsteins cows from 14 studies. Milk FA were classified according to their source: < 16-carbon FA from de novo synthesis in the mammary gland and > 16-carbon preformed FA originating from extraction from plasma. Mixed source FA (16-carbon FA) originate from de novo synthesis in the mammary gland and extraction from plasma. Data were analyzed using a mixed model multiple regression, including diet nutrient composition (% of DM of starch, NDF, forage NDF, 16:0, 18:0, 18:1, 18:2n6, 18:3n3 and total FA) and DMI and DMI^2 (kg/d), and quadratic terms as fixed effects. Cow, block, period, and study were included as random effects. Variables were removed from the model using backward elimination with significance criteria of $P < 0.05$. The best fitting prediction equations for the concentration and yield of milk FA are presented in Table 1. Our results demonstrate that DMI is important for predicting the concentration and yield of de novo and preformed milk FA. The dietary concentration of 18:2n6 is negatively associated with the concentration and yield of de novo and mixed FA, while 16:0 is positively associated with the concentration and yield of mixed FA.

Key Words: chemical composition, fatty acids, milk fat

Table 1 (Abstr. T238). Best fit equations for the concentration and yield of milk FA in relation to diet chemical composition

Variable	Concentration, g/100g FA			Yield, g/d		
	De novo	Mixed	Preformed	De novo	Mixed	Preformed
Intercept	39.8 ± 4.265	36.2 ± 11.900	33.8 ± 2.674	144 ± 57.645	−66.7 ± 13.111	361 ± 60.703
DMI (kg/d)	0.55 ± 0.111	—	−0.66 ± 0.153	19.2 ± 3.455	8.02 ± 0.924	4.99 ± 1.208
DMI × DMI	−0.01 ± 0.00197	—	0.008 ± 0.0027	−0.21 ± 0.0611	—	—
Starch (%)	0.36 ± 0.0481	−0.74 ± 0.194	—	—	−3.73 ± 1.618	—
NDF (%)	—	−0.60 ± 0.231	0.25 ± 0.0329	−1.51 ± 0.717	—	3.39 ± 1.266
Forage NDF (%)	−1.43 ± 0.240	2.05 ± 0.308	—	—	27.4 ± 7.858	—
Total FA (%)	−1.48 ± 0.108	—	−1.41 ± 0.139	—	—	—
16:0 (%)	—	2.93 ± 0.165	—	—	64.1 ± 4.018	—
18:0 (%)	1.43 ± 0.200	—	1.74 ± 0.273	—	—	—
18:1 (%)	2.02 ± 0.687	−2.93 ± 0.735	3.10 ± 0.938	—	−79.9 ± 24.495	—
18:2n6 (%)	−1.84 ± 0.403	−3.08 ± 0.507	8.65 ± 0.548	−56.1 ± 11.351	−54.4 ± 17.556	—
18:3n3 (%)	—	—	—	−25.5 ± 12.795	—	—
RMSE	1.31	1.52	1.79	22.5	50.1	31.5
R^2	0.91	0.86	0.91	0.96	0.88	0.91

T239 Fatty liver develops with nonuniform changes in hepatic choline-containing sphingomyelins and phosphatidylcholines. S. S. Samii[1,2], Y. Zang[2], W. A. Myers[1,2], E. Grilli[3], and J. W. McFadden*[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV,* [3]*University of Bologna, Bologna, Italy.*

The development of hepatic steatosis involves the enhanced hepatic uptake of fatty acids which induces sphingomyelin (SM) synthase activity. Increased hepatic SM levels may limit the availability of choline for the synthesis of phosphatidylcholine (PC), a glycerophospholipid required for liver triacylglycerol (TAG) secretion. Alternatively, the presence of inflammation provokes acid sphingomyelinase (i.e., sphingomyelin degradation) which is secondary to PC degradation and the subsequent synthesis of diacylglycerol (DAG). Our objective was to determine how bovine liver phospholipids and acylglycerols change during the progression of hepatic steatosis. Thirty multiparous Holstein cows were enrolled −28 d prepartum and fed diets formulated to meet or exceed requirements. Liver tissue was biopsied at d −28, 5, and 14, relative to parturition. Untargeted lipidomics was performed using quadrupole time-of-flight mass spectrometry. Multivariate analysis of normalized, auto-scaled lipidomic data included ANOVA and Pearson correlation coefficient procedures. Total hepatic lipid content increased by d 14 postpartum, relative to d −28 (9 versus 4% lipid; $P < 0.05$). Mass spectrometry monitored 30 SM, 35 PC, 76 TAG, and 24 DAG. Uniform increases in 19 DAG and 74 TAG were observed during the transition from gestation to lactation ($P < 0.05$). Moreover, total DAG was strongly correlated with liver lipid content and total TAG ($r = 0.74$ and 0.97, respectively; FDR <0.05). Nonuniform changes in hepatic SM and PC were observed. A total of 17 SM (e.g., SM 42:0 and 44:2) and 13 PC (e.g., PC 34:2 and 36:1) increased with time ($P < 0.05$), whereas 10 SM (e.g., SM 48:3 and 34:2) and 16 PC (e.g., PC 40:4 and 42:6) decreased with time ($P < 0.05$). Liver lipid content was positively correlated with 17 SM and 10 PC (FDR <0.05) and negatively correlated with 10 SM and 18 PC (FDR <0.05). The majority of DAG were inversely related to polyunsaturated PC (e.g., DAG 32:2 versus PC 42:5; $r = -0.84$, FDR <0.05). We conclude that bovine steatosis develops with a uniform accrual in hepatic DAG and TAG, as well as nonuniform changes in liver SM and PC.

Key Words: phosphatidylcholine, sphingomyelin, steatosis

T240 Methodological and feed factors affecting measurement of protein A, B, and C fractions, degradation rate, and intestinal digestibility of rumen-undegraded protein. D. M. Liebe*[1], J. L. Firkins[2], H. Tran[3], P. J. Kononoff[3], and R. R. White[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*The Ohio State University, Columbus, OH,* [3]*University of Nebraska, Lincoln, NE.*

When formulating dairy cow rations, characterization of protein in feeds requires estimation of protein degradation in both the rumen and intestine. The objective of this work was to evaluate experimental and feed related factors that affect characterization, using in situ, in vitro, or mobile bag techniques, of 0-h washout (A), potentially degradable (B), and undegradable (C) protein fractions, protein degradation rate (K_d), and digestibility of rumen undegradable protein (dRUP). Data sets of 136 studies on A, B, C, and K_d and 113 studies on dRUP were amassed from the literature. Mixed-effect linear models were used to relate these variables to methodological and feed factors while accounting for random differences among studies. Predictions of A, B, and C protein fractions were significantly ($P < 0.05$) influenced by CP and NDF interactions with sample grind size, bag pore size, incubation time, bag area, and sample-to-bag area ratio. For example, a 20% decrease in CP of a theoretical feed sample would increase A fraction prediction by 16.7%, but only 8.69% with bag pore size −1 SD below the mean. A shift in measurement method halves the predicted A fraction of the same feed. Similarly, reported K_d values were significantly ($P < 0.05$) influenced by CP interactions with sample grind size, bag area, and sample-to-bag area ratio. Feed variables and measurement variables influencing protein digestibility measures suggest that these analytical factors are likely associated with variance among differing methodologies and within unique samples of the same feed. When predicting dRUP, pepsin-acid incubation time and use of mobile bag method produced significantly different ($P < 0.05$) estimates compared with the traditional in vitro 3-step method. The use of mobile bag resulted in a 12% (±3.1%) higher estimate of dRUP compared with the in situ technique. In 618 and 977 samples, sample variation to sample mean ratio for ADF and pepsin-acid incubation time was 63% and 58%, respectively. Variation in feedstuff content and lack of standardization of methods used to measure protein disappearance lead to a lack of robustness in the measurements commonly employed.

Key Words: ruminal degradability of protein, in situ, in vitro

T241 Effect of betaine supplementation on rumen fermentation measures in Holstein dairy cows. H.-C. Hung*[1], C.-Y. Tsai[1], G. Chibisa[1], M. Chahine[1,2], M. McGuire[1], and P. Rezamand[1], [1]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*Twin Falls Research and Extension Center, University of Idaho, Twin Falls, Twin Falls, ID.*

Betaine, a trimethylglycine, is a natural ingredient of wheat and sugar beets. In the body, choline oxidation can lead to the formation of betaine. Betaine acts as an organic osmolyte and a methyl donor. It may also be fermented in the rumen. The objective of this experiment was to determine the effect of supplemental betaine on ruminal fermentation in dairy cows. The experiment included 3 rumen-cannulated Holstein dairy cows in a 3 × 3 Latin square design with 3 periods, 8 d each (1 d for in situ bags introduction and rumen fluid collection, 7 d wash-out), and 3 treatments of betaine at 0, 100, and 200 g placed into each Dacron bag and double sealed. Rumen fluid was collected and large mesh bags containing Dacron bags were introduced into the rumen at different time points (0, 1, 1.5, 2, 3, 6, 12, and 24 h). At each time point, 3 bags of each treatment were prepared. Dry and organic matter degradation was determined on dried, post in situ sample bags. Rumen fluid samples from different time points were analyzed for volatile fatty acid profile using gas chromatography. Data were analyzed using the Proc Mixed of SAS with significance declared at $P \leq 0.05$. Organic matter degraded greater ($P < 0.0001$) when betaine retention time was longer than 12 h. There were increases in the ratio of acetate to propionate with betaine supplementation (1.7, 1.9, and 1.8 ± 0.03, for 0, 100, and 200 g betaine, respectively; $P < 0.0001$) and molar proportion of isovalerate with betaine supplementation ($P < 0.005$). Significant decreases in pH (6.4, 6.3, and 6.2 for 0, 100, and 200 g betaine, respectively) and molar proportion of propionate, butyrate, and isobutyrate with betaine supplementation were observed ($P < 0.001$ for all).There were no detectable changes in the molar proportion of acetate (0.42, 0.43, and 0.42 ± 0.006) or valerate (0.05, 0.04, and 0.05 ± 0.005). Overall, changes in volatile fatty acid profile observed suggest the possibility of an effect of betaine supplementation on rumen microbial populations, which warrants further investigation.

Key Words: betaine, rumen fermentation, volatile fatty acid

T242 In situ ruminal dry matter and fiber degradability of distillers dried grains with solubles with varying fat content by lactating dairy cows. K. C. Krogstad[1], J. L. Anderson*[1], J. S. Osorio[1], and K. J. Herrick[2], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*POET Nutrition, Sioux Fall, SD.*

Our objective was to determine the effect of fat content in dried distillers grains with solubles (DDGS) on the rumen degradability of dry matter (DM) and neutral detergent fiber (NDF). A secondary objective was to evaluate the effect of types of bags used for ruminal incubation on DM digestibility. Three ruminally cannulated primiparous Holstein cows (33.2 ± 3.41 milk kg/d; 19.5 ± 1.40 dry matter intake kg/d; 601 ± 17.2 kg body weight) were used to determine the rumen degradable dry matter (RDDM) and rumen degradable neutral detergent fiber (RDNDF) from 6 different distiller's grains (DG1 to DG6) samples from different sources that varied in fat content. A soybean meal (SBM) sample was included as a control. The feeds were incubated in the rumen for 0, 2, 4, 8, 16, 24, 48, 72, and 120 h using F57 fiber filter bags with 25 µm porosity and Dacron bags with 50 ± 10 µm porosity from Ankom Technology. Ruminal rate of passage (Kp) of concentrates averaged 6.38 ± 0.21%/h among the 3 cows. Rumen degradation constants for DM and NDF were estimated using the NLIN procedures in SAS 9.4 and means were compared using PDIFF. The RDDM differed among the DDGS with DG1, DG4 and DG5 having greater RDDM and lower fat contents. The Dacron bags had more RDDM than the F57 filter bags. The RDNDF and NDF rate of digestibility (NDF K_d) were similar among the DDGS and all were less than SBM. Therefore, differences in RDDM in DDGS that vary in fat content are thought to be more related to rumen degradability of other nutrients such as protein rather than NDF.

Key Words: dried distillers grains with solubles, fiber, rumen degradability

T243 In vitro comparison of *Silphium perfoliatum* varieties and corn silage. S. W. Gee*[1], L. McNea[2], B. Gilroyed[2], and A. J. Carpenter[1], [1]*Deparment of Animal Biosciences, University of Guelph, Ridgetown, ON, Canada,* [2]*School of Environmental Sciences, University of Guelph, Ridgetown, ON, Canada.*

With rising feed prices and concern over competition between humans and animals for land utilization, forages that can be grown with lower resource utilization provide an opportunity for use in ruminant rations. One such potential crop is cup plant (*Silphium perfoliatum*), a perennial flowering plant that has low agronomic inputs and can be grown on marginal land. The objective of this experiment was to compare cup plant varieties to corn silage (CS) in vitro to isolate genotypes that may be pursued as forages for ruminants. Twelve genotypes of cup plant (varieties 1–12) were tested in batch culture with CS as a control. Substrates were dried at 50°C for 48 h and ground to 2 mm particle size. Strained rumen fluid was mixed with McDougall's buffer in a 2:1 ratio of buffer to rumen fluid. This inoculum (50 mL) was added to 125-mL serum bottles containing substrate (0.5 g) and one bottle containing inoculum alone. Sealed bottles were incubated in a shaking water bath at 39°C for 24 h. At 5, 10, and 24 h of incubation, gas production was measured by water displacement. Contents were transferred into 50-mL containers and dried at 100°C for 48-h; remaining dry matter was used to calculate dry matter disappearance (DMD). Measurements were replicated during 4 24-h periods. Means were compared against CS using the Dunnett test in PROC GLM of SAS 9.4. Differences were declared significant at $P \leq 0.05$, and tendencies were noted at $P < 0.10$. No differences were detected between any cup plant varieties and CS for gas production ($P \geq 0.70$) or DMD ($P \geq 0.65$). Three cup plant varieties were similar to CS in pH at 24 h ($P \geq 0.26$; pH = 6.38, 6.48, 6.5, and 6.49 ± 0.042 for CS and varieties 7, 8, and 11, respectively). Four cup plant varieties (variety 4, 9, 10, and 12) tended to differ in pH from CS ($0.1 \geq P > 0.05$; pH = 6.54, 6.54, 6.54, and 6.54 ± 0.042, respectively). The remaining 5 varieties had different pH from CS, ranging from 6.54 – 6.60 ($P \leq 0.05$; SEM = 0.042). These results indicate that varieties 7, 8, and 11 may be more suitable candidates than the other varieties investigated for future research examining the inclusion of cup plant in ruminant rations.

Key Words: cup plant, novel forage, sustainability

T244 In vitro evaluation of rumen-protected methionine sources. H. L. Diaz*[1], J. Albrecht[1], J. Linn[1], C. Soderholm[1], M. Van Amburgh[2], and D. Ross[2], [1]*Milk Specialties Global, Eden Prairie, MN,* [2]*Cornell University, Ithaca, NY.*

The objective was to evaluate the rumen stability (RS; % of CP) and TTCPDig (% of CP) of rumen-protected Met (EBMet) and 2 commercial Met sources using in vitro techniques. EBMet is a 34% DL Met coated with saturated FA. Statistical analysis was done using JMP® and means compared using Students *t*-test with significance at $P < 0.05$. Nitrogen content of the residues was determined by Kjeldahl. Study 1 assessed RS in an IV system. EBMet samples were incubated in a Daisy incubator in Dacron bags with rumen buffer and rumen fluid for 16 h and RS content of EBMet was determined to be 81.5% (SEM = 5.37). Study

Table 1 (Abstr. T242).

Item	DG1	DG2	DG3	DG4	DG5	DG6	SBM	SEM
DM, %	92.1	93.2	92.9	91.9	91.5	92.0	90.4	—
CP, % DM	30.9	30.0	28.7	31.2	29.9	32.0	22.2	—
RUP,[1] % CP	58.1	71.5	71.6	63.8	65.9	65.2	36.6	—
NDF, % DM	29.3	34.6	34.5	31.7	28.7	29.2	9.4	—
Ether extract, % DM	7.62	9.52	10.48	5.63	5.79	4.97	0.85	—
Dacron RDDM, % DM	58.4^{b}	49.7^{d}	46.4^{e}	59.1^{b}	59.9^{b}	55.1^{c}	66.0^{a}	1.90
F57 RDDM,% DM	45.1^{ab}	39.0^{bc}	34.1^{c}	47.8^{a}	44.5^{ab}	41.3^{b}	49.3^{a}	1.69
F57 RDNDF, % NDF	20.5^{b}	20.9^{b}	20.4^{b}	19.5^{b}	19.4^{b}	18.4^{b}	32.9^{a}	2.27
NDF K_d, %/h	1.81^{b}	1.97^{b}	1.86^{b}	1.72^{b}	1.66^{b}	1.49^{b}	4.14^{a}	0.22

$^{a–e}$Values with unlike superscripts differ by $P < 0.05$ using PDIFF.

[1]Ross method used for RUP estimation.

2 determined RS of EBMet samples, a pH sensitive polymer coated 76% DL Met product (Product A) and a rumen-protected 85% DL-Met (Product B). Samples were placed in flasks with 40 mL of rumen buffer and 10 mL of rumen fluid. Flasks were incubated at 39°C for 16 h under constant CO_2, then filtered with a 1.5 µm glass filter and N content of the residue was determined. The RS for EBMet, Product A and Product B was 88.6, 91.5 and 61.2% ($P < 0.1$, SEM = 7.14). Study 3 was conducted using an IV rumen + intestinal digestibility (ID) assay to assess TTCPDig in EBMet, Product A and Product B. Samples were incubated in rumen fluid + buffer and after 16 h of IV incubation acidified with 3M HCL to bring the pH to 2 and incubated for 1 h after addition of 2 mL of pepsin. Samples were neutralized with 2 M NaOH and an enzyme mix containing trypsin, chymotrypsin, lipase, amylase, and bile was added and incubated for 24 h at 39°C. Samples were filtered on a 1.5 µm glass filter and N content of the residue was determined. The EBMet, Product A and Product B showed a TTCPDig of 78.6, 91.7 and 50.9% ($P < 0.001$, SEM = 3.72). Study 4 was conducted to determine TTCPDig as in study 3 but in a different lab. The TTCPD differed among all 3 Met products, EBMet, Product A and Product B were 93.3, 85.9 and 79.7% ($P < 0.007$, SEM = 1.07), respectively. Methods were effective in determining differences between products with different types of Met protection. Data suggest EBMet is a rumen stable Met product with high ID as shown IV by RS and TTCPDig.

Key Words: methionine, amino acid, in vitro

T245 Effect of crude glycerin on milk yield and composition in early lactation Gyr × Holstein dairy cows. A. Suarez-Ariza[1], J. Pardo-Guzman[1], T. Garcia-Diaz[2], C. F. -De Rubio[1], C. Ortiz-Riobo[1], D. Paez-Bernal[1], and R. Castaneda-Serrano*[1], [1]*Universidad del Tolima, Ibague, Tolima, Colombia,* [2]*Univesidade Estadual de Maringa, Maringa, Parana, Brazil.*

The objective of this study was to evaluate the effect of crude glycerin (CG) supplementation on milk yield and composition in early lactation Gyr × Holstein dairy cows. Multiparous Gyr x Holstein dairy cows (n = 40), BW = 550 ± 25 kg were used during 90 d of lactation. Treatments were control (without CG), LG, MG, and HG with 500, 1000, and 1500 g CG per cow per day, respectively. CG contained 0.86 g/g glycerol. Cows were kept in a rotating pasture of *Brachiaria decumbens* and supplemented during milking twice daily (0600 and 15:00 h) with CG and concentrate, after of morning milking, cows was feeding with corn silage ad libitum during 3 h until afternoon milking. Then, cows were conducted into de paddocks with the *Brachiaria decumbens* pasture. The diet was formulated based on NRC (2001) recommendations for a 550-kg cow producing 20 kg/d of milk containing 34 g/kg of milk fat and 32 g/kg crude protein. CG in the current study was used as a glucogenic supplement to provide additional glucogenic substrates and no adjustment in NE_L concentration was made in the control diet. Milk yield were recorded daily from 4 d until 90 d of lactation. To composition analysis milk samples from each cow were collected each 30 d from each milking. It was observed that as the level of supplementation of CG increased, milk production increased (Linear $P < 0.05$). However, there was no effect ($P > 0.05$) of treatment on fat, protein, total solids and NUL. In conclusion the supplementation with CG in dairy cows Gyr x Holstein improves milk production and decreases lactose levels.

Table 1 (Abstr. T245). Effect of crude glycerin (CG) supplementation on milk yield and composition in Gyr × Holstein dairy cows

	Treatment[1]					*P*-value	
Item	C	LG	MG	HG	SEM	L	Q
Milk yield, L/d	14.20	16.08	15.95	16.73	0.12	0.001	0.098
Fat, %	2.92	3.04	2.63	2.55	0.30	0.624	0.256
Protein, %	2.91	2.82	2.97	3.00	0.07	0.344	0.988
Total solids, %	11.72	11.52	11.45	11.31	0.32	0.827	0.555
Lactose, %	5.08	4.88	5.05	4.9	0.04	0.021	0.001
NUL, mg/dL	14.25	14.20	9.98	12.33	1.31	0.123	0.201

[1]C = Control; LG = 500 g/cow per day CG; MG = 1,000 g/cow per day CG; HG = 1,500 g/cow per day CG.

Key Words: glycerol, gyrolando, supplementation in grazing

T246 An evaluation of the Molly cow model predictions of ruminal metabolism and nutrient digestion for dairy and beef diets. M. Li*, R. R. White, and M. D. Hanigan, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

Model evaluation, as a critical process of model advancement, is necessary to identify adequacy and consistency of model predictions. The objectives of this study were 1) to evaluate the accuracy of the Molly cow model predictions of ruminal metabolism and nutrient digestion when simulating dairy and beef cattle diets; and 2) to identify deficiencies in the representations of biology that could be used to direct further model improvement. A total of 229 studies (n = 938 treatments) including dairy and beef cattle data, published from 1972 through 2016, were collected from the literature. Root mean square errors (RMSE) and concordance correlation coefficients (CCC) were calculated to assess model accuracy and precision. Ruminal pH was very poorly represented in the model with a RMSE of 4.6% and a CCC of −0.004. Although VFA concentrations had negligible mean and slope bias, the CCC was 0.28 implying that further modifications with respect to VFA production and absorption are required to improve model precision. The RMSE was greater than 50% for ruminal ammonia and blood urea-N concentrations with high proportions of error as slope bias indicating that mechanisms driving ruminal urea N recycling were not properly simulated in the model. Only slight mean and slope bias were exhibited for ruminal outflow of NDF, starch, lipid, total N, and non-ammonia N, and for fecal output of protein, NDF, lipid, and starch, indicating the mechanisms encoded in the model relative to ruminal and total-tract nutrient digestion were properly represented. Except for ruminal outflow of non-ammonia, non-microbial N, all variables related to ruminal metabolism and nutrient digestion were more accurately predicted for dairy diets than for beef diets. Overall, ruminal pH was poorly simulated and contributed to problems in ruminal nutrient degradation and VFA production predictions. Residual analyses suggest ruminal ammonia concentrations need to be considered in the ruminal pH equation, and therefore the inaccuracies in predicting ruminal urea N recycling must also be addressed. These modifications to model structure will likely improve model performance across a wider array of dietary inputs and cattle categories.

Key Words: digestion, model evaluation, ruminal metabolism

T247 Lactation performance and feed efficiency of dairy cows fed freshly ensiled corn silage-based diets with exogenous amylase and protease. L. K. Shearer[1], J. L. Anderson*[1], J. S. Osorio[1], and K. Mjoun[2], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Alltech Inc., Brookings, SD.*

The objective of this study was to examine the effects of feeding exogenous amylolytic and proteolytic enzymes on lactation performance of dairy cows fed freshly-ensiled corn silage-based diets. At the start of feeding, corn silage had been ensiled for 48d. It was hypothesized that the addition of amylolytic and proteolytic enzymes would improve nutrient utilization and consequently lactation performance. Thirty-six Holstein cows (18 primiparous, 18 multiparous; 132 ± 48 DIM) were used in a 9-wk randomized complete block design study. Cows were blocked by milk yield, DIM, parity, and body weight. Treatments were a 40% (DM basis) corn silage TMR with (1) no enzymes (CON), (2) amylolytic enzymes (AMY; 10 g/hd/d), and (3) amylolytic and proteolytic enzymes (AMYP; 10 g/hd/d +15 g/hd/d). Cows were housed in a freestall barn and fed with a Calan Broadbent system to determine daily individual intakes. Cows were milked 2×/d with weights recorded. Milk samples were collected for compositional analysis every 3 wk. Data were analyzed using MIXED procedure of SAS 9.4 with repeated measures and means compared using Tukey's test. Significance was declared at $P \leq 0.05$. There was a treatment × week interaction ($P < 0.01$) for DMI and milk yield, but no clear trends were observed. Milk yield averaged 33.6, 33.8, 33.2 kg/d; SEM = 1.21 for CON, AMY, AMYP, respectively. The dry matter intake averaged 25.4, 25.2, 24.7 kg/d; SEM = 0.73. No differences ($P \geq 0.05$) were observed in feed efficiency (milk yield/DMI; 1.38, 1.38, 1.36; SEM = 0.05), yields of milk fat (1.00, 1.06, 0.93 kg/d; SEM = 0.05), protein (1.61, 1.62, 1.57 kg/d, SEM = 0.05), lactose (1.57, 16.5, 1.57 kg/d; SEM = 0.08), and SNF (2.91, 3.08, 2.88 kg/d; SEM = 0.13). There was a treatment × wk interaction ($P < 0.05$) for MUN (12.99, 12.64, 12.20 mg/dL; SEM = 0.62) with AMY and AMYP being slightly less in wk 9 compared with CON. Body weights and condition scores were similar among treatment with no treatment × week interactions. Under the conditions of this study, the addition of exogenous enzymes maintained lactation performance compared with CON.

Key Words: amylase, protease, lactation performance

T248 Effect of a prebiotic and essential oil based feed additive on the health and performance of dairy calves housed on Central Texas calf ranches. D. M. Paulus Compart*[1], C. M. Underwood[2], B. L. Trainer[2], P. Karnezos[1], and T. R. Liska[1], [1]*PMI Nutritional Additives, Arden Hills, MN,* [2]*Purina Animal Nutrition, Arden Hills, MN.*

A meta-analysis was conducted on 405 Holstein, Jersey, and Jersey-Holstein crossbred calves from 5 studies across 4 Central Texas calf ranches to evaluate the efficacy of a prebiotic and essential oil based feed additive on calf performance, morbidity, and mortality. Two hundred and one calves were fed a control diet (CON) based on each farm's standard operating procedure. The control diet consisted of 2 to 3 quarts of whole milk offered 2–3 times daily and the addition of supplements or antibiotics in the milk varied by location. Two hundred and four calves were fed 2 to 3 quarts of whole milk offered 2 yo 3 times daily with the addition of Surmount Feed Additive (PMI Nutritional Additives, Arden Hills, MN) added at a rate of 10 g per head per day (TRT). Data collected in each of the 5 studies included girth length, hip height, body weight, fecal scores, nasal scores, eye scores, and mortality. Total number of calves treated for health issues and total number of health treatments per calf (treatment days) were recorded in 4 of the studies. Data collection period lengths varied by farm and ranged from 4 to 10 weeks with initial data being collected upon calf arrival at its respective ranch at about 3 d of age. The MIXED procedure, FREQ procedure, and a Z-test of SAS 9.4 (SAS Institute Inc., Cary, NC) were used to identify differences between treatment groups. Performance values did not differ between calves on the CON versus TRT diet. No signifcant day by treatment effects were found for any performance parameters. Number of calves treated for health issues did not differ between treatment groups ($P = 0.56$), nor did number of treatment days ($P = 0.58$). Fecal and eye score values were treatment dependent ($P < 0.01$) with TRT calves having improved scores relative to CON calves. Calf mortality tended to be lower (P-value = 0.07) for TRT calves (10.2 and 5.9% for CON and TRT respectively). Overall, Surmount Feed Additive may be an effective tool for reducing morbidity and mortality on commercial calf ranches.

Key Words: feed additive, prebiotic, essential oil

T249 Effects of amino acids on ruminal gas production and fermentation in in vitro batch culture. X. Chen[1], S. E. Räisänen*[2], C. M. M. R. Martins[3], K. Nedelkov[4], and A. N. Hristov[2], [1]*Lanzhou University, Lanzhou, Gansu, China,* [2]*The Pennsylvania State University, University Park, PA,* [3]*University of São Paulo, Pirassununga, Brazil,* [4]*Trakia University, Stara Zagora, Bulgaria.*

The effect of 7 individual AA (Leu, Met, Phe, Trp, Val, His, and Lys) on ruminal gas production and fermentation was investigated in a batch culture in vitro experiment. All AA were examined at 2 levels: 20 and 60 mg/L incubation medium. Two separate incubations were conducted for 6 (group 1) or 12 h (group 2). Rumen inoculum to buffer ratio was 1: 2. Gas pressure was recorded and a gas sample analyzed for concentration every 3 h for both groups. Compared with blank (no AA), gas production was lower ($P \leq 0.03$) for Phe, Trp, Val, His and Lys (ranging from 48.2 to 57.6 mL vs. 60.7 mL). Gas production was not affected by treatment in group 2 ($P \geq 0.30$). Compared with blank (6.38%), all 7 AA had lower methane concentration in the gas phase (ranging from 4.61 to 5.66%, $P \leq 0.02$) in group 1. Methane concentration was also lower (ranging from 4.36 to 4.66% vs. 4.97% for the blank, $P \leq 0.03$) in group 2, excepted for His (4.76%) and Met (5.12%). Total VFA concentration did not differ between treatments ($P = 0.45$). In group 1, isobutyrate concentration was increased by Phe (0.32 µmol/mL, $P \leq 0.001$) compared with blank (0.23 µmol/mL), and in group 2, isobutyrate (0.25 µmol/mL, $P = 0.04$) and isovalerate (0.47 µmol/mL, $P = 0.05$) concentrations were decreased by His compared with blank (0.34 and 0.51 µmol/mL). Other individual VFA were not affected by treatment. Compared with blank (1.96 mmol/L), ammonia concentration was greater (average 2.31 mmol/L, $P \leq 0.002$) for the AA treatments in group 1. In group 2, AA decreased ammonia concentration (average 1.42 mmol/L), compared with blank (2.20 mmol/L), except for His and Leu, 1.71 and 1.77 mmol/L, respectively. Protozoal counts were lower for AA treatments, except for His, (ranging from 1.87 to 2.77×10^4/mL, $P < 0.001$) compared with the blank (4.10 and 2.90×10^4/mL for group 1 and 2, respectively). These results indicate that some AA may have an inhibitory effect on total gas, methane production and some individual VFA in an in vitro batch culture system, but do not affect total VFA production and have a variable effect on ammonia concentration, depending on dose and incubation time.

Key Words: amino acid, fermentation, in vitro

T250 Hepatic metabolism of propionate relative to meals for cows in the postpartum period. K. M. Kennedy* and M. S. Allen, *Michigan State University, East Lansing, MI.*

Our long-term hypothesis is that anaplerosis of the tricarboxylic acid cycle (TCA) by propionate stimulates hepatic oxidation of acetyl CoA, increasing hepatic energy charge and satiety. The objective of this study was to determine the short-term effects of propionate on liver metabolism in dairy cows relative to meals. Eight dairy cows (5–14 DIM) were used in a duplicated 4x4 Latin square design with a 2 × 2 factorial arrangement of treatments. Cows received a pulse dose to the rumen of 1.5 moles of propionic acid (PA) in a 500 mL solution or 500 mL of water (control; CON) either 1 h before or 2 h after feeding. Liver samples were collected before dosing and 10 min post-dosing. Liver samples were analyzed for propionyl CoA (P-CoA), acetyl CoA (A-CoA), fumarate, malate, oxaloacetic acid (OAA) and citrate. Data were analyzed with the Mixed Model option in JMP Pro 13. PA increased malate compared with CON both before (PA: 133 to 267 vs. CON: 162 to 151 nmol/g) and after (PA: 224 to 329 vs. CON: 292 to 227 nmol/g) meals (interaction, $P = 0.06$). PA tended to increase fumarate compared with CON both before (PA: 39.5 to 57.5 vs. CON: 43.7 to 43.0 nmol/g) and after (PA: 44.1 to 70.2 vs. CON: 55.3 to 54.9 nmol/g) meals (interaction, $P = 0.13$); likely resulting from the downstream accumulation of malate. OAA was not affected by treatment so conversion of malate to OAA may be a bottleneck for cows in the postpartum period with elevated NADH/NAD. PA tended to increase citrate compared with CON ($P = 0.12$) and the increase tended to be greater after feeding (PA: 62.0 to 110 vs. CON: 101 to 96.0 nmol/g) than before (PA: 59.4 to 91.9 vs. CON: 51.9 to 58.3 nmol/g; interaction $P = 0.12$). PA tended to decrease A-CoA compared with CON both before (PA: 8.87 to 5.35 vs. CON: 9.62 to 7.82 nmol/g) and after (PA: 7.84 to 4.04 vs. CON: 4.64 to 6.26 nmol/g) feeding (interaction, $P = 0.11$). This reduction, along with increased citrate concentration, indicates that anaplerosis from propionate absorption within the timeframe of meals increases TCA cycle activity and likely increases hepatic energy charge despite the potential bottleneck at the malate dehydrogenase reaction.

Key Words: metabolism, liver, propionate

T251 Fatty acid digestion in dairy cows fed different fat sources: A meta-analytic approach. V. L. Daley*[1], L. E. Armentano[2], P. J. Kononoff[3], J. M. Prestegaard[4], and M. D. Hanigan[4], [1]*National Animal Nutrition Program (NANP), University of Kentucky, Lexington, KY,* [2]*University of Wisconsin, Madison, WI,* [3]*University of Nebraska-Lincoln, Lincoln, NE,* [4]*Virginia Tech, Blacksburg, VA.*

The dynamics of fat digestion and metabolism in ruminants depends on the dietary fatty acid (FA) profile and microbial metabolism of FA in the rumen. Digestion of FA can vary widely between dietary fat sources. A meta-analytic study was conducted to estimate the apparent total-tract digestibility coefficients of FA ($DCFA_{TTa}$) in dairy cows. A total of 31 studies (142 treatment means) that reported digestion of FA were used. Data for diet composition, intake, and digestibility, were collected from 357 cows (BW = 597 ± 74.9 kg, DM intake = 20.3 ± 4.92 kg/d, milk yield = 31.4 ± 8.28 kg/d). Dietary sources of fat were grouped into 11 categories. The digestible FA intake was then regressed on FA intake. The mixed model included the random effect of study. Studies were weighted according to the inverse of their variance (SE). $DCFA_{TTa}$ was influenced ($P < 0.001$) by the source of dietary fat; however, the intercept was not significant ($P > 0.01$). Fat supplements with high levels of palmitic, stearic acids, or hydrogenated triglycerides, had markedly lower FA digestion compared with those that are rich in unsaturated FA (Table 1). Overall, cows that were fed higher levels of saturated FA had lower FA digestion. Digestion coefficients of FA are useful to estimate the amount of dietary FA that could potentially be digested by dairy cows. Future studies should focus on individual FA digestion in lactating dairy cows.

Table 1 (Abstr. T251). Coefficients of apparent total tract digestibility of fatty acids ($DCFA_{TTa}$)

Class	$DCFA_{TTa}$, g/g	SE
Basal diet	0.73	0.026
Oil seeds	0.73	0.041
Oil[1]	0.70	0.033
Blended triglycerides[2]	0.63	0.027
Tallow[3]	0.68	0.029
Mildly hydrogenated tallow triglycerides[4]	0.61	0.037
Hydrogenated triglycerides[5]	0.44	0.030
Ca-salts of palm FA[6]	0.76	0.027
Partially hydrogenated tallow FA[7]	0.69	0.022
Palmitic acid, ~85%[8]	0.73	0.077
Palmitic or stearic acid > 90%[9]	0.31	0.046

[1]>20% PUFA and > 65% UFA.

[2]<20% PUFA and >56% UFA.

[3]>36% MUFA and <56% UFA.

[4]>25% MUFA and <36% UFA.

[5]<20% MUFA and <25% UFA.

[6]>30% MUFA.

[7]<15% MUFA and <20% UFA.

[8]>7% MUFA and <10% UFA.

[9]<2% MUFA and <2% UFA.

Key Words: dairy cow, fatty acids, digestion

T252 Palmitic fatty acid supplementation decreased neutral detergent fiber digestibility in continuous culture fermentors. B. A. Wenner* and N. R. St-Pierre, *Perdue AgriBusiness, Salisbury, MD.*

Recent meta-analyses on feeding palmitic fatty acid (PFA) supplements to dairy cattle indicate consistent improvement in total-tract NDF digestibility. However, the mode of action for increased NDF digestibility remains unexplained. Our hypothesis was that PFA supplementation increases fiber digestibility within the rumen. In the present study, we fed either a control (CON) or a palmitic fatty acid (PFA) diet to dual-flow continuous culture fermentors (n = 8, 1.82L) to evaluate the effect of PFA supplementation on NDF digestibility and VFA production when PFA was supplemented rather than substituted for dietary NDF. The CON diet was a 50:50 forage:concentrate pellet mixture that provided 7.0 g CP, 14.6 g NDF, and 4.9 g starch at 37.2 g DM once daily with no supplemental fat (1.3 g EE/d). The PFA diet was a 50:50 forage:concentrate pellet mixture that provided 6.6 g CP, 14.5 g NDF, 5.2 g starch, and 1.1 g PFA at 37.6 g DM once daily (1.9 g EE/d). Two experimental periods lasted 11 d each, with 4 d of sample collection. Buffer dilution and solids passage rate were maintained at 7.0%/h and 5.0%/h, respectively. Data were analyzed using a mixed model including the fixed effect of treatment, the random effects of fermentor and period, and a repeated statement for sampling day. There was no effect ($P > 0.2$) of treatment on apparent organic matter digestibility. Feeding PFA decreased ($P < 0.01$) NDF digestibility by 2.72 percentage units. There was no effect ($P = 0.19$) of PFA on daily VFA production nor was there an effect ($P \geq 0.12$) of PFA on molar proportions of acetate, propionate, isobutyrate, valerate, or isovalerate. However, PFA increased

($P = 0.04$) butyrate molar proportion compared with the control (0.130 versus 0.124, respectively, SE: 0.002). Feeding PFA tended to decrease ($P = 0.07$) fermentor ammonia concentration by 1.08 mg/dL. These results contrast with published in vivo evidence of PFA improvement of NDF digestibility and indicate that the rumen microbial adaptation is an unlikely source for the effect of PFA on NDF digestibility. Future research should focus on other factors potentially affecting NDF digestibility when PFA is fed, including feed intake behavior and rumen retention time.

Key Words: palmitic fatty acid, fermentor, NDF digestibility

T253 Supplementation of sodium butyrate to post-weaned heifer diets: Effects on growth performance, nutrient digestibility, and health. E. M. Rice*, K. M. Aragona, and P. S. Erickson, *University of New Hampshire, Durham, NH.*

The objective of this study was to determine the effect of varying levels of sodium butyrate (SB) on growth, nutrient digestibility, and health of post-weaned heifers. Forty Holstein heifers (84 ± 2.8 d of age) with body weights (BW) of 100.9 ± 11.2 kg were enrolled in a 15 wk study and blocked by birth date in a randomized complete block design. Treatments were 100 g of soybean meal carrier served as a control (CON); 0.25 g SB/kg of BW + carrier (0.25SB); 0.50 g SB/kg of BW + carrier (0.50SB); 0.75 g SB/kg of BW + carrier (0.75SB). Treatments were hand-mixed into a total mixed ration daily. Initial BW, hip and withers heights, heart girth and body length were measured before treatment and every week thereafter. From blood samples collected, plasma urea nitrogen and BHB concentrations were determined before treatment and weekly thereafter. Fecal samples were taken before treatment and every other week for coccidia counts. Data collected before treatment were used as covariates in the final analysis using the MIXED procedure of SAS. Apparent total-tract nutrient digestibility was determined using chromium oxide. Heifers underwent this phase from 47 to 54 d on study. There was no effect of SB on nutrient digestibility, however, increasing SB levels had a tendency to improve digestibility of acid detergent fiber ($P = 0.08$). Daily DMI were not different between treatments. However, ADG were 1.16, 1.15, 1.17, and 1.24 kg/d ($P = 0.12$) for CON, 0.25SB, 0.50SB, and 0.75SB, respectively, which led to a trend in improved feed efficiency ($P = 0.08$). Sodium butyrate had a positive effect on average BW and overall BW gain ($P = 0.04$; $P = 0.02$, respectively). Sodium butyrate had no effects on skeletal growth and plasma urea nitrogen concentrations ($P > 0.05$). Concentrations of BHB increased with increasing SB levels ($P < 0.05$). Fecal samples indicated the presence of coccidia, but counts were inconsistent within treatments. Sodium butyrate supplementation offers positive results in growth performance and feed efficiency of post-weaned heifers.

Key Words: post-weaned heifer, sodium butyrate, growth

T254 Impact of active dry yeast on production parameters in Friesian × Holstein cows during early lactation. O. AlZahal*[1], E. Febery[2], and J. Dunne[2], [1]*AB Vista, Marlborough, United Kingdom,* [2]*Drayton Animal Health Ltd., Stratford-on-Avon, United Kingdom.*

The objective of this study was to investigate the effect of supplementing active-dry yeast (ADY) on performance of lactating dairy cattle. Forty Friesian × Holstein cows (30 DIM) were enrolled a random complete block study. Cows were housed at the Tree House Farm (Drayton Animal Health, United Kingdom) in individual pens bedded with wood shavings. Candidate cows were sorted by calving date and randomly allocated into 1 of 2 treatments, ADY (Vistacell, AB Vista, UK; 3g/d providing 60×10^{10} cfu) or control (carrier only). Cows had ad libitum access to water via individual drinkers and received ad libitum TMR once a day for 12 wk. The ADY was applied as a top-dress. The TMR consisted (DM basis) of maize silage (39%), grass silage (39%), soy hulls (5%), wheat DDGS (5%), and a protein mix containing minerals and vitamins. A sample of the TMR was collected weekly throughout the experiment, composited monthly, and submitted for chemical analysis. The TMR contained 36.2% DM, and 24.3% ADF, 40.1% NDF, 21.0% CP, 7.8% ash, and 16.1% starch on DM basis. Orts were measured 3 time per week for each cow and milk yield was recorded daily for each cow. A weekly milk sample was collected form each cow and submitted for milk component analysis. Data were summarized by week and analyzed using PROC MIXED of SAS (v. 9.4). The model included the fixed effects of treatment (control, ADY), week (1 to 12), block (1, 2) and their interactions. The model included a repeated measurement statement with cow as the subject. The supplementation of ADY increased ($P = 0.03$) average daily milk yield (34.3 ± 0.24 kg/d vs. 35.0 ± 0.24 kg/d, control vs. ADY; respectively). This increase in milk yield was associated with an increase ($P = 0.007$) in feed efficiency (1.97 ± 0.017 vs. 2.03 ± 0.017). Dry matter intake, fat (content and yield), and protein (content and yield) were not significantly different between treatments. The effect of week was significant for DMI and milk yield, indicating a change in these parameters over time, but none of these parameters had a significant interaction with week. In conclusion, ADY supplementation during early phase of production, improved milk yield and feed efficiency.

Key Words: dairy, active-dry yeast, lactation

T255 Comparison between dietary palmitic and palmitoleic acid effects on milk performance and gene expression of granulosa cells in early lactation cows. M. Plante-Dube*[1], I. Gilbert[1], R. Gervais[1], C. Robert[1], B. Vlaeminck[2], V. Fievez[2], and P. Y. Chouinard[1], [1]*Laval University, Quebec, QC, Canada,* [2]*Ghent University, Ghent, East Flanders, Belgium.*

In early lactation, high-yielding cows suffer from negative energy balance associated with an increase in nonesterified fatty acid (FA) concentrations in plasma and follicular fluid. Consequently, folliculogenesis and granulosa cell metabolism can be impaired, but the effects may depend on FA composition. We hypothesized that, in dairy cows, milk production, milk fat concentration, and expression of genes related to lipid and energy metabolism of the granulosa cells are affected differently by palmitic and palmitoleic acids. Twenty Holstein multiparous cows in late gestation were randomly assigned to 200 g/d of FA supplements enriched in i) palmitic acid (PA; Palmit 80; Natu'oil Services Inc.; >80% 16:0) in the rumen or ii) palmitoleic acid (POA; Sea buckthorn oil; New Directions Aromatics Inc.; 28% *c*9–16:1, 27% 16:0, and 22% *c*9–18:1) in the abomasum. The treatment period ranged from 19 ± 8 d before calving to 67 ± 4 d postcalving. On wk 2, 6, and 10 following parturition, milk yield was recorded and milk samples collected during 3 consecutive days and composited. Ovum pick up (OPU) sessions were carried out at 46 and 67 ± 3 d postcalving. At each OPU session, cumulus oocyte complexes and granulosa cells were recovered. Total RNA was extracted from granulosa cells and the response of gene expression associated with lipid metabolism was analyzed by RT-qPCR. A repeated measures analysis was conducted using the MIXED procedure of SAS to test the effects of treatment, days in milk and their interaction. Treatments did not affect milk yield (39.6 ± 2.2 kg/d; $P = 0.74$) or fat concentration (3.88 ± 0.14%; $P = 0.27$). Abundances of mRNA for stearoyl-CoA desaturase (SCD1, SCD5) and perilipin 2 in granulosa cells were not different between treatments ($P > 0.12$). Compared with PA, POA tended

to decrease the expression of gene encoding for insulin receptor (P = 0.08). Also, SCD5 mRNA abundance was increased from first to second OPU (P = 0.05) and a similar tendency was observed for perilipin 2 (P = 0.07). These results suggest that stage of lactation and dietary FA profile may affect metabolism of granulosa cells in early lactation cow.

Key Words: dairy cow, fat supplement, gene expression

T256 Milk and components response of dairy cows when supplemented with a rumen protected B vitamins blend during heat stress. T. F. Gressley*[1], I. C. Shivas[2], and H. Leclerc[3], [1]*University of Delaware, Newark, DE,* [2]*Renaissance Nutrition Inc., Roaring Springs, PA,* [3]*Jefo Nutrition Inc., St. Hyacinthe, QC, Canada.*

A trial was conducted at the University of Delaware dairy center to compare milk and component responses to supplemental rumen protected B vitamins (BV; Lactation VB, Jefo, 3 g/cow/d) supplying vitamins B5, B6, B7, B9 and B12 in replacement for non-protected vitamin B7 alone (B; 20 mg/cow/d). B was provided for > 30 d (Period 1) before milk testing, switched to BV for the following DHIA test (Period 2), then cows were switched back to B for period 3. Intake was not measured. Milk results were analyzed using individual cows (65) in a paired t-test, adjusted to a common 150 DIM using the following equations: Adjusted milk = milk + milk × (DIM − 150) × 0.0029; Fat % = fat percent − 0.00269 × (DIM − 150); Protein % = protein % − 0.00167 × (DIM − 150). Days adjusted milk and component yields were greater for Period 2 with the use of BV than for Period 1 (P < 0.05; Table 1).There was substantial heat during both periods. High temperatures on test days were 28 and 31°C for Periods 1 and 2, respectively. The highest temperature on the Period 3 test day was 22°C, and results were used to assess production without heat stress. The temperature humidity index was 79, 83, and 70 for Periods 1, 2 and 3, respectively. Comparison of adjusted milk and component yield responses for the 2 B periods indicated that milk losses were substantial when cows were under heat stress conditions (Period 1 Vs Period 3, Table 1). These results showed that BV increased milk and component yields over B during periods of heat stress. When compared with the period without heat stress, BV appeared to alleviate a portion of the loss in production that occurs during heat stress.

Table 1 (Abstr. T256).

	Heat stress		No heat stress		
	Period 1	Period 2	Period 3	P-value	
Item	Control (B)	Test (BV)	Control (B)	1 vs. 2	1 vs. 3
Milk, kg/d	33.7	37.5	39.4	0.001	0.001
ECM, kg/d	34.1	37.9	41.8	0.001	0.001
Fat, kg/d	1.23	1.33	1.50	0.004	0.001
Protein, kg/d	0.93	1.09	1.23	0.001	0.001
Fat, %	3.83	3.60	3.88	0.013	0.628
Protein, %	2.79	2.91	3.13	0.001	0.001

Key Words: rumen-protected B vitamins, heat stress, milk production

T257 Prepartum fatty acid blend alters subsequent reproductive performance. A. Van De Kerckhove[1], A. Delaquis[2], F. Mueller[3], T. Steen[4], J. Guyader[5], and A. Park*[6], [1]*Federated Co-operatives Limited, Saskatoon, SK, Canada,* [2]*La Coop Fédérée, Montréal, QC, Canada,* [3]*Kalmbach Feeds, Upper Sandusky, OH,* [4]*Tennessee Farmers, LaVergne, TN,* [5]*Neovia, Château-Thierry, France,* [6]*Cooperative Research Farms, Richmond, VA.*

Our objective was to determine if supplementing fatty acid blends (FAB) to transition dairy cows improves production and reproduction metrics in the next lactation. In trial 1, 249 cows were balanced by previous 305-d mature-equivalent milk yield and parity then randomly assigned to 1 of 3 dietary treatments: control, calcium salt FAB, or plant FAB. Cows were individually fed prepartum treatments 3 weeks ahead of expected calving date then individually fed a common diet postpartum with individual milk yield and composition out to d 70, and individual reproductive metrics for 5 services. In trial 2, 805 cows on 3 commercial farms with each farm having a pen fed either control (3 pens) or plant FAB dietary treatments (3 pens) in a randomized design from 3 weeks before expected calving date until calving. Milk yield until 90 DIM and reproductive metrics for 5 services were monitored. Data were analyzed using the PROC MIXED procedure (SAS) with differences noted at P < 0.05 and trends at P < 0.15. In trial 1, control cows gave more 3.5% FCM (1.4 kg / d; P < 0.15) and energy corrected milk (ECM - 1.3 kg / d; P < 0.15) than cows fed the plant FAB. Control cows had a higher fat yield (0.07 kg / d; P < 0.08) compared with plant FAB cows. Plant FAB cows tended to have lower milk fat percentage (0.09%; P < 0.12) and less ketosis (3.8%; P < 0.09) than control cows. Plant FAB cows increased confirmed pregnant on first service (12.6%; P < 0.09) over calcium salt FAB cows. Plant FAB cows had a greater number pregnant by 100 (16.2%; P < 0.03) and 125 (13%; P < 0.04) DIM versus control fed cows. In trial 2, milk yield was affected by parity with aged cows fed plant based FAB having greater ECM yield (1.32 kg/ d; P < 0.05), higher milk protein yield (0.05 kg / d; P < 0.01) and more milk fat yield (0.04 kg; P < 0.12). Plant FAB cows had fewer services per conception (0.18 services; P < 0.13), and tended to have higher pregnancy probability (5.72%; P < 0.07) by 75 DIM. Feeding the plant FAB prepartum appears to yield variable production and metabolic disease incidences but consistently enhanced reproductive metrics in the next lactation.

Key Words: transition, fatty acid, reproduction

T258 Effects of lipopolysaccharide dosing on ruminal bacterial community compositions in a dual-flow continuous culture system. X. Dai*, E. M. Paula, A. L. J. Lelis, L. G. Silva, V. L. N. Brandao, H. F. Monteiro, P. Fan, K. C. Jeong, and A. P. Faciola, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective of this study was to evaluate the effects of lipopolysaccharide (LPS) dosing on ruminal bacterial community compositions (BCC). Diets were randomly assigned to 6 fermentors in a duplicated 3 × 3 Latin square with three 11-d experimental periods that consisted of 7 d for diet adaptation and 4 d for sample collection. Treatments were control diet (CON); wheat and barley diet (WBD), to induce subacute ruminal acidosis (SARA); and control diet + LPS (LPSD). Fermentors were fed 72 g of DM/d. The forage:concentrate ratio of CON was 65:35. The WBD was achieved by replacing 40% of the DMI of the CON diet with equal amount of ground wheat and barley; the LPS concentration in LPSD was 200,000 EU, which was similar to that observed in cows with SARA. The SARA inducing and LPS dosing started at d 8. The ruminal bacterial samples were collected at 0, 2, 5, and 9 h after morning feeding in both solid and liquid fractions. The bacterial community was determined by sequencing the V4 region of the 16S rRNA gene using the Illumina MiSeq platform. Ruminal pH of WBD was lower than LPSD and CON (P = 0.05), and WBD was able to keep ruminal pH below 5.6 for 240 min every sampling day, successfully inducing SARA. Ruminal pH of LPSD was not different from CON, and both were above 6 for the entire experimental period. The ruminal BCC of LPSD was different from WBD and tended to differ from CON in the

solid fraction. In the liquid fraction, the ruminal BCC was different among treatments. Greater *Bacteroidetes* and lower *Firmicutes* were observed in WBD, while no difference was found between CON and LPSD. LPS dosing increased the relative abundance of *Succinimonas, Anaeroplasma*, *Succinivibrio*, *Succiniclasticum* and *Ruminobacter* but decreased the relative abundance of *Treponema*, *Coprococcus* and some *Prevotella* species, which are the main gram-negative bacteria associated with starch digestion. In conclusion, LPS dosing changed ruminal BCC, especially in liquid fraction and affected the growth of gram-negative bacteria associated with starch digestion in a dual flow continuous culture system.

Key Words: 16S rRNA sequencing, SARA, ruminal bacteria

T259 *Saccharomyces cerevisiae* fermentation products increase volatile fatty acid production in an in vitro rumen model on forage samples from five European countries. C. Reedy*, T. Kwan, T. Werner, J. Butler, and I. Yoon, *Diamond V, Cedar Rapids, IA*.

The objective of this study was to evaluate the effect of 2 *Saccharomyces cerevisiae* fermentation products: Diamond V Original XPC (XPC) and NutriTek (NutriTek) on in vitro VFA production on forage samples from 5 countries in Europe. Eight different forage samples were collected from the 5 countries: Poland [corn silage (CS PL) and legume forage (LF PL)]; Germany [corn silage (CS DE)]; United Kingdom [hybrid rye forage (HRF GB) and perennial rye forage (PRF GB)]; Czech Republic [mixed grasses with fescue (MGF CZ)]; and Netherlands [perennial rye forage (PRF NL) and corn silage (CS NL)]. To each serum bottle, treatments were included based on the recommended feeding rate for XPC (14 g/hd/d) and NutriTek (19 g/hd/d) along with 0.3 g of forage. Each serum bottle was inoculated with 40 mL of a 5% buffered rumen fluid from cows on a diet of 70% forage and 30% concentrate and was incubated for 24 h. Four experiments were performed to generate 10 replicates per treatment and diet. Each forage was analyzed separately using the GLM model of JMP, means were compared using Tukey analysis, and significance was defined as $P \leq 0.05$. Percent increases in acetate (A), propionate (P), butyrate (B), and total VFA (T) concentrations are reported in Table 1. Significant treatment effects were observed (indicated with *) in all forage samples tested after 24 h with few exceptions. In conclusion, XPC enhanced in vitro VFA production over Control and Nutritek further enhanced VFA production over XPC on all of the forage samples tested. Results suggest that XPC and NutriTek could enhance ruminal VFA production from these forages of European origin.

Key Words: NutriTek, forage, ruminal VFA

T260 Impact of *Saccharomyces cerevisiae* fermentation product (SCFP) on feed intake parameters and lactation performance of transition dairy cattle. K. E. Olagaray*[1], S. E. Sivinski[1], B. A. Saylor[1], J. A. Sauls[1], I. Yoon[2], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*Diamond V, Cedar Rapids, IA.*

The effects of a SCFP (NutriTek, Diamond V, Cedar Rapids, IA) on feeding behavior and milk production and composition were evaluated in transition cows. Treatments were control (CON; n = 30) or SCFP (n = 34; 18 g/d) TMR fed from −29 ± 5 to 42 d relative to calving. Cows were individually fed 3×/d prepartum (45% NDF, 14% starch) and 2×/d postpartum (32% NDF, 19% starch). Cows were milked 2×/d with samples 2×/wk for composition analysis and calculation of ECM, FCM, and energy balance (EB). Results were analyzed using the MIXED procedure of SAS. SCFP did not affect prepartum DMI, but tended to increase meals/d (8.66 vs. 9.27 ± 0.22 /d; $P = 0.06$) and reduced time between meals (2.26 vs. 2.09 ± 0.05 h; $P = 0.03$). A treatment × parity interaction ($P = 0.03$) reflected greater meals/d in SCFP vs. CON primiparous cows, but not multiparous cows. We observed no difference in prepartum meal weight or length. Similarly, SCFP increased postpartum meal count (11.4 v. 12.6 ± 0.45 /d; $P = 0.03$) and tended to reduce intermeal interval ($P = 0.07$), but did not affect meal weight or length. We observed no differences in milk, FCM, or ECM yields (all $P > 0.31$). Milk fat concentration was greater in SCFP ($P = 0.01$) with differences in wk 4 and 5 ($P < 0.05$) that tended to increase fat yield in these 2 wk, without affecting BCS, BW, or plasma fatty acid concentration (all $P > 0.35$). Milk lactose concentration tended to be greater for CON ($P = 0.06$) and milk urea nitrogen tended to be lesser for CON ($P = 0.06$), but there were no treatment effects on milk protein concentration or somatic cell count. Assuming equal digestibility, EB was lesser in SCFP than CON (−4.34 vs. −6.15 ± 0.74 Mcal/d; $P = 0.03$) with differences in wk 4 and 5. Energy density of diets calculated from observed ECM yield and BW change did not differ by treatment (1.90 vs. 1.83 ± 0.04 Mcal/kg DM for SCFP and CON; $P = 0.18$). In conclusion, SCFP increased meals/d and milk fat concentration in wk 4 and 5, although no effect on milk yield was detected.

Key Words: transition cow, feeding behavior

T261 Rumen-protected linseed oil supplementation: Energy status. J. D. Iorio*[1], E. E. Salado[2], R. A. Palladino[1,3], M. G. Maciel[2], Y. M. González Moreno[1], M. F. Olmeda[3], and D. Curletto[2], [1]*University of Buenos Aires, School of Agriculture, Buenos Aires, Argentina,* [2]*National Institute of Agricultural Technology, Santa Fe, Argentina,* [3]*Faculty of Agricultural Sciences-UNLZ, Buenos Aires. Argentina.*

Table 1 (Abstr. T259). Percent increases of ruminal VFA production by XPC and NutriTek

	XPC over Control				NutriTek over XPC			
Forage	A	P	B	T	A	P	B	T
CS PL	2.9	17.7*	6.4*	8.1*	6.1*	20.7*	4.8*	11.3*
LF PL	5.8*	7.8*	7.4*	6.6*	2.0	8.6*	4.1*	3.9*
CS DE	1.9	26.8*	3.3	10.4*	9.2*	27.2*	7.5*	16.5*
HRF GB	9.5*	13.3*	7.4*	10.0*	9.0*	18.4*	6.8*	1.6*
PRF GB	11.2*	14.3*	9.1*	12.1*	6.1*	13.1*	4.4*	8.0*
MGF CZ	10.0*	13.3*	11.1*	11.2*	5.6*	14.5*	1.3	7.9*
PRF NL	7.1*	7.0*	7.7*	7.0*	2.4	9.8*	1.8	4.9*
CS NL	2.4	27.3*	9.7*	11.2*	8.4*	31.7*	5.9*	17.2*

The aim of this research was to evaluate the effect of supplementing calcium salts from linseed oil (AG-Ca; rich in linolenic acid, C18:3n-3) to partially replace corn grain on energy status during early lactation. In a 10-wk assay, 38 primiparous and multiparous Holstein dairy cows (average ± SD; 55 ± 18.1 DIM, 594 ± 96.8 kg BW, 2.71 ± 0.4 BCS, 2.0 ± 1.4, and 38 ± 9.4 kg/d milk yield) were used in a complete randomized block design. Cows were assigned to one of 2 treatments (O3: AG-Ca supplementation or CON: control, no AG-Ca supplementation) and were blocked by DIM, number of lactation, BW and milk production. Samples of blood were analyzed for glucose (GLU), BHB, growth hormone (GH), insulin (INS) and insulin-like growth factor-1 (IGF-1) once every 15 d. The BW, BCS (1–5) and milk production were recorded weekly. Isoenergetic diets were formulated to meet requirements of a Holstein dairy cow of 650 kg BW with 40 DIM, producing 40.0 kg/d of milk with 3.6% fat (NRC, 2001). Diets (O3 and CON) included 12 kg DM of alfalfa pasture (Medicago sativa), 13.5 kg DM of TMR (63.5% corn silage, 18.0% soybean meal, 10.6% cracked corn and 7.9% alfalfa hay) and treatment concentrate (O3, 5.2 kg DM including 0.85 kg/d of AG-Ca (DM: 82,3% DM, 13,2% ashes, 86,8% EE and 35,82% C18:3n-3), commercial feed and cracked corn; CON, 5.9 kg DM including commercial feed and cracked corn) given at milking time (twice a day). Estimated total DMI was estimated by NRC (2001) while pasture DMI was estimated by subtracting TMR and concentrate DMI to Total DMI. Data were analyzed using the MIXED procedure of SAS with significance declared at $P \leq 0.05$. Supplementation did not change milk production (O3: 33.4 kg d-1 and CON: 33.8 kg d-1; $P > 0.10$), neither blood parameters (GLU: 0,59 vs. 0,60 g l-1, BHBA: 0,59 vs. 0,57 mmol l-1, INS: 0,91 vs. 0,95 ng ml-1, GH: 2.42 vs. 2.40 ng ml-1, IGF-1: 109.37 vs. 109.79 ng ml-1, for O3 and CON respectively; $P > 0.10$) nor BW (O3:584.27 kg and CON: 589.58 kg; $P > 0.10$) and BCS (O3: 2.72 and CON: 2.64). These results suggest that supplementation with AG-Ca of linseed oil could be an option to partially replace corn grain without affecting the energy status during the early lactation.

Key Words: linseed oil supplementation, energy status, milk production

T262 Effect of *Clostridium butyricum* sp. nov. and *Pichia kudriavzevii* sp. nov. on Holstein milk composition and yield. G. Gogul*[1], M. Striluk[1], C. Martino[1], A. Lago[2], and M. Embree[1], [1]*Ascus Biosciences, San Diego, CA,* [2]*DairyExperts Inc., Tulare,CA.*

Effect of an endomicrobial supplement (EMS) on dairy cow milk composition and yield was assessed. The EMS consisted of *Clostridium butyricum* sp. nov. and *Pichia kudriavzevii* sp. nov., injected at a total of 4×10^9 and 1×10^9 cells/day. Observations were collected from 16 multiparous, ruminally cannulated Holstein cows that were randomly split into a control (CON) and inoculated (INO) group. Study consisted of 3 periods: 10-d pre-treatment, 32-d treatment, and 10-d post-treatment. Cows were individually penned and fed a common TMR (17% CP, 27.1% NDF) twice daily. During morning feedings of TRT, INO received the EMS and CON received sterile PBS via rumen cannula. A composite milk sample per cow was collected at each milking on d 10 pre-TRT, and daily during the TRT and post-TRT periods. Milk composition was analyzed using near-infrared spectroscopy for crude protein, fat, and milk urea nitrogen (MUN) at the Tulare DHIA Laboratory. Data were analyzed by averaging daily values to produce weekly means for conducting repeated measures using the MIXED procedure of SAS. A composite rumen fluid sample was collected 18 times throughout the 52-d study to determine EMS colonization by sequencing the ITS and 16S rRNA V1-V3 hypervariable regions on the Illumina MiSeq Platform. EMS abundance of INO, compared with the CON, had increased on d 2 of TRT. Peak relative abundance of *C. butyricum* sp. nov. (1.4%) and *P. kudriavzevii* sp. nov. (5%) occurred at d 19 in INO. A tendency for a higher milk fat percentage for INO vs. CON group was observed ($P = 0.0991$). A treatment by week interaction was observed for milk yield ($P = 0.0025$), fat-corrected milk (FCM, $P = 0.0026$), energy-corrected milk (ECM, $P = 0.0019$), protein yield (PY, $P = 0.0012$), fat yield (FY, $P = 0.0880$), feed efficiency (FE, $P = 0.0671$) and rumen pH ($P = 0.0741$). Though treatment by week interaction was not significant, results indicate that under the conditions of this study, EMS containing *Clostridium butyricum* sp. nov. and *Pichia kudriavzevii* sp. nov., may have a positive effect on cow performance.

Key Words: endomicrobial supplement, *Clostridium*, *Pichia*

T263 Evaluation of different fiber contents in solid diets of pre-weaning dairy calves. M. Poczynek[1], G. F. Virginio Jr[1], A. P. Silva[1], A. F. Toledo[1], M. G. Coelho[1], M. D. Silva[1], G. B. Oliveira[2], and C. M. M. Bittar*[1], [1]*Dept. Of Animal Sciences, Colle of Agriculture Luiz de Queiroz (ESALQ), University of Sao Paulo, Piracicaba, Sao Paulo, Brazil,* [2]*Dept. Animal Production, FMVZ, UNESP/Botucatu, Botucatu, Sao Paulo, Brazil.*

The solid diet of calves is the main determinant for ruminal development. It must contain high digestible ingredients, however with adequate level of fiber to maintain ruminal health. However, levels of NDF for calf starters are still controversial. Forty males and female calves Holstein newborns were blocked according to sex and birth weight and distributed in 3 treatments: (1) conventional calf starter (16% NDF); (2) high fiber calf starter (25% NDF), with soybean hulls replacing part of the corn; and (3) conventional calf starter (16% NDF) plus free access to grass hay.

Table 1 (Abstr. T263). Performance of calves fed diets with different fiber contents

	Treatment				*P*-value[1]		
Item	16 NDF	25 NDF	HAY	SEM	T	A	T × A
Starter intake, g/d	319.9	435.0	305.3	61.07	0.27	<0.0001	0.045
Hay intake, g/d	—	—	18.6	5.99	—	0.007	—
Solid diet intake, g/d	322.5	435	321.6	60.72	0.32	<0.0001	0.044
Initial BW, kg	35.6	37.3	37.7	1.33	0.21	—	—
Final BW, kg	53.8	58.3	54.9	2.38	0.21	—	—
BW gain, g/d	308.8	350.2	314.1	35.24	0.59	<0.0001	0.868
Fecal score	2	1.9	1.9	0.11	0.83	<0.0001	0.799
Fecal pH	6.8	6.7	7.0	0.12	0.18	0.607	0.349

[1]T = treatment effect; A = age effect; T × A = treatment vs. age effect.

Animals received 4 L/d of milk replacer (MR) until gradual weaning at 56 d. Calves were weighed weekly and feed intake was recorded daily. The solid diets with higher levels of NDF did not negatively affect the starter or total solid diet intake nor the performance ($P > 0.05$). Daily gains are in agreement with NRC (2001) prediction for calves receiving 4 L/d of MR. All performance data were affected by the age of the calf ($P < 0.05$). Fecal score and pH were not affected ($P < 0.05$). These results show that solid diets with lower corn inclusions and consequently lower starch contents can result in similar weight gain as observed for conventional diets. Free access to hay did not reduce feed intake, leading to similar weight gain among groups with or without access to hay. Increasing the NDF of calf starter from 16 to 25%, including a high quality fiber source, have not reduced calves' performance.

Key Words: calf nutrition, NDF, weight gain.

T264 Crambe meal can completely replace soybean meal in diets for dairy cows. D. C. Moura, F. J. G. Vieira, R. M. Miranda, P. O. Cordeiro, L. Molossi, D. S. Souza, V. H. Zampieri, S. R. Soares, F. N. Viana, K. C. Alessi*, and A. S. Oliveira, *Dairy Cattle Research Lab, Universidade Federal de Mato Grosso-Sinop, Sinop, Mato Grosso, Brazil.*

Crambe meal (CM; *Crambe abyssinica* H.) is a byproduct obtained after oilseed extraction with high protein content and with great potential of use in the feeding of ruminants. We evaluated the effects of CM (CP = 38.2% DM; glucosinolates = 450 mg/kg DM) inclusion in partial total mixed ration (pTMR; 0, 4.5, 9 and 13.5% DM), as a replacement of soybean meal, on intake, total-tract diet digestibility and nitrogen metabolism of lactation dairy cows. Twelve crossbred Holstein × Zebu dairy cows (456 ± 91 kg BW) were used in a replicated 4 × 4 Latin squares design with 4 21 d experimental periods. The pTMR diets were isonitrogenous (CP = 13.0% DM) and offered ad libitum between 6:00 a.m. and 7:00 p.m. Between 7:00 p.m. and 6:00 p.m. the cows remained in pastures of *Panicum maximum* cv. Mombaça (NDF = 56.4% DM; CP = 9.04% DM). Milk yield and intake the pTMR were recorded from d 15 to 21 of each period. The milk samples were collected on d 17 and 18. Daily samples of feces from each animal were collected between d 17 and 21 for fecal excretion (using titanium dioxide as an external indicator) and pasture intake (using indigestible neutral detergent fiber after 288 ruminal incubation in situ). Spot urine samples were obtained 3 h supplement post-feeding on d 20. Data were analyzed using a mixed model (treatment as fixed effect; square, period and cows(square) as random effects). Treatment effects were decomposed on 3 orthogonal contrast: linear, quadratic, and cubic effects. Inclusion of CM did not affect pTMR intake ($P = 0.173$; 11.47 ± 0.20 kg DM/d), pasture intake ($P = 0.185$; 0.90 ± 0.07 kg DM/d), CP intake ($P = 0.481$; 1.49 ± 0.01 kg DM/d), organic matter total-tract digestibility ($P = 0.254$; 0.749 ± 0.01 g/g), CP total-tract digestibility ($P = 0.545$; 0.747 ± 0.02 g/g), ruminal microbial protein synthesis ($P = 0.348$; 0.83 ± 0.08 kg/d), milk yield ($P = 0.462$; 13.29 ± 0.24 kg/d), nitrogen (N) milk ($P = 0.566$; 64.2 ± 1.3 g/d), milk urea-N ($P = 0.178$; 10.6 ± 0.94 mg/dL), N urinary excretion ($P = 0.717$; 90.9 ± 1.9 g/d) and N milk efficiency ($P = 0.622$; 0.268 ± 0.01 g N milk/g N intake). Crambe meal can be used up to 13.5% of DM in pTMR without affecting intake, total-tract diet digestibility and nitrogen metabolism of dairy cows.

Key Words: brassica, intake, glucosinolate

T265 Dietary strategies to optimize milk production and composition of dairy goats fed a high-concentrate diet. S. Dion*[1], M.-E. Brassard[1], J. Levesque[2], D. E. Rico[2], R. Gervais[1], and P. Y. Chouinard[1], [1]*Université Laval, Québec, QC, Canada,* [2]*Centre de recherche en sciences animales de Deschambault, Québec, QC, Canada.*

In early lactation, dairy goats undergo changes in rumen fermentation that can impair milk fat synthesis, due mainly to high levels of dietary concentrates. This study was undertaken to compare the potential of dietary lipid supplements of different fatty acid (FA) compositions to affect milk performance when early lactation dairy goats are fed a high-concentrate diet. Thirty Alpine goats were enrolled at kidding, housed in pens with Calan gate feeders, and received a total mixed ration with a forage-to-concentrate (F:C) ratio of 55:45 on a dry matter (DM) basis. On the last 4 d of this 23 ± 5 d pretreatment period, data and samples were collected and used as covariates. Goats were then allocated to 1 of 10 blocks according to milk fat concentration. During a period of 41 d, goats received: CTL) a basal diet with a F:C ratio of 45:55; PALM) CTL + 2% (DM basis) of a by-product of palm oil industry, as a source of 16:0; FLAX) CTL + 7% of extruded linseed, as a source of 18:3 n-3. Collection periods were carried out on d 7 to 10, 17 to 20 and 38 to 41. Because no differences were observed between the last 2 collection periods, data were combined, and treatment effects were compared with the Tukey adjustment for multiple pairwise comparisons. As expected, in CTL group, feeding the high-concentrate diet reduced milk fat concentration by 19% as compared with pretreatment period ($P < 0.01$). Dry matter intake (3.37 kg/d; $P = 0.53$) and milk yield (4.82 kg/d; $P = 0.22$) were not different between treatments. As compared with CTL, goats fed PALM or FLAX had a greater milk fat concentration (3.92^a and 3.90^a vs. 3.60^b %). Milk fat from goats receiving PALM had a greater concentration in 16:0 (29.8^a %) as compared with CTL (26.7^b %) and FLAX (21.5^c %), whereas a greater concentration of 18:3 n-3 was observed when goats were fed FLAX (1.22^a %) as compared with CTL (0.73^b %) and PALM (0.66^b %). Results indicate that feeding lipid supplement increases milk fat concentration and that milk fat composition is affected according to the FA profile of the lipid supplement.

Key Words: milk fat, extruded flaxseed, palmitic acid

T266 Effects of a novel rumen-undegradable protein source on performance of lactating Holstein cows. M. I. Rivelli*[1], M. J. Cecava[2], P. H. Doane[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*ADM Research Division, Decatur, IL.*

Feeding rumen-undegradable *p*rotein (RUP) is a practical strategy to supplement essential amino acids to high producing cows and reduce N losses to the environment. The objective of this study was to determine the effects of a novel RUP source (ADM) on performance and nutrient digestion by Holstein cows. A total of 18 Holstein cows [BW (653.0 ± 96.5 kg); DIM (123 ± 50 d)] were assigned to 1 of 3 treatments in a replicated 3 × 3 Latin Square Design with experimental periods 28d in length. Periods were divided in an adaptation phase (d 1 to 21), and measurement phase (d 22 to 28). Treatments were CON, cows were fed a control diet (16% CP, 70% RDP, and 30% RUP) without a RUP source; AMP, cows fed a diet with RUP source 1 (16% CP, 60% RDP, and 40% RUP); and ADM, cows fed a diet with RUP source 2 (16% CP, 60% RDP, and 40% RUP). Data were analyzed using the MIXED procedure of SAS. Two treatment orthogonal contrasts were used. Contrast 1 (CONT1): CON compared with ADM and Contrast 2 (CONT2): AMP compared with ADM. There were no treatment differences for either contrasts (CONT1 or CONT2) for DMI as a percent of BW, BCS, or fat-corrected milk (3.5%) ($P > 0.10$). Milk protein and lactose yields were higher for cows in CON (1.14 ± 0.02 kg/d and 1.78 ± 0.03 kg/d, respectively) than AMP (1.08 ± 0.03 kg/d and 1.68 ± 0.03 kg/d; $P = 0.02$ and $P = 0.03$, respectively; CONT1). However, milk lactose concentration and

casein as a percentage of protein were higher for cows in ADM (4.75 ± 0.01% and 76.62 ± 0.17%, respectively) than AMP (4.70 ± 0.02% and 76.22 ± 0.18%; $P = 0.01$ and $P = 0.03$, respectively; CONT2). Cows in ADM (14.21 ± 0.4 mg/dL) had lower MUN concentration compared with cows in CON and AMP (15.88 ± 0.5 mg/dL and 16.08 ± 0.4 mg/dL; $P < 0.05$, CONT1, and $P < 0.05$, CONT2). Cows in ADM (0.36 ± 0.02mg/dL) had lower BHB plasma concentrations than CON and AMP (0.42 ± 0.02 mg/dL; $P < 0.05$, CONT1, and $P < 0.05$, CONT2). Cows in ADM had the lowest treatment BHB plasma concentrations on d 26 (0.34 ± 0.02mg/dL) compared with d 22 (0.38 ± 0.03 mg/dL). In conclusion, performance of cows in ADM was more similar to Cont than those of AMP and seem to have lower protein breakdown (i.e.; deamination) than cows in CON and AMP.

Key Words: rumen-undegradable protein, casein, BHB

T267 Effects of source and level of forage neutral detergent fiber on feeding behavior of Holstein and Jersey cows. O. I. Santana*[1,2], M. E. Uddin[1], and M. A. Wattiaux[1], [1]*Department of Dairy Science. University of Wisconsin-Madison, Madison, WI,* [2]*INIFAP, Pabellon, Aguascalientes, Mexico.*

Our objective was to assess 2 levels of forage neutral detergent fiber (FNDF) in the diet primarily as either alfalfa silage (AS) or corn silage (CS) on feeding behavior of Holstein (H) and Jersey (J) cows. Twent-four lactating primiparous cows (12 H, and 12 J; 606 ± 40 and 407 ± 43 kg of BW; 106 ± 17 and 112 ± 15 DIM, respectively) were the experimental units in a triplicated split-plot Latin square design with 2 × 2 factorial arrangement of treatments to evaluate 4 diets: Low FNDF (LF; 20% of diet dry matter (DM)) and high FNDF (HF; 26% of diet DM) with AS and CS NDF in ratios of either 70:30 or 30:70. All diets contained 30% NDF, 16.5% crude protein, 1.52 Mcal NEl/kg, and 24% starch (DM basis). On d 18 and 19 of each 28-d period, samples of TMR and individual orts were taken to determine particle size distribution using the Penn State Particle Separator (3-sieve and pan). Rumination time (RT) was measured using Hi-Tag rumination collars and data flow software with a resolution of 2 min. As expected, H had greater DM intake (DMI) than J cows (22.7 vs. 17.1 kg/d). However, as a percentage of BW, DMI tended to be greater for J than H cows (4.1 vs. 3.7, $P = 0.08$), and was greater for cows fed LF than HF diets (4.1 vs. 3.8), as well as for cows fed CS compared with AS diets (4.0 vs. 3.8). Particle size distribution as a percentage of DMI (estimated by the difference of DM retained on each sieve for TMR offered and orts) did not differ between breeds and FNDF sources, but physical effective DM (particles >1.18 mm) was greater for cows fed HF than LF diets (80.8 vs. 71.2% of DMI). Breed did not influence RT (492 ± 23 min/d), but J had greater RT than H cows when expressed as min per kg of DMI (31.3 vs. 20.6), FNDF intake (137 vs. 90), and BW (1.2 vs. 0.75). There were no effect of FNDF source on RT, but cows fed HF had greater RT than cows fed LF diets (511 vs. 473 min/d, 28.0 vs. 23.9 min/kg of DMI, 120 vs. 108 min/kg of FNDF, and 1.1 vs. 0.96 min/kg BW). In conclusion, breed and FNDF source did not influence feeding behavior, but HF diets resulted in greater intake of large particles and RT.

Key Words: breed, particle size, rumination

T268 Monensin modifies fermentation profile and affects the innate immune response in the rumen. E. Trevisi*[1], F. Riva[2], A. Minuti[1], M. Mezzetti[1], J. F. S. Filipe[2], P. Bani[1], and M. Amadori[3], [1]*Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy,* [3]*Cellular Immunology Laboratory, IZSLER, Brescia, Italy.*

Monensin is a ionophor that selects against gram-negative rumen bacteria and increases the propionic acid proportion in the rumen, the main precursor of gluconeogenesis in the liver. Changes of rumen microbiota and fermentation pattern can also affect the permeability of epithelium. In previous studies, the inflammatory profile (leukocyte infiltration, cytokines and Ig content) of rumen fluids was suggested as a marker of these changes. Based on these findings, we investigated monensin effect on fermentation patterns and innate immune responses in the rumen of lactating cows. Forty-three dairy cows, 13 primiparous (PR) and 30 multiparous (ML), were allocated to 2 homogeneous groups: Kex received monensin by a controlled release capsule (approx. 335 mg/d, Kexxtone, Elanco Animal Health, UK) 21 d before expected calving and Ctrl (control). Fluid samples were collected with a rumen probe at 30 d in milk, 6 h after feed distribution. An aliquot was used for volatile fatty acids and ammonia analyses and one was used for flow cytometry and molecular assays. The prevalence of T and B lymphocytes and myeloid leukocyte cells was determined by monoclonal antibodies to CD3, surface IgM and an epitope expressed by the majority of monocytes and granulocytes in peripheral blood (IL-A24), respectively. Data were analyzed by one-way ANOVA, considering treatment and parity fixed effects. Kex cows showed a lower concentration of ammonia (58.7 vs 93.3 mg/L; $P < 0.05$), a higher level of propionate (24.5 vs 22.4 mol/100 mol; $P < 0.10$) and a reduced acetate:propionate ratio (2.5 vs 2.8; $P < 0.10$). Kex cows showed a lower B lymphocytes proportion compared with Ctrl (1.66 vs 2.50%; $P < 0.05$), in particular in PR cows. The T lymphocytes content of rumen fluid tended to be lower in Kex PR cows compared with Ctrl PR ($P < 0.1$), but no effect appeared in ML. These results confirmed monensin effects on rumen fermentations and a greater response of the immune system in the rumen of Ctrl cows. Moreover, the assessment of the rumen leukocytes activity under stressful physiological conditions could help to evaluate the impact of diet strategies on animal wellness.

Key Words: leukocyte, rumen, ionophore

T269 Effects of microbial inoculum composition on rumen microbial ecology of dairy calves. L. M. Cersosimo*, W. Radloff, and G. I. Zanton, *US Dairy Forage Research Center, Madison, WI.*

The objective of this study was to determine if microbial inoculum composition affects dairy calf rumen microbial ecology. Holstein bull calves (n = 20) were removed from their dam at birth and individually housed in calf hutches with sand bedding. Responses were studied using a randomized complete block design with repeated measures and a 2 × 2 factorial arrangement of treatments: stomach intubation of 50 mL of rumen fluid (RF) as autoclaved RF, bacterial-enriched RF (BE), protozoal-enriched RF (PE), or 50 mL of each BE and PE. Inocula were prepared fresh by differential centrifugation of blended RF+solids (BE) or gravimetric sedimentation of RF (PE) from a rumen content composite taken from 4 rumen fistulated, primiparous Holstein cows (mean ± SD: 101 ± 5 DIM, 37.8 ± 2.0 kg milk/d). BE was microscopically confirmed to be free of ciliates, while PE contained $2.9 \pm 2.2 \times 10^5$ cells/mL. Calf RF was sampled and inocula was administered on 1 d/wk during wk 3–6 of age by stomach intubation; RF sampling immediately preceded inoculation. Data were analyzed as repeated measures with BE, PE, and the interaction as contrasts of interest with $P < 0.05$ as significant. Calf DMI and ADG were 0.4 ± 0.08 and 0.9 ± 0.1 kg/d, respectively and not different by treatment. At 3 wk, all calves were fauna-free and calves that did not receive PE remained fauna-free through wk 6. Ciliate protozoa were observed in RF from 6, 8, and 6 PE calves (n =

10) at wk 4, 5, and 6, respectively; ciliate concentrations averaged 1.5 × 10^4/mL in these calves. Butyrate molar percent was greater in BE calves (10.8 *vs* 8.3 ± 0.8), while other VFA molar percentages were not affected by treatment; ruminal NH_3 was lower in PE calves (3.3 *vs* 6.8 ± 1.0 mM). Rumen microbial α and β diversities (by targeted PCR of the V4 region of the 16S rRNA gene and sequencing with Illumina MiSeq) did not differ by treatment or wk. PE calves had greater relative abundances of the genus *Prevotella* (33 vs. 18 ± 5.3%) and phylum Proteobacteria (12.4 vs. 2.3 ± 3.2) at wk 4 and 5, respectively. These findings demonstrate the potential for inoculation with BE and PE to affect the ruminal environment.

Key Words: protozoa, bacteria, calf

T270 Determination of optimal inclusion level of bioactive *Olea europaea* extract in promoting secretion of GLP-1 and its effects on growth and health in dairy calves. S. Y. Morrison*[1], I. R. Ipharraguerre[2], R. C. Zapata[3], P. K. Chelikani[3], F. Rosa[4], J. S. Osorio[4], J. J. Pastor[5], F. Bargo[5], M. Blanch[5], and J. K. Drackley[1], [1]*University of Illinois, Urbana, IL,* [2]*University of Kiel, Kiel, Germany,* [3]*University of Calgary, Calgary, AB, Canada,* [4]*South Dakota State University, Brookings, SD,* [5]*Lucta S. A., Barcelona, Spain.*

A bioactive extract from *Olea europaea* (OBE) can stimulate secretion of glucagon-like peptide-1 (GLP-1) and GLP-2, through bile acid receptor TGR5. Increasing circulation of GLP-2 promotes beneficial effects on intestinal function, which can be compromised in preruminant calves. The aims of this study was to determine the optimal inclusion level of OBE to promote maximal secretion of GLP-1 and determine the effects of OBE on health and growth of Holstein calves. Experiment 1 was a 5 × 5 Latin square design with 5 doses of OBE (0, 60, 90, 120, and 180 mg/kg of BW) administered in 5 periods to 5 calves. Blood was sampled at 0, 15, 30, 60, and 90 min relative to dosing to determine plasma GLP-1 concentration using an ELISA kit. In experiment 1, the maximal [GLP-1] after dosing was greatest at 60 mg/kg of BW vs. 0 mg/kg of BW (P = 0.04) 60 min after dosing. Experiment 2 was a RCBD with 2 doses of OBE (0 or 60 mg/kg of BW) with 44 calves/treatment. Intake of milk replacer (MR), starter, and water were recorded daily. Body weight and stature measurements were recorded weekly. Blood was sampled (wk 2) to determine [GLP-1]. Total RNA was isolated from fecal samples (wk 2) for RT-qPCR analysis. Data were analyzed using the GLIMMIX procedure in SAS. In experiment 2, there were no differences in MR, starter, and water intakes, body weight, or growth measurements (P > 0.10). The likelihood of occurrence of scours or medication did not differ between treatments. Calves given OBE had numerically (P = 0.11) fewer average days medicated for digestive issues in the first 3 wk. In experiment 2, prefeeding concentrations of GLP-1 tended (P = 0.06) to be higher in OBE fed calves but the delta change after feeding tended (P = 0.10) to be less for OBE fed calves. Expression of *TNFA* was similar, but *NFKB1* expression differed between 0 vs. 60 mg/kg of BW OBE (−4.62 vs. −2.41 ± 0.54; P < 0.01), respectively. In conclusion, OBE had few positive effects on growth or health of calves during the preweaning period or through weaning.

Key Words: bioactive extract, glucagon-like peptide-1, calves

T271 Relationship between near-infrared reflectance spectroscopy and in situ fiber-related analyses of corn silage hybrids. M. T. Harper*[1], G. Roth[1], C. Canale[2], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Cargill Animal Nutrition, Shippensburg, PA.*

Near-infrared reflectance spectroscopy (NIRS) analyses of forages are routinely used to predict forage quality and, consequently, animal performance. In this study, corn silage hybrids ensiled for up to 150 d (n = 74) were used to assess the relationships between NIRS NDF-related analyses conducted by 2 commercial laboratories and in situ NDF degradability variables. The in situ analysis involved 3 ruminally-cannulated cows (DMI, 26.8 ± 1.0 kg/d; milk yield, 39.8 ± 2.96 kg/d). Corn silage samples, dried and ground through a 4-mm sieve, were incubated in the rumen of the cows for up to 72 h and separately for 240 h (indigestible NDF, iNDF). A separate data set of experiments conducted at The Pennsylvania State University (n = 483 observations) was used to evaluate the relationships of undigested NDF (uNDF) of TMR or corn silage and in vivo total-tract NDF/ADF digestibility, DMI, and milk production. The relationships between in situ NDF degradability variables [b, potentially degradable NDF (mean ± SD; 95 ± 5.9%); c, rate of NDF degradation (1.14 ± 0.29%/h); ED, effective NDF degradability (36 ± 3.7%; at 2%/h passage rate); 24, 48, and 72 h NDF degradability (19.4 to 55.9, ± 5.40 to 3.68%); and iNDF (11.1 ± 1.37% of DM)] and NIRS analyses [NDF, % of DM (37 ± 2.5%); lignin, % of DM (3.3 ± 0.26%); NDF 24, 30, 48, or 240 h degradability (from 13.0 to 82.5%, ± 0.90 to 4.75%); total-tract NDF digestibility (34.1 ± 5.14%); and uNDF, % of DM or NDF (9.1 ± 1.37 and 24.5 ± 2.58%, respectively)] were non-existent or poor ($R^2 \leq 0.10$), except between iNDF and NDF ($R^2 \leq 0.24$; P < 0.001). The relationship of uNDF with lignin and 30-h NDF degradability was high (R^2 = 0.73 and 0.88, respectively; P < 0.001). The relationship of uNDF and in vivo NDF or ADF digestibility was moderate (R^2 = 0.29 and 0.43; P < 0.001). There was no relationship between uNDF and DMI or milk yield (P > 0.63). Overall, the relationship between NIRS fiber degradability analyses and in situ NDF degradability was poor; uNDF was moderately related to in vivo fiber digestibility, but had no relationship with DMI or milk yield in dairy cows.

Key Words: fiber degradability, corn silage, dairy cattle

T272 Relationships of TMR factors with sorting of prefresh dry period rations and postpartum subclinical ketosis in dairy herds fed anionic diets prepartum. A. L. Kerwin*, C. M. Ryan, A. Richards, and T. R. Overton, *Department of Animal Science, Cornell University, Ithaca, NY.*

The objective was to (1) identify relationships between prefresh diet sorting, as measured by herd urine pH coefficient of variation (pHCV), and management and TMR factors related to the initially fed (0 h) and 6 h post-feeding (6 h) rations, and (2) determine whether pre-fresh sorting factors are associated with herd level prevalence of hyperketonemia (herdBHB), defined as the percentage of fresh cows with BHB ≥1.2 mmol/L. Data were collected from commercial dairy herds (n = 23) fed pre-fresh rations supplemented with anions in a cross-sectional study design. Pre-fresh TMR particle size was analyzed at 0 h and 6 h, urine pH was measured on ~15 multiparous prefresh cows at 6 h postfeeding, and whole blood BHB concentrations (Nova Vet, Nova Biomedical, Waltham, MA) were measured on ~15 multiparous fresh cows. In SAS v. 9.4, univariable relationships were explored for each predictor with the outcomes phCV and herdBHB using PROC REG. Variables associated with pHCV (P < 0.20) were offered to a multivariable regression model. A backward selection method was used and variables remained if P < 0.10. The final model (P < 0.001) had 3 remaining variables for predicting pHCV; average herd urine pH (avepH), ash (%DM) at 0 h (Ash0h) and percent of DM at 0h (DM0h). The parameter estimates are presented in Table 1. The model resulted in an adjusted R^2 = 0.69. For interpretation purposes, a 1-unit increase in avepH, Ash0h, and DM0h results in the pHCV to increase by 4.73, 1.44, and decrease by 0.44,

respectively. No relationship existed between herdBHB and pHCV (R^2 = 0.06), avepH (R^2 = 0.01), or the percentage of TMR on the top sieve of the Penn State Particle Separator at 0h (R^2 = 0.00). This study provides insight on factors that may influence ration sortability, as reflected in the variation of the herd urine pH. The avepH was the most influential variable on pHCV and we did not find any biologically plausible prepartum variables to influence fresh cow health, as determined by herdBHB.

Table 1 (Abstr. T272).

Variable	Parameter estimate	SE	*P*-value
Intercept	−12.02	7.65	0.13
avepH	4.73	0.99	<0.001
DM0h	−0.44	0.10	0.007
Ash0h	1.44	0.47	<0.001

Key Words: sorting, prefresh TMR, urine pH

T273 Effects of lysophospholipids on rumen fermentation and bacterial population in dairy cows: In vitro and in vivo. C. Lee[1], D. L. Morris[1], J. M. Hettick[1], S.-H. Kim[1], J. E. Copelin[1], and I. Kwon*[2], [1]*Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH,* [2]*Easy Bio Inc., Seoul, South Korea.*

The current study examined lysophospholipids (LPL) on rumen fermentation and bacterial populations in vitro and in vivo. An in vitro batch culture was conducted and the substrate used for the incubation was a ground dairy diet (55% forage and 45% concentrate). Dietary treatments were CON (control), L-LPL, (0.025% LPL in dietary DM; Lipidol Ultra, EASY BIO, Inc.); M-LPL, (0.05%), and H-LPL (0.075%). The incubation was conducted for 48 h in triplicate and the incubation was repeated. All data were analyzed using the MIXED procedure of SAS (incubation as random). Supplementation of LPL tended to increase (linear $P = 0.058$) propionate as % of total VFA and decrease (linear $P = 0.06$) A:P ratio. In addition, LPL increased (linearly $P < 0.01$) ammonia concentration. An in vivo experiment was conducted with 8 cannulated lactating cows in a repeated 4 × 4 Latin square design. Cows were fed a basal diet (CON) supplemented with MON (16 mg/kg in dietary DM), M-LPL or H-LPL. Rumen contents were collected on 2 consecutive days to determine pH, ammonia, VFA, and bacterial population. Bacterial populations were determined using bacterial tag-encoded FLX amplicon pyrosequencing. All data were analyzed using the MIXED procedure of SAS (group and cow within group as random). A decrease (linear $P = 0.03$) in acetate and numerical increase ($P = 0.13$) in propionate as % of total VFA resulted in a tendency for a linear decrease ($P = 0.09$) in the A:P ratio with increasing LPL. Rumen bacterial population was significantly altered by MON vs. CON. However, minimal effects of LPL on microbial population were observed. Increasing LPL in the diet did not affect ($P > 0.14$) any bacterial populations at phylum level. However, LPL decreased (linear $P = 0.05$) the proportion of *Rikenella* and *Paludibacter*. The proportion of *Fibrobacter* tended to be greater ($P = 0.09$) for LPL vs. CON. In conclusion, the pattern of rumen fermentation altered by LPL was generally agreed between in vitro and in vivo. Despite the changes in rumen fermentation by LPL vs. CON, there were minimal effects on bacterial population in the rumen.

Key Words: lysophospholipid, rumen fermentation, bacterial population

T274 Feed, nitrogen and energy conversion efficiencies of lactating Holstein and Jersey cows fed 2 levels and 2 sources of forage neutral detergent fiber. M. E. Uddin*, O. I. Santana, D. D'Huyvetter, T. Wickert, and M. A. Wattiaux, *Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

Our objective was to determine the effects of replacing alfalfa silage (AS) NDF with corn silage (CS) NDF at 2 levels of forage NDF (FNDF) on performances and efficiencies of Holstein (H) and Jersey (J) cows. Twelve H and 12 J cows (primiparous and mid-lactation) housed in tie-stalls, fed once and milked twice daily, were fed 4 total mixed rations in a 2 × 2 factorial arrangement of FNDF level (20 and 26% of DM) and FNDF sources (70:30 and 30:70 ratio of AS:CS NDF). All diets had identical crude protein (16.5% of DM) and starch (24% of DM). The design was a split plot triplicated 4 × 4 Latin square with breed in the main plot and diet in subplots. Data presented here were collected in wk 3 and 4 of each 28-d period. Data were analyzed using proc Mixed of SAS. Effects presented here had $P < 0.05$. Holstein cows had greater dry matter intake (DMI, 22.5 vs. 16.8 kg/d), milk yield (MY, 33.2 vs. 21.3 kg/d), fat protein corrected milk (FPCM, 32.7 vs. 24.9 kg/d), fat yield (FY,1.37 vs. 1.12 kg/d), protein yield (PY, 0.97 vs. 0.76 kg/d), and lactose yield (LY, 1.61 vs. 1.01 kg/d) but lower fat (4.13 vs. 5.28%) and protein (2.93 vs. 3.56%) than J cows. Cows fed low FNDF diet had greater DMI (20.5 vs. 18.8 kg/d), MY (27.7 vs. 26.7 kg/d), FPCM (29.5 vs. 28.2 kg/d), FY (1.27 vs. 1.22 kg/d), PY (0.89 vs. 0.83 kg/d), LY (1.35 vs. 1.27 kg/d), protein (3.28 vs. 3.20%) and lactose (4.83 vs. 4.77%) but lower feed efficiency (FE, 1.35 vs. 1.43 kg MY/kg DMI; 1.45 vs. 1.52 kg FPCM/kg DMI), net energy of lactation (NEL) efficiency (0.71 vs. 0.74 Mcal milk NEL/NEL intake) and N efficiency (0.26 vs. 0.27 milk N/N intake) than cows fed high FNDF diet. Cows fed AS based diet had lower DMI (19.1 vs. 20.3 kg/d), MY (26.9 vs. 27.6 kg/d), FPCM (28.3 vs. 29.3 kg/d), FY (1.22 vs. 1.26 kg/d), PY (0.84 vs. 0.88 kg/d), LY (1.29 vs. 1.33 kg/d) and protein (3.22 vs. 3.27%) but greater FE (1.42 vs. 1.36 kg MY/kg DMI) than cows fed CS diet. In conclusion, cows fed high FNDF and high AS based-diets were more efficient than their counterpart, but breed did not affect efficiencies.

Key Words: alfalfa silage, corn silage, cow breed

T275 Effects of *Saccharomyces cerevisiae* fermentaion product supplementation during the periparturient period on rumen pH of dairy cows fed postpartum diets differing in starch content. W. Shi*[1], C. E. Knoblock[1], I. Yoon[2], and M. Oba[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Diamond V, Cedar Rapids, IA.*

The objective of this study was to determine the effects of feeding a *Saccharomyces cerevisiae* fermentation product (SCFP; NutriTek, Diamond V, Cedar Rapids, IA) during the periparturient period on rumen fermentation of dairy cows fed diets differing in starch content during the first 3 wk after calving using a randomized block design. From wk 4 before the expected calving date to 3 wk after calving, 18 ruminally cannulated multiparous Holstein cows were fed diets without (CON; n = 9) or with SCFP (SCFP; n = 9). All cows were fed a common basal diet (1.43 Mcal NE_L/kg DM; 13% starch) before calving. Cows within each treatment (CON or SCFP) were fed either a low- (LS; 21% starch; n = 9) or high-starch (HS; 27% starch; n = 9) diet from wk 1 to 3 after calving. Rumen pH was measured using a submersible continuous ruminal pH measurement system and VFA profile was analyzed by gas chromatography. Data were analyzed using the FIT model procedure of JMP (SAS Institute Inc., Cary, NC). Duration of pH below 5.8 was longer (406 vs. 195 min/d; $P = 0.04$) for cows fed the LS diets than the HS diets on d 6 after calving. The LS cows tended to have higher

molar proportion of acetate (60.6 vs. 58.2 mol/100 mol; $P = 0.08$) and lower molar proportion of propionate (23.1 vs. 25.2 mol/100 mol; $P = 0.10$) on d 21 to 23 after calving. Supplementation of SCFP tended to increase the area below pH 5.8 (54.1 vs. 16.7 pH × min/d; $P = 0.09$) than CON on d 10 to 13 after calving, but tended to increase minimum rumen pH (5.64 vs. 5.44; $P = 0.07$) and tended to decrease duration of pH below 5.8 (116 vs. 323 min/d; $P = 0.10$) on d 21 to 23 after calving. These findings suggest that feeding low starch diets during the immediate postpartum period may not necessarily decrease the risk of sub-acute ruminal acidosis (SARA; pH <5.8) of dairy cows fed a low-energy close-up diet, and that SCFP supplementation has inconsistent effects on rumen pH after calving but may reduce the risk of SARA at the end of calving transition period.

Key Words: calving transition, dietary starch, *Saccharomyces cerevisiae* fermentation product

T276 Ruminal protein degradation of faba bean (*Vicia faba* L. major): Effect of variety and pelleting temperature. F. Hassanat*[1], R. Pilote[2], S. Claveau[2], and C. Benchaar[1], [1]*Sherbrooke Research and Development Center-Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [2]*Agrinova, Alma, QC, Canada.*

The objective of this study was to examine the effect of 2 varieties (Minica and Primo) and pelleting temperature (untreated, 60°C, and 84°C) of fava bean (FB) on in situ ruminal degradation of CP. For this purpose, 4 rumen cannulated dairy cows were used in a completely randomized block design with a 2 × 3 factorial arrangement of treatments. Samples of FB were ground (2-mm), weighed (5-g) in triplicate in polyester bags, and incubated in the rumen for 0, 2, 4, 8, 16, 24, 48 and 72h. Ruminal degradation kinetic parameters of CP were determined using a nonlinear model: $P = a + b\ (1 - e^{-c(t\ -\ L)})$, where P = disappearance at incubation time t (h), a = soluble and rapidly degradable fraction (%), b = slowly degradable fraction (%), c (/h) = fractional rate of disappearance of the fraction b, and L = lag time (h). Effective rumen degradability (ERD) was calculated as: $a + [bc/(c + k_p)] \times e^{(-kpL)}$ with k_p = ruminal fractional passage rate of 6.1%/h. Data were analyzed using the MIXED procedure (SAS) with variety, pelleting temperature and variety × pelleting temperature interaction as fixed effects while cow was a random effect. Significance was declared when $P \leq 0.05$. Compared with untreated treatment, pelleting FB (Minica and Primo) at 60°C increased (+26%) the soluble fraction (a), while the extent of increase was higher for Primo than for Minica (+17 vs. + 9%) when pelleted at 84°C (interaction variety × pelleting temperature; $P = 0.01$). The fraction (b) was similar between the 2 varieties and was lower ($P < 0.01$) for the FB pelleted at 60°C (20%) and 84°C (27%) than for the untreated FB (36%). The rate of disappearance (c) of the fraction (b) was unaffected by experimental treatments. An increase ($P < 0.01$) in ERD of CP was observed when FB (Minica and Primo) were treated at 60°C and 84°C, but the magnitude of increase was higher for Minica (+14%) than for Primo (+8%) when treated at 60°C (interaction variety × pelleting temperature; $P = 0.03$). We conclude that pelleting FB at 60°C and 84°C increased CP solubility and consequently, enhanced ERD of CP. The extent of the effect of pelleting temperature on CP degradability varied with the variety of FB used.

Key Words: faba bean, pelleting temperature, CP degradation in situ

T277 In vitro assessment of oil releasing extent from calcium salt of fat supplements in different sites of gastrointestinal tract. P. Peravian[1], M. Dehghan Banadaky*[2], H. Mirzai[3], P. Rezamand[4], and H. Khalilvandi[5], [1]*University of Tehran, Tehran, Tehran, Iran,* [2]*College of Agriculture and Natural Science, University of Tehran, Alborz, Karaj, Iran,* [3]*Zanjan University, Zanjan, Iran,* [4]*Animal and Veterinary Science Department, University of Idaho, Moscow, ID,* [5]*University of Urmia, Urmia, Iran.*

The purpose of this study was the evaluation of oil releasing extent (ORE) in fat calcium salt supplements (Persiafat) in simulated culture of gastrointestinal tract (GT) < rumen, abomasum, small intestine (SI) >. Treatments included 1.5 g fish oil, 1.5 g flaxseed oil, 1.5 g blended oil, 1.5 g mixture of fish and flaxseed oil. Rumen fluid (RF) was collected from fistulated cow 4 h after feeding. Anaerobic environment was provided by RF filteration in textile and using CO_2 gas. Six bottles were used for incubation in each site of GT. 1.5 g fat calcium salt and 50 mL rumen fluid were added in each bottle, pH was adjusted to 6.4 and incubation was done in 39°c with 100 rpm for 24 h in batch culture. Bottle components were filtered in textile after incubation and fat extraction was done by Folch (chloroform/methanol (2/1)) method. ORE in abomasum and SI was measured by preparing 3.2 g pepsin (pH = 1.2) and 10 g pancreatin (pH = 6.8) with specific solution for abomasum and SI in order. Incubation time was 3 h for both abomasum and SI. The steps of measuring ORE was done according to rumen but petroleum solution was utilized rather than Folch. The results showed that percent of oil releasing between treatments in rumen were significant ($P < 0.01$) (15.64, 19.93, 14.8, 11.11 for treatment 1–4 respectively), however the highest was for flaxseed and the lowest was for blended oil. As it was expected, low pH in abomasum dissociated the linkage between calcium and fatty acid so the highest ORE was in abomasum in compare of other sites of GT (90% <) and the percent of oil releasing between treatments were not significant ($P > 0.05$). (87.02, 93.79, 94.84, 94.40 for treatment 1–4 respectively). In small intestine treatments affect ORE ($P < 0.01$) (6.84, 8.14, 5.17, 7.99 for treatment 1–4 respectively) and the highest was for flaxseed and the lowest was for fish oil. According to results of this study, calcium salt of fatty acid could be used with minimum effects on rumen fermentation because lowest oil releasing happend in rumen in compare of other protection methods.

Key Words: gastrointestinal tract, calcium salt of fatty acid, oil releasing extent

T278 Supplementation of rumen-protected lysine (AjiPro-L) during the close-up dry period affect prepartum feed intake and lactation performance in dairy cows. S. Ishimaru*[1], H. Funo[2], M. Nakamura[2], I. Shinzato[3], Y. Ohta[3], K. Nakagawa[3], A. Haruno[3], T. Obitsu[1], and T. Sugino[1], [1]*The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima, Japan,* [2]*Shimane Prefectual Livestock Technology Center, Izumo, Japan,* [3]*Ajinomoto Co., Tokyo, Japan.*

This study evaluated the effect of supplementation of rumen-protected (RP) lysine, AjiPro-L (Ajinomoto Co. Inc., Tokyo), during the close-up dry period on nutrient metabolism and lactaion performance in early lactating cows. Twenty pregnant Holstein dairy cows (8 primiparous and 12 multiparous) were housed in individual stalls during the last 21 d of gestation. From 21 d before expecting calving to parturition, cows were blocked by parity and randomly assigned 1 of the following 2 treatments: (1) control diet [CP: 13.3% of DM, NE_L: 1.36 kcal/g of DM, metabolizable lysine: 81 g/d; Con]; (2) RP lysine supply for control diet [metabolizable lysine: 97g/d; Lys]. After calving, all cows were managed in freestall barns; TMR was provided 3 times daily and milking done twice daily. Blood samples were collected from the jugular vein at −21, −14, 0 (calving date), 7, 21, and 35 d. A mixed model was used to determine the effects of treatment and time as repeated measures, and their interaction. Dry matter intake (DMI) was higher for Lys (12.4

kg/d; LSM) than for Con (10.4 kg/d), during the prepartum period (P = 0.03). After calving, DMI tended to be higher for Lys (14.5 kg/d) than for Con (11.6 kg/d) during the first 21 d. In addition, milk yield tended to be greater for Lys (27.9 kg/d) than for Con (24.2 kg/d). Milk fat and lactose yield were greater for Lys (1.46, 1.41 kg/d) than for Con (1.17, 1.19 kg/d), respectively (P = 0.02). Furthermore, liver functionality index, (calculated using plasma concentrations of albumin, cholesterol and bilirubin) tended to be higher for Lys than for Con after calving (P = 0.08), whereas plasma hormone concentrations (ghrelin, insulin-like growth factor 1, growth hormone, glucagon and insulin) did not differ between treatment groups. In conclusion, RP lysine supplementation during the close-up dry period increased DMI in the peripartum period, resulting in enhanced lactating performance, and may offer benefits in terms of liver functionality in early lactating cows.

Key Words: rumen-protected lysine, dry period, metabolism

T279 Effects of feeding reduced-fat distillers grains with and without monensin on nitrogen, phosphorus, and sulfur utilization and excretion in dairy cows. D. L. Morris*, S.-H. Kim, and C. Lee, *Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH.*

This study investigated effects of high inclusion of reduced-fat corn distillers grains with solubles (RFDG) with and without monensin on utilization and excretion of N, P, and S. The experiment was conducted with 36 Holstein cows in a randomized block design for 11 wk. Cows were blocked by parity, and days in milk and assigned to the following diets: (1) CON, a control diet; (2) DG, CON with RFDG included at 28.8% (% of DM) replacing soybean meal, soyhulls, and supplemental fat and P; (3) DGMon, DG with monensin at 20 mg/kg of DM. Milk and spot urine and fecal samples were collected in wk 3, 7, and 11 to determine dietary N, P, and S utilization and excretion. Data were analyzed with a model that included the fixed effect of diet, week, and 2-way interaction, and random effect of block and cow within block. Repeated measures were modeled. Compared with CON, DG and DGMon did not change crude protein content (17.6%) but increased rumen undegradable protein, P (0.48 vs. 0.36%) and S content (0.41 vs. 0.21%; DM basis). As a result, DG vs. CON decreased (P < 0.01) plasma and milk urea-N concentration, and urinary N excretion. Although P intake, plasma P concentration, and urinary and fecal P excretion increased (P < 0.05) for DG vs. CON, milk P secretion was not affected. Intake of S was greater (P < 0.01) for cows fed the DG vs. CON, resulting in greater (P < 0.03) plasma total S and sulfate concentration and urinary and fecal S excretion. However, milk S secretion was not affected by DG compared with CON. Monensin supplementation to the DG diet did not affect N intake, utilization, and excretion except that apparent N digestibility was lower (P < 0.04) compared with DG. In addition, DGMon did not affect P and S utilization and excretion compared with DG. This study suggests that a high inclusion of RFDG can decrease urinary N excretion but increase manure P and S excretion. However, monensin supplementation to a high-RFDG diet, overall, had minimal effects on N, P, and S utilization and excretion in lactating dairy cows.

Key Words: reduced-fat distillers grains, monensin, nutrient excretion

T280 In vitro screening of technical lignins for their antifungal activity against fungi isolated from spoiled hay. D. C. Reyes*[1], S. L. Annis[2], S. A. Rivera[1], D. S. Argyropoulos[3], J. J. Perry[4], C. Wu[6], S. Alparslan[1], D. Gomez[1], D. DePippo[1], M. S. Castillo[5], and J. J. Romero[1], [1]*Animal and Veterinary Sciences, University of Maine, Orono, ME,* [2]*School of Biology and Ecology, University of Maine, Orono, ME,* [3]*Department of Forest Biomaterials, North Carolina State University, Raleigh, NC,* [4]*Food Science and Human Nutrition, University of Maine, Orono, ME,* [5]*Department of Crop and Soil Science, North Carolina State University, Raleigh, NC,* [6]*Department of Animal and Food Sciences, University of Delaware, Newark, DE.*

The objective of this experiment was to screen a set of technical lignins for their antifungal activity in vitro. Treatments evaluated (4% wt/vol) were (1) Kraft lignin (UW), (2) treatment (TRT) 1 acetone insoluble fraction (AI), (3) TRT 1 hexane insoluble (HEX), (4) TRT 1 Hexane soluble (PI), (5) alkali Kraft lignin (AKL), (6) Na lignosulfonate (NaL), (7) Mg lignosulfonate (MgL), (8) ammonium lignosulfonate (AMOL), (9) propionic acid (PRP), and (10) Control (untreated). *Mucor circinelloides*, *Penicillium solitum*, *Aspergillus amoenus*, and *Debaryomyces hansenii* were isolated from spoiled alfalfa and timothy hay. The experiment had a complete randomized block design (CRBD, 4 runs) and a factorial arrangement of 3 molds (MLD) × 10 TRT. A CRBD was used to test for the yeast (*D. hansenii*). Data were analyzed with PROC GLM (SAS 9.4); when an interaction was present, the SLICE option was used. Differences were declared at $P \leq 0.05$. We found an interaction effect of MLD × TRT (P < 0.001). For *A. amoenus* we observed 100 ± 2.77% antifungal activity for PRP and NaL, followed by MgL (40.9%) and AKL (12.1%, $P \leq 0.05$). Inhibition by other TRT was <5%. For *M. circinelloides* the most effective TRT were PRP and NaL (100 ± 2.77%), followed by MgL (73%) and AKL (49.7%; $P \leq 0.05$). Inhibition by other TRT was <10%. For *P. solitum* the most effective TRT were PRP and NaL (100 ± 2.77%), followed by MgL, (28.1%), and PI (8.1%; $P \leq 0.05$). Inhibition by other TRT was <1%. For NaL, PRP, and HEX there were not differences across MLD. However, for MgL, different inhibitions were observed across *M. circinelloides*, *A. amoenus*, and *P. solitum*, (72.9, 40.9, and 28.1 ± 2.77%, respectively; $P \leq 0.05$) as well for AKL (49.7, 12.1, and −8.0 ± 2.77%, respectively; $P \leq 0.05$). Overall, *M. circinelloides* was the most sensitive mold, followed by *A. amoenus,* and *P. solitum*. For *D. hansenii*, we identified PRP, NaL, and MgL as the most effective TRT with 100 ± 3% antifungal activity, followed by AKL, AMOL, PI, and UW (9.8, 9.9, 10.6, and 8.4% respectively; $P \leq 0.05$). Inhibition by other TRT was <4%. PRP, NaL, MgL, and AKL were found to have the most antifungal activity.

Key Words: hay, spoilage, technical lignins

T281 Effects of dietary cation-anion difference (DCAD) on acid-base status and DMI in primigravid cows. R. Zimpel*[1], M. B. Pondexter[1], A. Vieira-Neto[1], E. Block[2], C. R. Staples[1], W. W. Thatcher[1], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*Arm and Hammer Animal Nutrition, Princeton, NJ.*

The objective was to determine if reduction in DMI by diets with negative DCAD is mediated by inclusion of acidogenic products (AP), content of Cl, or by changes in acid-base (AB) status. The hypothesis was that the decrease in DMI is mediated by metabolic acidosis and not by feeding AP. Ten nulliparous Holstein cows at 148 ± 8 d of gestation were enrolled in a duplicated 5 × 5 Latin square design. The DCAD of diets and AB status were manipulated by incorporating an AP or adding salts containing Cl, Na, and/or K to the diets. Treatments were a base diet (BD) containing 55% corn silage, 10% grass hay, and 35% concentrate, without any added AP or salts (T1; K = 1.42%, Na = 0.04%, Cl = 0.26%; DCAD = 196 mEq/kg); the BD with added 1% NaCl and 1% KCl (T2; K = 1.83%, Na = 0.42%, Cl = 1.23%; DCAD = 194 mEq/kg); the BD with added 7.5% AP and 1.5% $NaHCO_3$ and 1% K_2CO_3 (T3; K = 1.71%, Na = 0.54%, Cl = 0.89%; DCAD = 192 mEq/kg); the BD with added 7.5% AP (T4; K = 1.29%, Na = 0.13%, Cl =

0.91%; DCAD = −114 mEq/kg); and the BD with 7.5% AP, 1% NaCl, and 1% KCl (T5; K = 1.78%, Na = 0.53%, Cl = 2.03%, DCAD = −113 mEq/kg). Periods lasted 14 d with the last 7 d used for data collection. Data were analyzed by the MIXED procedure of SAS for a replicated Latin square design. Feeding behavior was evaluated for 12 h/d in the last 2 d of each period. Contrasts evaluated included the effects of AP (AP: T1 vs. T4), AB status (AB:T2+T3 vs. T4+T5), and addition of Cl salts to an alkalogenic diet (ClAlk: T1 vs. T2) or to an acidogenic diet (ClAc: T4 vs. T5). Feeding acidogenic diets induced a compensated metabolic acidosis and reduced DMI but correcting the metabolic acidosis reestablished DMI in dry cows. The decrease in DMI in diets with negative DCAD was mediated by metabolic acidosis and not by addition of AP or Cl salts.

Table 1 (Abstr. T281).

Item	T1	T2	T3	T4	T5	SE
DMI, kg/d*§	10.3	10.2	10.2	9.7	9.5	0.2
DMI, % of BW*§	1.76	1.75	1.74	1.68	1.64	0.03
Urine pH*§‡	8.1	7.9	7.9	5.7	5.6	0.06
Blood pH*§‡	7.450	7.436	7.435	7.420	7.416	0.005
Blood HCO_3^-, m*M*†§	25.9	25.5	25.8	24.3	23.7	0.3
Eating bouts, n/12h*§	6.8	6.3	7.1	5.8	6.1	0.4
Intermeal interval, min*§	90.8	99.3	84.7	111.5	104.0	6.8

*AP ($P < 0.05$); §AB ($P < 0.05$); ‡ClAlk ($P < 0.05$); †ClAc ($P < 0.05$).

Key Words: acid-base status, DMI, DCAD

T282 D-Lactate metabolism in dairy cows under risk for subacute ruminal acidosis. L. E. Hernández-Castellano*, A. C. Storm, T. Larsen, and M. Larsen, *Department of Animal Science, Aarhus University-Foulum, Tjele, Denmark.*

D-Lactate has been proposed as biomarker for subacute ruminal acidosis (SARA). This study aimed to evaluate D-lactate absorption and metabolism in dairy cows under high or low risk for SARA. Six lactating Holstein dairy cows fitted with ruminal cannula and indwelling catheters in major splanchnic blood vessels were used. Animals were randomly assigned to either a SARA diet (80:20 concentrate to forage; 370 g starch/kg dry matter) or a control diet (50:50 concentrate to forage; 225 g starch/kg dry matter) in a cross over design with 28-d periods. On d 20, 8 hourly sample sets of fluid from the medial and ventral rumen, and blood from an arterial, portal and hepatic vein were collected, starting 30 min after the morning feeding. After 4 h relative to feeding, 1.75 kg of ground barley was administrated via the ruminal cannula in all cows. Net D-Lactate releases from the portal drained viscera (PDV) and the total splanchnic tissues (TSP) were calculated. The MIXED procedure of SAS was used considering sampling time as repeated measure. No differences were detected between groups for DMI (20.3 ± 0.8 kg/d; $P > 0.05$) and milk yield (30.5 ± 1.2 kg/d; $P > 0.05$). In the first 4 h after feeding, ventral rumen pH did not differ between treatments ($P > 0.05$). Medial rumen pH and D-lactate concentration in ventral and medial rumen constantly decreased in both groups after morning feeding ($P < 0.05$), but did not differ between treatments ($P > 0.05$). Arterial D-lactate as well as the net PDV release of D-lactate decreased after feeding ($P < 0.05$), but did not differ between treatments ($P > 0.05$). The D-lactate release from TSP decreased in both groups after feeding ($P < 0.05$), but did not differ between treatments ($P > 0.05$). The direct rumen administration of ground barley at 4 h after feeding increased D-lactate release from TSP in both groups ($P < 0.05$), but the increase did not differ between treatments ($P > 0.05$). The other variables were not affected by either time after administration or diet ($P > 0.05$). In conclusion, ruminal D-lactate absorption and metabolism did not differ in cows fed the SARA diet compared with the control. The lack of differences between experimental groups may indicate adaptation of the microflora to high starch diets.

Key Words: dairy cow, D-lactate, ruminal acidosis

T283 Effects of close-up dietary energy level and supplementing rumen protected lysine on blood β-hydroxybutyrate concentration and milk production in transition cow. G. D. Delelesse[1], L. Ma[1,2], F. Wang[1], Q. Jiang[1], and D. Bu*[1,3], [1]*Institute of Animal Science, State key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China,* [3]*Hunan Co-Innovation Center of Safety Animal Production, Changsha, Hunan, China.*

The objective of this study was to investigate the close-up dietary energy level and supplementing of rumen-protected lysine on blood β-hydroxybutyrate level, dry matter intake, milk production and composition in transition cows. Sixty-eight multiparous Holstein cows were allocated to 4 treatments arrange in a 2 × 2 factorial arrangement with dietary NE_L level (1.37 Mcal/kg vs 1.53 Mcal/kg: HE vs LE) and addition of rumen protected lysine (from Ascor ChimiciSrl, 40 g or 0 g: Blys or CK) as 2 factors. All cows were randomly allocated into 4 treatment groups (HEBlys, HECK, LEBlys and LECK) after balanced in their expected calving date, previous milk yield and body condition score. All cows were fed with the same NE_L (1.34 Mcal/kg DM) diet in dry off period. Rumen protected-lysine was top dressed at 40 g/d/cow to total mixed ration 3 weeks before to 3 weeks after calving. After calving all cows received the same NE_L (1.69 Mcal/kg DM) diet. Blood samples were collected at −21, −14, −7, 0, 3, 7, 14 and 21 d relative to calving and blood β-hydroxybutyrate concentration was measured. Amount of feeds offered and refused for individual cows were collected and measured 4 d per week. Milk samples were collected once per week and analyzed for milk composition. Data were analyzed using proc mixed SAS (SAS institute, Inc., Cary NC). Feeding high NE_L to close up cows decreased the concentration of β hydroxybutyrate (0.55 mol/mL vs 0.37 mol/mL, $P < 0.0001$) in pre-partum cows. Rumen protected lysine improved postpartum dry matter intake (18.9 kg/d vs 19.5 kg/d, $P < 0.0001$) and decreased β-hydroxybutyrate concentration (1.62 mol/mL vs 0.95 mol/mL, $P < 0.01$). Either energy or bypass lysine and their interaction did not affect milk yield, and percentage of milk fat and protein. Cows fed HE together with Bypass lysine reduced in β-hydroxybutyrate concentration (1.09 mol/mL vs 0.81 mol/mL, $P < 0.02$) in postpartum cows and tended to produce more milk. This revealed that feeding high-energy diet together with rumen protected lysine favorable in improving dry matter intake and lower β-hydroxybutyrate levels in transition cow.

Key Words: β-hydroxybutyrate, rumen-protected lysine, transition cow

T284 Effects of supplementing active dry yeast, a blend of probiotic bacteria, or the combination on the performance and total-tract digestion of growing steers. T. A. Batchelder*, Y. Liang, E. Davis, and M. A. Ballou, *Texas Tech University, Lubbock, TX.*

Impacts of feeding live yeast, probiotic bacteria, or a combination on the performance and apparent total-tract digestion in 3 mo old steers. Experimental design was an incomplete replicated 4 × 4 Latin square

design with 4 replicates and 3 periods. Sixty-four calves were blocked by initial BW and randomly assigned within blocks to a pen with 3 calves per pen. Dietary treatments were arranged as a 2 × 2 factorial and included: Control, no supplement; Yeast, supplemented with 1.0 × 1010 cfu/d live *Saccharomyces cerevesiae*; Probiotic, supplemented 2.5 × 109 cfu/d of a blend of *Enterococcus faecium* and *Lactobacillus casei*; or Combination, supplemented with both the Yeast and Probiotic treatments. Periods consisted of a 10 d adaptation period followed by a 4 d collection period. Individual BW were measured at the beginning and end of each collection period and feed intake daily. Fresh fecal samples were collected from the surface of each pen 8 times during the collection period and composited for subsequent nutrient analyses and calculation of apparent total-tract nutrient digestibility using iNDF240. The model included the fixed effects of Yeast, Probiotic, and their interaction. Period was included as a random intercept and treatment nested within pen was the subject of the repeated statement. There was a tendency ($P = 0.057$) for calves fed Yeast to have increased DMI (4.44 vs 4.22 ± 0.44 kg/d). The ADG of all treatments tended ($P = 0.054$) to be greater when compared with the Control (1.41, 1.44, 1.39, and 1.26 ± 0.16 kg/d for Yeast, Probiotic, Combination, and Control, respectively). There were no differences ($P \geq 0.189$) in apparent NDF or CP digestibility, but there was a tendency ($P = 0.064$) for a Yeast × Probiotic interaction on ADF digestibility (30.9, 32.0, 29.8, and 29.7 ± 0.90% for Yeast, Probiotic, Combination, and Control, respectively). Data indicate that supplementing growing steers with specific active dry yeast or probiotic bacteria can influence fiber digestibility and improve performance, but the mechanisms of action may be different.

Key Words: probiotic, rumen, yeast

T285 Rumen disappearance of carvacrol and anethole in lactating dairy cows. J. Oh*[1], M. Harper[1], P. Smith[1], E. Wall[2], and A. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Pancosma, Geneva, Switzerland.*

Information on rumen disappearance of phytonutrients is needed to assess their potential post-ruminal effects in ruminants. The objective of this study was to investigate rumen disappearance rates and escape of 2 phenolic phytonutrients, carvacrol (CAR) and anethole (ANE), in 2 experiments using dairy cows. Each experiment involved 4 mid-lactation Holstein cows (average ± SD; 26 ± 2.8 kg/d DMI; 41 ± 3.1 kg/d milk yield; 134 ± 8.5 d in milk) and consisted of 3, 14-d experimental periods. In Exp. 1, CAR was administered into the rumen of the cows at 3 doses: 0.5, 1.0, and 2.0 g/cow in periods 1, 2, and 3, respectively. Chromium-EDTA was used as a rumen fluid passage rate marker. On the first day of each experimental period, CAR and Cr-EDTA solutions were administered intraruminally in each cow through the rumen cannula at the time of feeding. Rumen fluid samples were collected from 4 locations in the rumen at 0 (background), 0.5, 1, 2, 6, 12, and 24 h after CAR administration. The same procedure was conducted for ANE in Exp. 2; ANE doses were the same as for CAR. Concentration of CAR and ANE in ruminal fluid were fitted to a single exponential decay model ($y = a \times \exp[-b \times x]$). The rate of degradation of CAR or ANE was found as: k_{CAR} or $k_{ANE} - k_{Cr}$, where k_{Cr} is the slope of decline in Cr concentration in ruminal fluid (on average 0.20 ± 0.07 h^{-1}) and k_{CAR} and k_{ANE} are slopes of decline in CAR and ANE concentrations, respectively. Rumen escape was calculated as: $k_{Cr} \div (k_{Cr} + kd)$, where kd is the rate of degradation of CAR or ANE. Rumen disappearance rates of CAR were 0.29 ± 0.05, 0.31 ± 0.07, and 0.34 ± 0.08 h^{-1} and those of ANE were 1.43 ± 0.29, 1.52 ± 0.43, and 1.42 ± 0.38 h^{-1} for the 0.5, 1.0, and 2.0 g/cow doses, respectively. The rates of degradation of CAR were estimated at 0.15 ± 0.07, 0.13 ± 0.06, and 0.09 ± 0.07 h^{-1} and those of ANE were 1.24 ± 0.27, 1.31 ± 0.41, and 1.24 ± 0.43 h^{-1}, respectively. Rumen escape of CAR was 47.2, 59.3, and 73.8% and that of ANE was 13.7, 14.8, and 14.2%, respectively. In this study, rumen escape of CAR and ANE in lactating dairy cows was from 13.7 to 73.8%, depending on dose and compound.

Key Words: carvacrol, anethole, rumen escape

T286 A meta-analysis on intestinal digestibility of long-chain fatty acids in lactating dairy cows. J. de Souza*[1], H. Leskinen[2], K. J. Shingfield[4,2], P. Huhtanen[3], and A. L. Lock[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Animal Genomics, Green Technology, Natural Resources Institute Finland (Luke), Jokioinen, Finland,* [3]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden,* [4]*Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK.*

The objective of our meta-analysis was to determine intestinal digestibilities for individual long-chain fatty acids (FA) in lactating dairy cows. Our analysis utilized individual observations (n = 79) of lactating Nordic Red dairy cows from 6 Latin square or switch-back design studies. Digesta flow measurements were conducted using omasal sampling with a triple-marker system and FA were determined in omasal and fecal samples. We estimated the intestinal digestibility of long-chain FA (16:0, 18:0, *cis*-9 18:1, 18:2n-6, 18:3n-3 and total FA) using a Lucas test with a regression of the omasal flow of FA (g/kg DMI) on absorbed FA (g/kg DMI); slopes indicate true digestibility and intercepts endogenous synthesis. Mixed model regressions were developed between the variables of interest taking into account experiment, period within experiment, and cow within experiment as random factors. Based on the Lucas tests, estimated intestinal digestibility coefficients were 0.78, 0.70, 0.83, 0.77, 0.75, and 0.75, for 16:0, 18:0, *cis*-9 18:1, 18:2n-6, 18:3n-3 and total FA, respectively (Table 1). Increasing 16:0 omasal flow (43 to 145 g/d) did not affect 16:0 digestibility, while increasing 18:0 omasal flow (36 to 1306 g/d) linearly decreased 18:0 digestibility (%) [93 ± 2.16 – 0.02 ± 0.003 × 18:0 flow (g/d), $P < 0.001$, $R^2 = 0.69$]. Increasing *cis*-9 18:1 flow (6 to 130 g/d) increased in a quadratic manner *cis*-9 18:1 digestibility [56.5 ± 2.91 + 0.68 ± 0.13 × *cis*-9 18:1 flow (g/d) - 0.004 ± 0.0001 × *cis*-9 $18:1^2$ (g/d), $P < 0.001$, $R^2 = 0.45$]. Our results demonstrate that 18:0 has a lower digestibility coefficient compared with 16:0 and unsaturated FA, which may be associated with lower solubility of this FA, limits in emulsification capacity as flow increases, and a larger flow compared with other FA.

Table 1 (Abstr. T286). Influence of omasal flow of FA (g/Kg DMI) on absorbed FA (g/kg DMI) estimated by mixed model regression analysis (FA= A + BX_1)

Variable	A	SE	*P*-value	B	SE	*P*-value	R^2
16:0	−0.28	0.12	0.08	0.78	0.021	<0.001	0.97
18:0	2.49	0.87	0.05	0.70	0.024	<0.001	0.98
cis-9 18:1	−0.15	0.03	0.01	0.83	0.014	<0.001	0.99
18:2n-6	−0.034	0.012	0.01	0.77	0.016	<0.001	0.98
18:3n-3	−0.012	0.004	0.05	0.75	0.013	<0.001	0.99
Total FA	1.50	1.20	0.28	0.75	0.021	<0.001	0.96

Key Words: fatty acids, digestibility, meta-analysis

T287 Effect of rumen-protected B vitamins and choline supplementation on feed intake, milk production and liver health of transition dairy cows. E. I. Morrison*[1], H. Reinhardt[1], J. J. Loor[2], H. Leclerc[3], and S. J. LeBlanc[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*University of Illinois, Urbana, IL,* [3]*Jefo, St. Hyacinthe, QC, Canada.*

Dairy cows may benefit from dietary supplementation with rumen-protected B vitamins and choline (RPBC) during the transition period. The objective was to determine the effect of a commercially available RPBC supplement on feed intake, milk yield, and gene expression and fat infiltration in the liver. Holstein dairy cows (n = 50) in the University of Guelph research herd were randomly assigned to receive 100 g/cow/d of RPBC (Jefo; choline, riboflavin, folic acid and B12 microencapsulated in a lipid matrix) or placebo (lipid supplement), fed in a common TMR from 3 wk before calving through 21 DIM. Cows were in freestall pens with individual feed bins (Insentec). DMI was measured daily and blood samples were collected weekly. Liver biopsies were taken at 4 and 14 ± 1 DIM to measure triacylglycerol (TAG) concentrations and expression of 28 genes selected to represent relevant aspects of liver metabolism. Data were analyzed with multivariable mixed linear regression models. All results are LS means ± SEM. DMI did not differ ($P > 0.2$) between RPBC and control (prepartum: 13.7 vs. 13.4 ± 0.5 kg/d; 0 to 21 DIM: 14.6 vs. 13.9 ± 0.70; 22 to 28 DIM: 17.9 vs. 16.9 ± 0.74). Mean blood BHB was lower at wk 3 in RPBC (0.6 vs 0.9 ± 0.12 mmol/L, $P < 0.01$) with no difference between treatments for mean blood concentrations of fatty acids (wk −1 or 1) and BHB at wk 1 or 2. Liver TAG was lower in primiparous cows at 4 DIM in RPBC (2.0 vs. 4.4 ± 1.2%, $P = 0.05$) but not at 14 DIM (2.2 vs. 3.2 ± 0.97%, $P = 0.34$) with no treatment effect for multiparous cows (4.6%, $P > 0.75$). Acyl-CoA oxidase 1, palmitoyl had lower mRNA abundance in RPBC with no difference between treatments for the other genes, but the expression of half of the genes assessed differed with DIM. Accounting for parity, DIM, fat and protein percentages, repeated test days, and a random effect of cow, cows that received RPBC tended to produce more milk across the first 3 DHIA tests (41.2 vs. 38.0 ± 1.4 kg/d, $P = 0.11$). The supplementation of RPBC may provide benefits to liver health and milk yield.

Key Words: rumen-protected B vitamin, liver TAG, transition cow

T288 Production performance in lactating dairy cows fed treated corn stover pelleted with soybean meal or distillers grains. K. L. Ostendorf*[1] and K. F. Kalscheur[2], [1]*University of Wisconsin, Madison, WI,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

The nutritive value of corn stover can be improved by an application of alkaline treatment and combining it with a protein source. The objective was to evaluate the effect of feeding a treated corn stover pelleted with either soybean meal (SBM) or dried distillers grains with solubles (DDGS) on lactation performance in dairy cows. Forty-five lactating Holstein cows (113 ± 39 DIM) were assigned to 1 of 3 diets in a randomized complete block design with a 2-wk adaptation period followed by a 10-wk experimental period. Cows were blocked by DIM and milk production. Dietary treatments were one of the following: (1) control diet with no pellets (CON), (2) treated corn stover-SBM pelleted diet (SBM Pellet), or (3) corn stover-DDGS pelleted diet (DDGS Pellet). Inclusion rate of pellets in the treatment diets was 17% of the total ration on a DM basis and pellets replaced a combination of soybean hulls (75.3%), soybean meal (20%), and limestone (4.7%). Data were analyzed using the MIXED procedure of SAS. Cows fed CON had greater DMI (30.2 vs. 27.2 and 25.4 kg/d; $P < 0.001$) and milk production (50.4 vs 46.5 and 45.1 kg/d; $P < 0.001$) compared with cows fed SBM pellet and DDGS pellet diets. Milk yield did not differ between pellet diets ($P = 0.30$). However, DMI was greater for cows fed the SBM pellet diet than cows fed the DDGS pellet diet ($P = 0.04$). Milk protein percentage was greater for cows fed CON compared with SBM and DDGS pellet diets (2.88 vs. 2.82 and 2.79; $P < 0.01$) and tended to have increased milk fat percentage (3.67 vs. 3.60 and 3.40; $P = 0.07$). Protein source added to the pellet (SBM vs. DDGS) tended to effect milk fat yield (1.67 vs. 1.55 kg/d; $P = 0.07$) and milk fat percentage (3.60 vs. 3.40; $P = 0.06$). Milk urea nitrogen was greater for cows fed CON compared with SBM and DDGS pellet diets (13.0 vs. 11.9 and 11.4 mg/dL; $P < 0.001$) and tended to be greater for cows fed the SBM pellet versus cows fed the DDGS pellet ($P = 0.06$). Overall, cows fed the CON diet had the highest performance. However, the source of protein used in the treated corn stover pellets may have an effect on lactation performance.

Key Words: corn stover, pellet, protein

T289 Conjugated linoleic acid, but not α-linolenic acid, improved energy balance in dairy cows fed a diet with reduced n-3 fatty acid content during the late lactation and transition period. H. M. Hammon*[1], L. Vogel[1], M. Gnott[1], C. Kröger-Koch[1], J. M. Weitzel[1], A. Tröscher[2], and A. Starke[3], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*BASF SE, Lampertheim, Germany,* [3]*Clinic for Ruminants and Swine, Faculty of Veterinary Medicine University of Leipzig, Leipzig, Germany.*

Corn silage (CS) based diets result in low α-linolenic acid (ALA) and conjugated linoleic acid (CLA) supply in dairy cows. The present study investigated the effects of replenishing essential fatty acids (EFA), especially ALA, and CLA in dairy cows fed a CS based diet on performance and energy metabolism during the late lactation and transition period. Holstein cows (n = 40) were fitted with abomasal tubes placed through rumen cannula and were daily supplemented either with coconut oil (45.5% C12:0; 16.9% C14:0; CNTR, 76g/d), linseed (53.9% C18:3) and safflower oil (62.0% C18:2) (EFA, 78 and 4g/d), Lutalin (CLA, *c*9, *t*11 and *t*10, *c*12, 10g/d each) and EFA+CLA from wk 9 antepartum (ap) to wk 8 postpartum (pp). A CS-based TMR was fed for the last 3 mo of gestation and early lactation. Plasma concentrations of metabolites and hormones were measured in blood samples taken on d 63, 42, 35, 21, and 10 ap and weekly from d 1 until d 56 pp. Data were analyzed using the MIXED procedure of SAS with treatment and time as fixed effects. DMI tended to be lower in CLA ($P < 0.1$) than in EFA (wk 7) and in CNTR (wk 8). Milk yield in late lactation declined in all groups but not EFA ($P < 0.01$). CLA supplementation reduced milk fat ($P < 0.01$) in CLA and EFA+CLA. Energy balance was improved ($P < 0.01$) pp in both CLA treated groups. Milk citrate concentration pp was higher ($P < 0.01$) in CLA than in EFA and in CNTR. Glucose concentration on d 21 pp was higher ($P < 0.05$) in CLA than in EFA. Plasma concentration of NEFA was higher ($P < 0.05$) in CNTR (d 21 and 28 pp) and EFA (d 21 pp) than in CLA and EFA+CLA. Plasma insulin concentration was highest on d 1 pp in CLA ($P < 0.05$) and was higher during the transition period in CLA than CNTR ($P < 0.05$). Our data confirmed the reduced milk fat content and improved energy balance in cows treated with CLA due to milk fat depression by *t*10,*c*12 CLA. Elevated milk citrate might point at reduced NADPH synthesis for fatty acid synthesis in the mammary gland of the CLA cows and metabolic changes in blood plasma support the improved energy balance in CLA cows.

Key Words: cow, fatty acid supply, essential fatty acids

T290 Effect of zearalenone hydrolyase ZenA on zearalenone kinetics in the rumen. M. Aleschko[1], M. Killinger[1], A. Höbartner[1],

B. Doupovec[1], J. Faas*[1], N. Reisinger[1], D. Schatzmayr[1], I. Kröger[2], V. Neubauer[2], Q. Zebeli[2], and W.-D. Moll[1], [1]*Biomin Research Center/ Biomin Holding GmbH, Tulln, Austria,* [2]*Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria.*

Zearalenone (ZEN) is one of the most frequently detected mycotoxins in cereals and feed all over the world. Among farm animals, it is thought that ruminants are less sensitive to ZEN. However, the α-ZEL metabolite produced in the rumen has higher estrogenic potential than ZEN. There is evidence that the ZEN degrading enzyme zearalenone hydrolyase (ZenA) exhibits activity against ZEN by forming the non-estrogenic hydrolyzed zearalenone (HZEN) as the primary reaction product. In this study, we evaluated the effect of ZenA as a feed additive on ZEN kinetics in the rumen.In a 10-d feeding trial, 4 rumen-cannulated Holstein Friesian cows (nonlactating, non-gestating) were used to assess the kinetics of ZEN, depending on the experimental day, all animals received either (1) a corn portion containing 5mg of ZEN, (2) a corn portion containing 5mg of ZEN supplemented with ZenA, or (3) a uncontaminated corn portion(control). Rumen fluid and fecal samples were taken each day at various time points and ZEN, α-ZEL, and HZEN concentrations were determined using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) analysis. For statistical analysis, a *t*-test was performed if requirements of normal distribution and homogeneity of variances were met. Otherwise, a non-parametric test (Mann-Whitney U test) was used. When animals received ZEN contaminated feed, ZEN, α-ZEL and HZEN was detected in the rumen fluid at a concentration of 25.04 nmol/L, 2.42 nmol/L and < LOQ, respectively. When animals received ZEN contaminated feed supplemented with ZenA, ZEN, α-ZEL and HZEN was detected in the rumen fluid at a concentration of 2.18 nmol/L, < LOQ and 41.75 nmol/L, respectively. Detected ZEN ($P = 0.001$) and α-ZEL ($P < 0.001$) levels were significantly lower and HZEN ($P < 0.001$) levels significantly higher after administration of ZenA. Fecal samples showed the same significant ($P < 0.01$) shift from the estrogenically active ZEN and α-ZEL to the non-toxic HZEN. We conclude that ZenA applied as a feed additive was effective in transforming ZEN to HZEN in the rumen of dairy cows and it is therefore, a promising approach to prevent toxic effects of ZEN contaminated feed.

Key Words: zearalenone, rumen, hydrolase

T291 Impact of feeding a highly available source of calcium and magnesium on serum concentrations and urinary and excretion in prefresh dairy cattle. B. N. Ellison[1], S. J. Taylor[2], A. T. Byrd*[3], and J. K. Bernard[4], [1]*Applied Biotechnologies Inc., Evansville, WI,* [2]*Celtic Sea Minerals Ltd., Carrigaline, Cork, Ireland,* [3]*Feedworks, Cincinnati, OH,* [4]*University of Georgia, Tifton, GA.*

A crossover design field trial was conducted to investigate the effect of feeding a source of rumen soluble Ca (30%) and Mg (6%) from calcareous marine algae (CMA) on serum and urinary concentrations of Ca and Mg compared with those achieved from finely ground limestone and magnesium oxide (CONT). Periparturient cows (Holsteins and Jerseys) synchronized for seasonal breeding regimen were used (n = 67 control and n = 76 test) in the trial. Diets were formulated to provide 30 g/d of supplemental Ca (17% of total) and 6 g/d supplemental Mg (10% of total) from either CONT or CMA for a minimum of 10 d before calving. Cows that did not consume diets for a minimum of 10 d before calving were not enrolled in the trial. Urine samples were collected daily before calving (average 2.6 d) for analysis of Mg, Ca, and creatinine, which was used as a marker for calculating urine volume based on 29 mg creatinine excreted per kg BW. Blood was collected at 12 h post calving and analyzed for serum total and ionized Ca and Mg. Blood Ca and Mg values were affected by both breed and lactation number, thus breed and lactation were included in the final statistical model as covariates to evaluate the effect of treatment. Cows fed CMA had increased serum concentrations of total Ca ($P = 0.027$) and ionized Mg ($P = 0.020$), but no differences were observed in ionized Ca or total Mg ($P > 0.10$). Urinary concentrations of Ca and Mg were higher ($P = 0.01$) for cows fed CMA which results in increased ($P < 0.0001$) excretion of Mg implying that metabolic Mg tissue pools have been saturated. No differences ($P > 0.10$) were observed in Ca excretion: 12.48 and 12.95 g/d for CONT and CMA, respectively. Regression analysis predicted serum Mg using urine Mg excretion as follows: urine Mg = −0.34 + 2.5 Serum Mg ($P < 0.023$, $R^2 = 0.08$). No differences were observed for other metabolites. These results suggest Mg bioavailability was greater for CMA relative to conventional MgO, however, no improvements in serum Ca were observed.

Key Words: calcareous marine algae, magnesium, calcium

T292 Replacing dietary starch with a combination of sugar and soluble fiber in combination with soybean oil alters fermentation in continuous culture. L. E. Koch[1], B. M. Koch[1], R. N. Klopp[1], M. J. Oconitrillo[2,1], R. Hughes[1], M. Courey[1], A. Sackett[1], T. C. Jenkins[1], and G. J. Lascano*[1], [1]*Clemson University, Clemson, SC,* [2]*Earth University, Limon, Mercedes, Costa Rica.*

The current study was designed to investigate the effect of feeding diets with or without soybean oil and the replacement of a portion of the starch with high sugar (Su) and high soluble fiber (SF) on in vitro fermentation. We hypothesized that high Su or high SF would improve fermentation parameters in continuous cultures when challenged with soybean oil and high starch. An experiment was conducted using 8 dual-flow continuous culture fermentors. Treatments included soybean oil (High PUFA; HF) or no soybean oil (Low PUFA; LF), low (LSu; 4% DM) or high sugar (HSu; 9% DM), and low or high soluble fiber (SF; 6 or 12% DM). This resulted in 8 unique treatments, 2 PUFA levels: LF (2.5% EE) and HF (5% EE) and to a combination of Su and SF sequence [LSuLSF (4% Su, 6% SF); LSuHSF (4% Su, 12% SF); HSuLSF (9% Su, 6% SF); and HSuHSF (9% Su, 12% SF)] within PUFA level administered according to a split-plot, 4 × 4 Latin square design. Cultures were run for 4 periods of 10 d, with fresh inoculum used at the start of each period. Statistical analysis was conducted using the MIXED procedure of SAS. High PUFA-fed fermentors had lower digestibility coefficients for NDF, ADF, DM, and OM ($P = 0.01$), but did not alter VFA production or pH. Mean culture pH was higher ($P < 0.01$) in the HSu vs LSu-fed fermentors (6.14 vs. 5.95 ± 0.13). Mean pH was not altered by SF, but lactate tended ($P = 0.06$) to be lower for HSF versus LSF (9.35 vs. 9.61 ± 1.93). There was a tendency ($P = 0.09$) for total VFA to be increased with HSu treatments ($P = 0.09$). In addition, there was a significant reduction ($P = 0.02$) in propionate and an increase ($P = 0.03$) in butyrate concentrations with Su. Inclusion of SF exhibited an increase in the proportions of acetate ($P < 0.01$), and a reduction of propionate ($P < 0.01$).The results of this study imply that replacing a portion of the starch with sugar or SF may improve rumen fermentation.

Key Words: sugars, soluble fiber, continuous culture

T293 Dietary supplementation of *Scutellaria baicalensis* extract during early lactation decreases milk somatic cells and increases whole lactation milk yield in dairy cattle. K. E. Olagaray*[1], M. J. Brouk[1], L. K. Mamedova[1], F. Robert[2], E. Dupuis[2], M. Zachut[3], and B. J. Bradford[1], [1]*Kansas State University, Manhattan,*

KS, [2]CCPA Group, Janze, France, [3]Agriculture Research Organization, Volcani Center, Rishon Lezion, Israel.

Multiparous Holstein cows (n = 122) were used in a randomized block design to determine the effect of *Scutellaria baicalensis* extract (SBE) on 305-d milk yield and milk markers of inflammation and metabolic function. Treatments were 1) control, 2) 5-d administration of SBE (SBE5), and 3) 60-d administration of SBE (SBE60). Treatments were included in a treatment pellet that was identical to the control pellet except for the extract, both provided via an automated milking system beginning on d 1 of lactation. Milk samples were collected on d 1, 3, and once during d 5–12 of lactation, followed by weekly sampling until 120 d in milk. Milk samples collected in the first 2 wk were used for biomarker analysis (haptoglobin, β-hydroxybutryate [BHB], and glucose-6-phosphate [G6P]) and all samples were used for component analysis. Milk production, programmed pellet allocation, and actual provision of both pelleted feeds were recorded daily. Treatment effects were evaluated by contrasts between control and SBE5 and control and SBE60 for both the treatment (TP; wk 1–9) and carryover periods (CP; wk 10–37). Total pellet allocated was greater for SBE60 in both the TP ($P < 0.01$) and CP ($P = 0.02$), but was not different for SBE5 during either period (both $P > 0.12$). No treatment effects were observed for milk haptoglobin, BHB, or G6P (all $P > 0.88$). SBE5 did not alter milk yield ($P = 0.60$) or milk components ($P > 0.10$). SBE60 increased whole-lactation milk yield ($P = 0.03$). SBE60 increased milk lactose and fat yields ($P < 0.05$) and tended to increase milk protein yield ($P = 0.09$) during TP, and each increased during CP ($P < 0.05$). Somatic cell count decreased in SBE60 during TP ($P = 0.02$) but not CP ($P = 0.13$). Mastitis incidence tended to differ by treatment ($P = 0.06$), being lesser for SBE5 ($P = 0.04$) and SBE60 ($P = 0.05$) vs. control. SBE60 tended to decrease the hazard of leaving the herd compared with control and SBE5 ($P = 0.07$). In conclusion, supplementation of postpartum dairy cows with SBE for 60 d was effective at increasing whole lactation milk yield.

Key Words: bioactive, polyphenol, transition cow

T294 Energy partitioning in primiparous Holstein cows with or without grazing pastures in early lactation. A. Jasinsky*, D. A. Mattiauda, M. Ceriani, A. Casal, and M. Carriquiry, *Facultad de Agronomia, Universidad de la República, Paysandu, Paysandu, Uruguay.*

The aim of the study was to compare energy partitioning between heat production (HP) and retained energy, and efficiency of dairy cows fed with different strategies during early lactation. At calving, 18 primiparous cows (528 ± 40 kg BW; 3.2 ± 0.2 BCS; fall calving) were assigned in a randomized block design to either (G0) total mixed ration (TMR) ad libitum (55% forage: 45% concentrate; offered 17 kgDM/d) or (G1) grazing of alfalfa (6-h grazing in 3-d strips; 20 kgDM/d) + 70% of ad libitum TMR intake (12 kgDM/d) during 65 d postpartum. All cows consumed at each milking, 2.0 kgDM/d of a grain-soybean meal concentrate. Cows were milked twice a day; milk yield was measured daily, milk composition once a week, and BW and BCS every 14 d. The HP was measured by the O2-pulse technique at 42 ± 3 d postpartum, and retained energy in milk and in body tissue (estimated from BW and BCS changes) were used to calculate energy balance from 29 to 57 ± 3 d postpartum. Metabolizable energy (ME) intake was estimated as HP + total retained energy. Residual HP was calculated as the difference between measured and predicted (based on NRC coefficients) HP. Data were analyzed with a mixed model including treatment as fixed and block as random effect. Milk yield tended to be greater ($P = 0.09$) for G0 than G1 cows, while its composition was not affected by treatment. Cow BW did not differ but BCS was greater for G0 than G1 cows ($P = 0.01$; 2.9 *vs*. 2.6 ± 0.06). Total retained energy was greater for G0 than G1 cows ($P < 0.01$; 92.7 *vs*. 84.8 ± 1.7 MJ/d) as both, energy in milk and tissue, were greater ($P \leq 0.04$) in the former ones. Whole-animal HP (90.7 vs. 94.4 ± 4.0 MJ/d) and calculated ME intake (182.2 vs. 179. Seven ± 4.8 MJ/d) did not differ between G0 and G1 cows. Residual HP (as % of ME intake) tended to be 10% less ($P = 0.06$) for G0 than G1 cows. Adjusted energy efficiency tended to be greater for G0 than G1 cows ($P = 0.08$; 0.51 vs 0.47 ± 0.01). Results indicated G0 cows were more efficient, secreting more energy in milk and retaining more energy in body tissue, as maintenance requirements increased around 10% by the inclusion of grazing in G1 cows.

Key Words: energy expenditure, nutrition, dairy

T295 In situ ruminal starch disappearance kinetics of hull-less barley, hulled barley, and corn grains. G. Ferreira*, Y. Yang, C. Teets, W. Brooks, and C. Griffey, *Virginia Tech, Blacksburg, VA.*

The objective of this study was to compare ruminal starch disappearance rates of hull-less barley, hulled barley, and corn grains. Five different genotypes were used for each of the 2 barley types. In addition, each of these genotypes was grown in 2 different locations and years, resulting in a total of 10 independent barley samples for each of the 2 barley grain types. Also, 5 different genotypes of corn grain were obtained from a commercial seed company. After grinding to pass through a 4-mm screen of a cutter mill, 3.6 g of grain were placed into porous bags, which were then incubated in the rumen of 2 ruminaly cannulated cows for 0, 4, 8, 12, 24, and 48 h. Corn grains showed greater instant ruminal dry matter (DM) disappearances than barley grains (26.3 and 15.0%, respectively). Instant ruminal DM disappearances did not differ between hulled and hull-less barley grains. Ruminal DM fractional disappearance rate was greatest for hull-less barley grains, medium for hulled barley grains, and lowest for corn grains (10.6, 8.5, and 5.9%/h, respectively). Ruminal DM half-life was shortest for hull-less barley (5.1 h) and longest for hulled barley and corn grains (6.6 h). Ruminal DM half-life did not differ between hulled barley and corn grains. Corn grains showed greater instant ruminal starch disappearances than barley grains (22.5 and 8.3%, respectively). Instant ruminal starch disappearances did not differ between hulled and hull-less barley grains. Ruminal starch fractional disappearance rates were greatest for hulled barley grains, medium for hull-less barley grains, and lowest for corn grains (15.1, 13.7, and 6.9%/h, respectively). Ruminal starch half-life was shortest for hulled and hull-less barley grains (4.4 h) and longest for corn grains (6.6 h). Ruminal starch half-life did not differ between hulled barley and hull-less barley grains. In conclusion, this study showed that starch from hull-less barley grains has a ruminal half-life similar to that of hulled barley grains and shorter to that of corn grains.

Key Words: barley, starch half-life, cereal grains

T296 Evaluating polyhalite as an anionic source to reduce dietary cation-anion difference and urine pH of prepartum dairy cows. G. Ferreira*[1], C. L. Teets[1], and R. J. Meakin[2], [1]*Virginia Tech, Blacksburg, VA,* [2]*Sirius Minerals Plc, Scarborough, United Kingdom.*

Polyhalite is a hydrated sulfate of potassium, calcium and magnesium. Based on its anionic properties, we hypothesized that feeding polyhalite mineral would decrease urine pH when fed to nonlactating cows before calving. The objective of this study was to determine if the mineral polyhalite is an effective means to decrease urine pH when fed to pre-partum dairy cows. At 21 d before expected calving date (ECD), 45 Holstein and Jersey dairy cows were transferred from the far-off to the close-

up nonlactating groups, respectively. Close-up cows were housed in a common pen bedded with wood shavings, and they were fed a common diet that contained (DM basis) 48% corn silage, 25% grass hay, and 27% concentrate and 14.4% CP, 44.7% NDF, and 32.1% NFC concentrations. The granular free-flowing anionic test product was provided in the pelleted concentrate and included in the diet at a rate equal to 3.9% DM (i.e., ~500 g/day), yielding an estimated DCAD of −20 mEq/100 g DM. On d 21, 14, 7, and 0 relative to ECD, urine samples were collected, and urine pH was immediately determined with a portable pH meter. Urinary pH was statistically evaluated as repeated measures using the MIXED procedure of SAS. For this analysis, cow was considered the experimental unit from which the effect of time since feeding polyhalite on urine pH was evaluated using repeated measures. The model included the random effect of cow (44 df), the fixed effect of day (3 df), and the random residual error. Feeding a close-up diet containing ~500 g/d of polyhalite mineral significantly reduced urine pH of nonlactating dairy cows ($P < 0.01$). Urine pH of the far-off cows at 21 d before ECD was 8.26, while urine pH of the close-up cows at 14, 7, and 0 d relative to ECD were 5.18, 5.12, and 5.15, respectively. In conclusion, feeding ~500 g/day of polyhalite mineral in close-up diets is an effective means to decrease urine pH when fed to pre-partum dairy cows.

Key Words: polyhalite mineral, DCAD, urine pH

T297 Rumen fermentation characteristics, microbial ecology and haematological parameters of yearling calves fed *Enterolobium cyclocarpum* (Jacq.) Griseb. leaf meal-based diets. B. A. Ayilara*, C. I. Onwuka, A. O. Oni, and M. O. Arigbede, *Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.*

The current study was undertaken to evaluate the rumen fermentation characteristics and hematological parameters of yearling calves fed agro- industrial by-products (wheat offal, palm kernel meal and cassava peels) supplemented with *Enterolobium cyclocarpum* leaves (ECLM) at different inclusion levels. ECLM foliage randomly harvested, sun dried and ground in a mill with 2mm sieve were included in concentrate ration formulated with the agro- industrial by-products at different levels. The experiment was carried out at the Cattle Unit of the Teaching and Research Farm Directorate of Federal University of Agriculture, Abeokuta, Nigeria. Sixteen yearling calves were used for the study in a 90 d experiment. The calves were grouped into 4 and randomly assigned to 4 treatment groups with 0, 5, 10 and 15% ECLM inclusion levels in a completely randomized design with *Panicum maximum* fed as basal diet. The statistical proceedure used is ANOVA. The ruminal pH, rumen ammonia concentration, total volatile fatty acids, acetic and butyric acids were significantly ($P < 0.05$) affected by increasing levels of ECLM in the diets. The pH value and NH_3 concentration were highest at 0% ECLM (6.93) and (9.67mg/100mL) respectively and decreased ($P < 0.05$) with increasing levels of ECLM while the total VFAs increased with increase in ECLM with 10% ECLM having the highest value (129 mmol/100mL) and 0% ECLM having the lowest value (107.67mmol/100mL). ECLM inclusion ($P < 0.05$) increased the total viable bacteria count with 10% ECLM having the highest value (26.27×10^7) and 0% ECLM having the least value (19.63×10^7). ECLM inclusion increased the bacteria population ($P < 0.05$), while protozoa population ($P < 0.05$) decreased and fungal zoospore populations across the treatments were similar. The red blood cells, serum glucose, serum protein and blood urea increased significantly ($P < 0.05$) with increasing level of ECLM and were highest at 10% but reduced at 15% ECLM inclusion level. In conclusion, *Enterolobium cyclocarpum* leaves could be used as supplement with other cheap agro-industrial by-products up to 10% level of inclusion in ruminant feeding.

Key Words: agro-industrial by-products, *Enterolobium cyclocarpum*, calves

T298 Ruminal escape and duodenal appearance of *N*-acetyl-L-methionine in lactating dairy cows. S. Sharp[1], M. A. Fagundes[1], J.-S. Eun*[1,2], J. O. Hall[1], J. S. Park[2], and J. O. Moon[2], [1]1*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT,* [2]*Biotechnology Research Institute, CJ CheilJedang, Suwon, South Korea.*

The *N*-acetyl-L-Met (NALM) is a Met derivative produced via protection of L-Met α-amino group with an *N*-acetyl group. The NALM has been shown to be bioavailable and capable of replacing the dietary requirement for Met in animals and humans. The efficacy of the NALM to provide a source of Met depends on its resistance to microbial degradation in the rumen, its rapid escape from the rumen with digesta, and its subsequent absorption and metabolism to Met in the small intestine. The objective of this study was to quantify the gastrointestinal availability of the NALM by measuring the ruminal escape and intestinal appearance in lactating dairy cows. The current experiment was conducted as a 3 × 3 Latin square design using 3 lactating dairy cows with cannula in the rumen and duodenum. Each period consisted of 10-d adaptation followed by 2 d of sampling. A dose of 0 (control), 30, and 60 g/d of NALM in powder form was placed under the rumen raft at the time of feeding every day. On the first day of sampling, a liquid phase marker (Co-EDTA) was also administered as a bolus dose into the rumen at the time of administration of the NALM. Blood and ruminal and duodenal contents were then taken at hours 0, 1, 3, 6, 9, 12, and 24 post-feeding. A nonlinear regression model was used to determine fractional rate constants for the passage of the liquid marker and the disappearance of NALM from the rumen. There was no difference between treatments for liquid passage and ruminal NALM passage rate. Ruminal escape as a percentage of the dose was numerically higher for the low dose (69.1%) than the high dose (46.2%), but no statistical difference was found ($P = 0.44$). Duodenal NALM appearance as a percentage of the dose did not differ ($P = 0.21$) between the low (2.16%) vs. the high dose (3.40%). In addition, NALM treatments at 30 and 60 g/d did not affect ruminal fermentation profiles. In conclusion, overall results from the current study suggest that NALM was effectively protected against microbial degradation in the rumen, particularly at the low dose and extensively digested in the small intestine of lactating dairy cows.

Key Words: *N*-acetyl-L-methionine, ruminal escape, duodenal appearance

T299 Effects of replacing canola meal with solvent extracted camelina meal on ruminal fermentation in a dual-flow continuous culture system. V. Brandao*, L. Silva, E. Paula, H. Monteiro, X. Dai, A. Lelis, A. Faccenda, A. Avila, and A. Faciola, *University of Florida, Gainesville, FL.*

We aimed to assess whether solvent extracted camelina meal (SCAM) could replace canola meal (CM) in lactating dairy cows' diets, and its effects on ruminal fermentation in a dual-flow continuous culture system. Diets were isonitrogenous (16%CP) and randomly assigned to 6 fermentors in a replicated 3 × 3 Latin square with three 10-d experimental periods consisting of 7 d for diet adaptation and 3 d for sample collection. Treatments were 0 (20.6% CM), 50 (10.3% CM), and 100% SCAM (0% CM) inclusion, replacing CM as the protein supplement.

Diets contained 55:45 forage:concentrate, and fermentors were fed 72 g of DM/d. On d 8, 9, and 10 of each period samples were collected for analyses of pH, VFA, NH_3, digestibility, and AA flow. Statistical analysis was performed using the MIXED procedure of SAS, and linear and quadratic effects of SCAM inclusion were assessed. Partial data are presented in Table 1. Total VFA and pH were not affected by treatments. Acetate concentration decreased ($P = 0.01$), while propionate increased ($P = 0.01$) with SCAM inclusion. Concentration of NH_3-N decreased ($P = 0.01$), and NAN increased ($P = 0.05$) with SCAM inclusion. Bacterial efficiency tended ($P = 0.06$) to be greater on 100% SCAM diet. Arg increased ($P = 0.02$) with SCAM inclusion. Overall AA flow was not affected by diet. Replacing CM with SCAM changed ruminal fermentation, increasing propionate and NAN, and decreasing NH_3-N, which may improve energy status and nitrogen utilization. Inclusion of SCAM did not change most AA flow, indicating that it can be a potential replacement for canola meal.

Table 1 (Abstr. T299).

	Camelina (%)				*P*-value		
Item	0	50	100	SEM	Trt	Lin	Quad
VFA, % of total							
Acetate	63.7	57.2	54.9	1.01	0.01	0.01	0.01
Propionate	19.4	25.9	27.9	0.81	0.01	0.01	0.01
Butyrate	14.1	13.6	14.2	1.08	0.35	0.83	0.16
Valerate	1.56	1.72	1.72	0.10	0.01	0.01	0.05
Total BCVFA,[1] m*M*	0.79	0.99	0.81	0.13	0.01	0.82	0.01
Flow, g/d							
NAN	1.53	1.60	1.68	0.06	0.05	0.04	0.94
Bacterial N	0.71	0.67	0.73	0.06	0.36	0.61	0.19
NANMN[2]	0.79	0.86	0.88	0.06	0.32	0.16	0.67
Arg	0.53	0.59	0.62	0.02	0.02	0.01	0.60
His	0.21	0.22	0.22	0.01	0.69	0.45	0.70
Lys:Met	3.03	2.97	3.05	0.02	0.07	0.76	0.03

[1]BCVFA = branched-chain VFA.

[2]NANMN = nonammonia nonmicrobial nitrogen.

Key Words: amino acid, nitrogen metabolism

T300 Replacing dietary starch with a combination of sugar and soluble fiber in combination with soybean oil alter lactating performance in Holstein dairy cows. L. E. Koch[1], B. M. Koch[1], R. N. Klopp[1], S. M. Hussein[1], M. J. Oconitrillo[2,1], R. Hughes[1], M. Courey[1], A. Sackett[1], T. C. Jenkins[1], and G. J. Lascano*[1], [1]*Clemson University, Clemson, SC,* [2]*Earth University, Limon, Mercedes, Costa Rica.*

Milk fat depression (MFD) is a multifactorial disorder that can be easily induced with high levels of polyunsaturated fatty acids (PUFA). However, little is known about how sugar or soluble fiber can improve the conditions of MFD and if performance can be enhanced. The aim of the current study was to investigate the effect of feeding diets with or without soybean oil and the replacement of a portion of the starch for sources of high Su and or high SF, on milk production. We hypothesized that the treatments with high Su or high SF would improve milk production performance when challenged with soy oil. Eight multiparous Holstein cows were randomly assigned to treatments, consisting of soybean oil (High PUFA; HF) or no soy oil (Low PUFA; LF), low (LSu; 4% DM) or high sugar (HSu; 9% DM), and low or high soluble fiber (SF; 6 or 12% DM). This resulted in 8 unique treatments, 2 PUFA levels: LF (2.5% EE) and HF (5% EE) and to a combination of Su and SF sequence [LSuLSF (4% Su, 6% SF); LSuHSF (4% Su, 12% SF); HSuLSF (9% Su, 6% SF); and HSuHSF (9% Su, 12% SF)] within PUFA level administered according to a split-plot, 4 × 4 Latin square design (21 d periods). Data were analyzed using the MIXED procedure of SAS with PUFA, period within PUFA, Su, and SF were fixed effects. High PUFA-fed animals had reduced milk fat yield, composition, and fat corrected milk ($P < 0.01$). In addition, MUN was reduced substantially with the high PUFA treatments ($P < 0.01$). There was no effect of sugar on DMI or milk fat production, but there was an interaction for DMI between PUFA level and SF ($P = 0.01$) where DMI decreased as SF level increased in the high PUFA-fed animals. Milk yield was increased with LSu-fed animals ($P < 0.01$), and there was also greater feed efficiency observed for LSu ($P = 0.01$). The results of the current study imply that replacing a portion of the starch with Su or SF does not ameliorate characteristic symptoms of MFD, but can alter DMI, milk production, and feed efficiency.

Key Words: sugars, soluble fiber, unsaturated fatty acids

T301 Energy utilization of lactating Jersey cows consuming diets containing hydrolyzed feathermeal. J. V. Judy and P. J. Kononoff*, *University of Nebraska-Lincoln, Lincoln, NE.*

A study using indirect calorimetry and 16 lactating multiparous Jersey cows (88.4 ± 22.7 DIM; mean ± SD) was conducted to evaluate the utilization of energy in cattle consuming diets containing hydrolyzed feathermeal (HFM). Using a replicated 4 × 4 Latin square design with 35-d periods, treatments included a control (CON) diet, which did not contain HFM, and treatment diets containing 3.3% (DM basis) HFM (LoFM), 6.6% HFM (MdFM), and 9.9% % (DM basis) HFM (HiFM). Diets were formulated such that HFM replaced bloodmeal, and animal energy losses in heat, feces, urine, and milk were collected. A mixed statistical model was used to analyze data with treatment and period as fixed effects and cow within square a random effect. Increasing the concentration of HFM resulted in a quadratic effect ($P = 0.041$) on DMI (19.6, 20.2, 20.3, and 19.1 ± 0.765 kg for CON, LoFM, MdFM, and HiFM respectively) and a similar trend ($P = 0.084$) was observed for milk yield (31.7, 32.0, 31.9, and 29.7 ± 1.258 kg). Increasing the concentration of HFM resulted in an increasing linear effect ($P = 0.026$) on the concentration of milk fat (5.35, 5.23, 5.45, and 5.54 ± 0.216 kg for CON, LoFM, MdFM, and HiFM respectively) but did not affect ($P = 0.694$) the yield of milk fat, which averaged 1.68 ± 0.069 kg/d. Increasing the concentration of HFM tended to linearly reduce ($P = 0.063$) the concentration of milk protein (3.34, 3.29, 3.23, 3.23 ± 0.098 for CON, LoFM, MdFM, and HiFM respectively) and linearly reduced ($P = 0.004$) the yield of milk protein (1.05, 1.05, 1.02, 0.96 ± 0.41 kg). The inclusion of HFM did not affect ($P = 0.158$) ECM which averaged 39.3 ± 1.415 kg. Apparent total-tract digestion of CP was linearly reduced ($P < 0.001$) with increasing HFM (63.5, 60.2, 58.4, 57.1 ± 0.81% for CON, LoFM, MdFM, and HiFM respectively). The concentration of NEL tended to increase quadratically ($P = 0.068$) (1.18, 1.23, 2.27, and 1.26 ± 0.04 Mcal/d for CON, LoFM, MdFM, and HiFM respectively). Results suggest that HFM is a palatable feedstuff, that when used in replace of bloodmeal may result in diets with a greater concentration of energy and maintain FCM, but protein availability may be reduced and this may negatively affect the production of milk protein.

Key Words: indirect calorimetry, hydrolyzed feathermeal, energy

T302 Effects of supplemental β-carotene to lactating Holstein cows on production and rumen fermentation. K. M. Aragona* and P. S. Erickson, *University of New Hampshire, Durham, NH.*

The objective of this experiment was to determine the effects of 500 mg of supplemental β-carotene on milk protein production and rumen fermentation. Sixteen multiparous lactating Holstein cows were blocked by DIM and randomly assigned to 1 of 4 treatments in a replicated 4 × 4 Latin square design. Two diets were fed: 9% RDP diet that contained (DM basis) 9.2% grass silage, 38.6% corn silage, 11% alfalfa hay, 25.7% energy mix, 11% protein mix, 2.2% mineral-vitamin premix, 1.6% protected fat and 0.6% bloodmeal, and an 11% RDP diet that contained (DM basis) 9.2% grass silage, 38.4% corn silage, 12% alfalfa hay, 23% energy mix, 2.2% mineral-vitamin premix, 1.6% protected fat, 0.9% bloodmeal, 0.5% urea and 12.3% soybean meal. Five hundred mg of β-carotene was top-dressed daily. Previously, incubating β-carotene in rumen fluid resulted in lower NH_3-N and higher microbial protein, indicting it may increase utilization of NH_3-N, leading to increased growth of microbes. If found to be beneficial, dairy producers would supplement β-carotene and be able to reduce purchased protein costs, therefore increasing profitability. Limiting crude protein can also prove to be beneficial by reducing nitrogen loss to the environment. Results are shown in Table 1. In this study, β-carotene did not maintain milk protein production when supplemented to a 9% RDP diet, compared with an 11% RDP diet. Therefore, based on these data, it is not recommended to supplement β-carotene to lactating cows under these dietary conditions.

Key Words: dairy cow, milk production, β-carotene

T303 Variability in urine pH and macro-mineral concentrations in dairy herds from northern Italy. P. Colturato[1] and A. N. Hristov*[2], [1]*Team Paragon, Crema, Italy,* [2]*Department of Animal Science, The Pennsylvania State University, University Park, PA.*

The objective of this study was to assess variability in urine macro-mineral concentrations as a tool to detect the risk of metabolic diseases in dairy cattle related to mineral imbalance. The data set used in the analysis included data from 200 dairy farms from northern Italy with average milk yield of 35 ± 2.5 L/d. Urine samples from 2,700 cows were analyzed for pH, anions (Cl, sulfate, phosphate, nitrate), and cations (Ca, Mg, Na, K, ammonium). Cows were categorized into 3 distinct groups: (1) a close-up group (−30 to −5 d before calving; n = 970), (2) a start-up group (from +3 to + 30 DIM; n = 898), and (3) a lactating group (from 70 to 120 DIM; n = 510). Data for milk production, metabolic diseases after calving, and the use of anionic salts in close up rations were collected at each farm. Urine samples were analyzed using an ionic chromatography system and adjusted using a refractometer system. Concentrations of major relevant cations and anions in the close-up group for farms that fed an anionic diet were (mmol/L, mean ± SE): Na, 64.1 ± 10.3; K, 266 ± 13.8; Mg, 23.6 ± 1.19, Ca, 6.9 ± 0.70; Cl, 118 ± 10.1; sulfate, 20.1 ± 1.68; phosphate, 0.57 ± 0.09, and pH = 8.16 ± 0.45 and for farms that did not feed anionic diets were: 58.9 ± 5.37, 274 ± 8.2, 17.7 ± 0.96, 1.97 ± 0.26, 70.0 ± 5.7, 14.2 ± 1.23, 1.10 ± 0.31, and 8.06 ± 0.11, respectively. Urine concentrations of Mg, Ca, Cl, and sulfate were higher ($P \leq 0.04$) for the anionic vs. non-anionic diets. Concentrations of these ions and pH in the start-up group were 107 ± 2.9, 231 ± 2.8, 20.1 ± 0.35, 2.8 ± 0.13, 67.8 ± 2.32, 13.4 ± 0.55, 1.18 ± 0.13, and 8.09 ± 0.01, respectively and in the lactation group: 108 ± 3.5, 248 ± 3.6, 23.7 ± 0.52, 2.8 ± 0.30, 82.6 ± 2.80, 16.8 ± 0.88, 0.90 ± 0.19, and 8.21 ± 0.01, respectively. Pearson correlations between urine analyses and incidences of metabolic diseases (r = −0.04 for phosphate to −0.22 for Ca) and milk production (r = −0.06 for Ca to 0.25 for Na) were weak. Data from this analysis suggest that feeding anionic diets in the close-up period increased urinary excretion of Mg, Ca, Cl, and sulfate in dairy cows.

Key Words: urine, anion, dairy cattle

Table 1 (Abstr. T302).

	Treatment					*P*-value		
Item	9%RDP	9%RDP+BC	11%RDP	11%RDP+BC	SEM	RDP	BC	Int
DMI, kg/d	26.6	26.8	27.5	26.8	0.56	0.006	0.11	0.02
Milk yield, kg/d	43.4	44.0	44.4	43.6	1.62	0.32	0.56	0.02
ECM, kg/d	49.4	49.8	50.4	49.5	1.34	0.38	0.59	0.16
4% FCM, kg/d	42.2	42.6	42.9	42.3	1.11	0.67	0.80	0.31
ECM/DMI, kg/d	1.85	1.85	1.84	1.85	0.04	0.90	0.80	0.79
Fat, %	3.91	3.91	3.84	3.91	0.14	0.34	0.39	0.40
Fat, kg/d	1.66	1.67	1.67	1.66	0.04	0.86	0.91	0.65
Protein, %	3.37	3.36	3.42	3.43	0.06	<0.0001	0.75	0.67
Protein, kg/d	1.44	1.45	1.50	1.47	0.04	0.0003	0.18	0.04
SNF, %	9.07	9.06	9.12	9.10	0.06	0.008	0.18	0.85
SNF, kg/d	3.92	3.96	4.04	3.94	0.13	0.09	0.29	0.01
MUN, mg/dL	8.38	8.62	12.81	12.75	0.33	<0.0001	0.54	0.33

ADSA Southern Branch Graduate Student Oral Competition

261 Effects of a *Megasphaera elsdenii* oral drench on rumen pH, feed intake, and milk yield in lactating dairy cows. G. Mazon*[1], C. Holcomb[1], J. M. Bewley[2], and J. H. C. Costa[1], [1]*University of Kentucky, Lexington, KY,* [2]*CowFocused Housing, Bardstown, KY.*

The objective of this study was to evaluate the effects of an oral drench with a direct-fed microbial (*Megasphaera elsdenii* NCIMB 41125; Lactipro Advance, MS Biotec, Wamego, KS) on rumen pH, feed intake, and milk yield of lactating dairy cows. The study was conducted at the University of Kentucky Coldstream Dairy from March to May 2017. Eight late-lactation (238.5 ± 54.7 DIM) Holstein cows were enrolled in a crossover trial divided into 2 experimental periods. Each period lasted 8 d with a 4-wk washout period. Upon enrollment, cows were randomly assigned to either an *M. elsdenii* (LACT) or distilled water (CON) drench. Drenching occurred on d 1 of the experimental period. On d 5 of the experimental period, cows received a challenge grain mix rich in highly fermentable carbohydrates to induce subacute ruminal acidosis (SARA). Rumen pH was recorded using the smaXtec rumen bolus in 10-min intervals, feed intake was measured using Insentec feed bins, and milk yield was recorded using the AfiLab automatic meter. Daily time and area under the curve (AUC) were calculated using 2 different pH thresholds to define SARA condition, pH below 5.8 or 5.6. A mixed linear model was used to determine the relationships of treatment and rumen pH, feed intake, milk yield, AUC, and time spent in SARA condition during the trial. Throughout the trial, LACT cows had greater overall mean rumen pH (LACT = 6.23 ± 0.01, CON = 6.11 ± 0.01; $P < 0.01$). In addition, LACT cows consumed more feed on an as-fed basis (LACT = 41.3 ± 2.5, CON = 37.4 ± 2.5 kg/d; $P = 0.04$) and had greater milk yield (LACT = 28.7 ± 1.6, CON = 26.1 ± 1.6 kg/d; $P < 0.01$). Cows on LACT drench had lesser AUC below pH 5.8 (LACT = 20.9 ± 27.3, CON = 81.4 ± 27.4; $P = 0.01$), and AUC below pH 5.6 (LACT = 6.8 ± 13.9, CON = 37.3 ± 13.9; $P = 0.03$). In addition, LACT cows spent less time below pH 5.8 (LACT = 1.8 ± 1.4, CON = 4.7 ± 1.4 h/d; $P < 0.01$), and pH 5.6 (LACT = 0.6 ± 0.9, CON = 2.7 ± 0.9 h/d; $P = 0.01$). Overall, cows drenched with *M. elsdenii* had greater rumen pH, experienced shorter and less intense SARA, and had greater milk yield.

Key Words: Lactipro, direct-fed microbial, subacute ruminal acidosis

262 Dietary effects on rumen VFA transporter abundance in preweaned dairy calves. T. T. Yohe*, R. R. White, M. D. Hanigan, H. Schramm, H. L. M. Tucker, C. L. M. Parsons, B. D. Enger, N. R. Hardy, and K. M. Daniels, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Diet is known to affect rumen growth and development. Calves fed an all liquid diet have smaller and less developed rumens and may have a decreased ability to absorb volatile fatty acids (VFA) compared with calves fed liquid and dry feed. However, it is unknown how these differentially developed rumens would respond when challenged with a defined VFA buffer. The objective of this study was to assess effects of 2 different feeding programs on VFA transporter abundance in preweaned calves. Neonatal Holstein bull calves (n = 12) were individually housed and randomly assigned to 1 of 2 treatment diets. Treatment diets were milk replacer only (MRO; n = 6) or milk replacer and starter (MRS; n = 6); diets were isoenergetic (3.87 ± 0.06 MCal of ME/d) and isonitrogenous (0.17 ± 0.003 kg/d of apparent digestible protein). Milk replacer was 22% CP, 21.5% fat (DM basis) while the textured calf starter was 21.5% CP (DM basis). Feed and ad libitum water intakes were recorded daily; body Tgrowth was measured weekly. Calves were exposed to a defined VFA buffer (acetate: 143 m*M*, propionate: 100 m*M*, butyrate: 40.5 m*M*) 6 h before euthanasia on d 43 ± 1. Rumen tissues were obtained from the ventral sac region and processed for morphological and immunohistochemical analyses of the VFA transporters monocarboxylate transporter 1 (MCT1) and 4 (MCT4). Data were analyzed using PROC MIXED in SAS 9.4 with the following effects: fixed = treatment (including week and interaction for repeated measures) and random = calf nested within treatment. Body growth did not differ between treatments, but empty reticulo-rumens were heavier in MRS than MRO calves (0.67 vs 0.39 ± 0.04 kg; $P = 0.001$) and MRS calves had larger papillae area (0.76 vs 15 ± 0.08 mm^2; $P = 0.001$). No differences between treatments in protein abundance of MCT1 and MCT4 per unit area were observed. These results indicate that the extrapolated increase in total abundance of MCT1 and MCT4 in MRS calves is not due to increased transporter density per unit area. Modeling of VFA absorption data will help determine the proportion of VFA undergoing protein-mediated transport via MCT1 and MCT4 versus those that are passively diffused.

Key Words: dairy, calf nutrition, ruminant physiology

264 Lameness incidence in dry cows depends on assessment frequency. H. K. Eriksson*, R. R. Daros, M. A. G. von Keyserlingk, and D. M. Weary, *Animal Welfare Program, Vancouver, BC, Canada.*

Lameness remains one of the greatest welfare challenges for the dairy industry, but there is little consensus on how best lameness can be surveyed on farm. This longitudinal study evaluated how measures of lameness incidence change with sampling frequency. Weekly visits to 6 farms in British Columbia were performed between May and December 2017, and 93 animals without previous history of severe claw horn lesions were repeatedly locomotion scored from 8 weeks prepartum until calving. Lameness was scored using a 1 to 5 numerical rating; cows were considered lame when they scored 3 (mildly lame) on 2 consecutive visits, or ≥4 (severely lame) on one occasion. The weekly data were used to create 3 data sets representing 1) weekly assessment, 2) assessment every other week, and 3) assessment every third week. Incidence of lameness was 31% when using the complete data set (i.e., weekly scores), versus 19% and 11% for assessments every 2 and 3 weeks respectively. Farm incidence ranged between 18 and 50% (median 30%), 6–50% (23%) and 0–50% (6%) when assessment occurred every 1, 2 and 3 wk. Differences in farm incidence depending on assessment frequency was tested using paired sample *t*-tests. Compared with weekly visits, farm incidence was significantly lower when assessment occurred every 2 ($t(5) = 3.5$) or 3 weeks ($t(5) = 4.2$). Of the animals that became lame during the dry period, average time from the first visit until diagnosed as lame was 20.5, 24.1, and 25.1 d when scoring was performed weekly, every other week and every 3 weeks respectively. These results suggest that (1) a high proportion of the animals become lame during the dry period, (2) estimates of lameness incidence are dependent upon sampling frequency, with low sampling rates likely leading to underestimation of the number of animals becoming lame, and (3) frequent assessment shortens the time before lameness is diagnosed.

Key Words: gait, transition cow, lameness

266 Educating dairy producers to systematically evaluate the cows they cull. A. Moorman*[1,2], T. F. Duffield[1,2], M. A. Godkin[3], J. Rau[4], and D. B. Haley[1,2], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, ON, Canada,* [3]*Ontario Ministry of Agriculture, Food and Rural Affairs, Elora, ON, Canada,* [4]*Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

The purpose of this study was to assess the effectiveness of an evaluation form designed to aid dairy producers to evaluate cows being culled, and to prevent the transport of cows that may be unable to withstand the stress of transport. The objectives of this pilot project were to educate dairy producers about Canadian transport requirements; have producers use the evaluation form to prevent the transport of unfit cows; assess the efficacy of the form; and to encourage the use of standard operating procedures for evaluating cull cows. A total of 16 practicing bovine veterinarians and 54 Ontario dairy producers participated in this study. Veterinarians and producers were sent a pre-study survey to assess their knowledge and attitudes regarding culling cows, 10 cull cow evaluation forms, 2 scoring keys (for both body condition and lameness), and later, a post-study survey, to determine whether their knowledge and attitudes had changed. Veterinarians provided training on proper use of the evaluation form, and the producers completed an evaluation form for every cow they culled from the herd from January to August 2017. The evaluation form included space to record the cow's body temperature, body condition score, gait, CMT score, last drug treatment date, last milking date, removal date and destination. Data analysis is underway to determine the efficacy of the form and opportunities for form improvement. SAS software was used to perform a Wilcoxon signed rank test to determine whether the responses given by participants differed between completed pre- and post-study surveys. Preliminary data analysis indicated that there was a tendency for producers to report higher confidence in their ability to assess cows before transport after using the evaluation form ($P = 0.06$). There was an increase in veterinarian's reported confidence in their ability to help their dairy clients assess cows before transport ($P < 0.05$). The potential implications of this research project include providing producers with an effective tool to help assess their cattle before transport, and to encourage greater veterinary involvement with their clients.

Key Words: culled cow, transport

268 Effect of selenium-enriched hay on Se concentration in blood and milk, immune function, and performance in dairy cows during the transition period. S. Jaaf*[1], M. Mezzetti[2,1], A. Krueger[1], B. Batty[1], J. Belveal[1], M. Premi[2,1], J. Foster[1], E. Trevisi[2], G. Bobe[1], C. Estill[1], and M. Bionaz[1], [1]*Oregon State University, Corvallis, OR,* [2]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Supplementation with selenized yeast before parturition improves Se and antioxidant status of dairy cows during early lactation. Agricultural biofortification is a cost-effective method for selenium supplementation of beef cows and their offspring. In this study, we evaluated the effect of supplementing dairy cows with sodium-selenate fertilized alfalfa from 40 d prior parturition to 2 weeks postpartum on whole blood and milk level of Se and other trace minerals, immune competence, and performance. After blocking for breed, 12 dairy heifers (6 Holsteins and 6 Jerseys) fed ad libitum with a TMR based on grass silage (0.14 mg Se/kg DM) were randomly assigned to a TRT group supplemented with 1 kg DM/100 kg BW with alfalfa enriched with Se (3.2 mg/kg DM) or a CTR group supplemented with low Se alfalfa (0.4 mg/kg DM). Cows were monitored daily for health status, dry matter intake, activity, and milk yield and weekly for body weight and body condition score. Carrageenan skin test was performed throughout the experiment. Whole blood and milk were used to determine Se and other trace minerals by ICP-MS. White blood cells count and hematocrit were assessed. Phagocytosis and leukocytes differentials were measured by flow cytometer. Milk yield and components were measured at each milking using Afimilk system and samples were collected to assess milk composition by a DHIA lab. Data were analyzed by GLIMMIX of SAS with the fixed effect of treatment, breed, time and their interactions and cows as random effect. After 4 weeks into the trial, Se concentration in whole blood increased 2-fold ($P < 0.0001$) in TRT vs. CTR (210.4 vs. 105.9 ng/mL) that resulted in higher ($P < 0.01$) Se in milk (42.1 vs. 24.9 ng/mL). Milk yield was higher in TRT vs. CTR (20.2 vs. 18.2 kg/d, $P < 0.05$). No other differences were detected. Our preliminary results point out that agricultural Se biofortification of dairy cows during the close-up period is an effective and cost-efficient management tool to improve Se in blood and milk. Se supplementation improved milk yield but had not

measurable effect on leukocytes profile and peripheral immune response in primiparous cows during the peripartum.

Key Words: agricultural biofortification, immune response, dairy cows

269 Statistical validation of a geometric approach to image analysis of anatomical traits. C. McVey*[1], J. Velez[2], and P. Pinedo[1], [1]*Colorado State University, Fort Collins, CO,* [2]*Aurora Organic Dairy, Boulder, CO.*

With milk records and treatment histories increasingly supplemented with insights from genomic and sensor technologies, the future of dairy will be an incredibly data rich one. Yet a cow's conformation and structure, the cornerstones of most dairy evaluations, are still largely assessed by eye. The goal of this research was to establish the foundational algorithmic framework needed to extract objective and reproducible measures of anatomical traits from standard quality 2D images acquired in a typical production environment. The training data set consisted of facial images from 108 mature Holstein cows acquired on 3 separate days while restrained at the feedbunk. The standard human facial mesh was adapted to a bovine model, and key anatomical points extracted from each cow image using the MatLab GInput tool. Eye shape was then quantified using 2 schemes: normalized distances between all pairwise combination of points, and a novel geometric approach to biometric extraction that emphasized angles and projection lengths. Compared with the simple normalized distances, the geometric biometrics showed on average a 10% reduction in measurement error associated with human error in point annotation. Geometric measures also demonstrated less correlation among themselves than the normalized distance measures, a desirable trait for the development of stable linear predictive models. To assess the resilience of these metrics to variations in image quality, measurement error across days was regressed against measures designed to reflect changes in image scaling, frame position, and face angle. For the geometric measures, average total correlation between measurement error and measures of image quality was less than 5%, with no correlations exceeding 25%. For normalized distances, average total correlation between measurement error and measures of image quality with 15%, with multiple metric having over 50% of their measurement error attributed to subtle changes in image attributes. These results suggest that a geometric approach to anatomical biometrics could provide a more robust and consistent means of extracting detailed quantitative measures of physical traits from farm quality images.

Key Words: image analysis, dairy

270 Factors associated with veal calf morbidity on an Ontario grain-fed (red) veal operation. K. J. Scott*, D. L. Renaud, T. F. Duffield, and D. F. Kelton, *University of Guelph, Guelph, ON, Canada.*

Health status at arrival at veal facilities may be an important predictor of calf morbidity and performance, as substantial disease occurs early in the calves' growing period on veal operations. The objective of this prospective cohort study is to explore associations between health attributes measured at arrival at a veal rearing facility and morbidity risk. Calves were evaluated immediately at arrival using a standardized health scoring system (Calf Health Scorer App), blood was collected to measure serum total protein (STP), and weight was measured at arrival. A mixed logistic regression model was built to evaluate factors associated with morbidity <21 d following arrival. Between January and July 2017, a total of 590 Holstein calves of unknown age were evaluated. In the first 21 d following arrival, 68% of calves were treated for either respiratory disease or diarrhea, and 5% died. There were 5 variables significant in the final model evaluating the association between morbidity and conditions identified at arrival. Calves without a sunken flank, those that weighed more at arrival, or those that had a higher concentration of STP had reduced odds of morbidity. Calves that were moderately dehydrated had a reduced odds of being treated for disease compared with a non-dehydrated calf. More specifically, 38% of calves presented with sunken flank, 25% were below 95lbs upon arrival, failure of passive transfer was present in 32% of the calves, and moderate dehydration was present in 7% of the calves. The results of this study indicate that risk factors for morbidity can be identified upont arrival at a veal facility, and interventions may be warranted to reduce morbidity. Future work will evaluate the efficacy of interventions to reduce the prevalence of these risk factors before arrival, so that veal calf health and welfare can be improved.

Key Words: male calves, dairy, morbidity

271 Effects of prebiotic supplementation on gut health, cellular immune function and performance of dairy calves. R. Gardinal[1], C. A. F. Oliveira[1], J. F. A. Koch[1], B. Mazzer*[1], F. de Oliveira Roberti[1], A. M. Rocha[1], and V. Vetvicka[2], [1]*Department of Research and Development, Biorigin Company, Lençóis Paulista, SP, Brazil,* [2]*Department of Pathology, University of Louisville, Louisville, KY.*

The commensal gut microbiota plays an important role for the host. Many carbohydrates from yeast cell wall, such as mannanoligosaccharides are used in livestock as gut prebiotic, acting as substrates for beneficial bacteria in the gut and purified β-glucans as immune response enhancers. This study aimed to evaluate if Prowean Rumi (PWR; Biorigin, Brazil) – a new generation of functional carbohydrates from yeast cell wall – is able to modulate the gut environment and enhance cellular immune function. The study was performed at University of Louisville, Kentucky. A total of 30 dairy calves with 20 d of age were randomly and equally assigned to 2 treatments: 1) Control (C; n = 15), no supplementation; 2) PWR (n = 15), fed 100 mg of PWR kg^{-1} of body weight added to milk during 28 d. The body weights were recorded weekly to quantify the average daily gain (ADG). At the end of experiment peripheral blood sample were collected and assays employing phagocytosis of synthetic polymeric microspheres were carried as described by Vetvicka et al. (2014). Short-chain fatty acids (SCFA) in feces were measured by PCR at the last day of experiment. Data were analyzed by ANOVA using the PROC MIXED of SAS 9.1 with fixed dietary effect, time effect, interaction between diet and time. Our results showed that addition PWR potentiated phagocytic activity ($P < 0.05$) of peripheral blood monocytes (21.8 vs. 24.6%) and neutrophils (25.4 vs. 28.9%) and increased ($P < 0.05$) SCFA concentration (105.5 vs. 111.5 mmol/L) in feces compared with control animals, demonstrating prebiotic effect and a more efficient immune response. Moreover, calves supplemented with PWR showed higher ($P < 0.05$) ADG and final body weight compared with control animals, demonstrating that modulation of the intestinal environment can result in higher absorption of nutrients. In conclusion, PWR can improve gut health, cellular immune function and performance of dairy calves supplemented.

Key Words: gut health, prebiotic, dairy calves

272 Evaluating the relationship between hoof lesions and culling of dairy cows. B. O. Omontese*[1], R. Bellet-Elias[1], A. Molinero[1], G. Catandi[1], R. Casagrande[1], Z. Rodriguez[1], R. S. Bisinotto[2], and G. Cramer[1], [1]*Department of Veterinary Population Medicine, University*

of Minnesota, St. Paul, MN, [2]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.*

Objective of this study was to evaluate the relationship between hoof lesions (HL) and culling in lactating dairy cows. Jersey cows were enrolled at 20 ± 3 DIM (d20). At d20, cows were evaluated for body condition score (BCS; high ≥3.50, moderate = 2.75 to 3.25, low ≤2.50) and were examined for presence of claw horn HL (sole ulcers, toe ulcer, white line disease), sole hemorrhage (SH), infectious HL (digital dermatitis, foot rot), and injury. At 120 ± 3 DIM (d120), cows were reevaluated for presence of HL and BCS. Culling data (died or sold) were collected until 250 DIM and cows that left the herd were right censored. To evaluate the relationship between HL present in early lactation and culling, lesion status at d20 was categorized into (1) no HL (noHL; n = 1,197) or with HL (wHL; n = 442) and (2) HL category as claw horn (CLW; n = 113), SH (n = 280), infectious (INF; n = 36), and injury (INJ; n = 13). To assess the relationship between development of new HL and culling, cows were classified as healthy (no HL at d20 and d120; n = 308), cured HL (presence of any HL at d20 and no HL at d120; n = 72), new HL (no HL at d20 and presence of any HL at d120; n = 597), or chronic HL (presence of any HL at d20 and d120; n = 226). Association between HL and culling was assessed using Kaplan Meier curves and Cox's proportional hazard regression. Overall, 13.4% cows (n = 221) were culled and there was no relationship between HL at d20 and culling (HR: 1.21; 95% CI: 0.91 to 1.61). Of all the lesion categories assessed in this study, cows with CLW had a greater culling hazard compared with healthy cows (HR: 1.52; 95% CI: 1.01 to 2.27; $P < 0.01$). Furthermore, cows with INF lesions tended to have greater culling hazard compared with healthy counterparts (HR: 1.72; 95% CI: 0.94 to 3.12; $P = 0.07$). Overall, 93% of new HL was SH. Cows that developed new HL had a smaller culling hazard compared with healthy cows (HR: 0.42; 95% CI: 0.22 to 0.78; $P < 0.01$). In conclusion, preexisting CLW and INF lesions at d20 were associated with increased hazard of culling whereas development of new HL between 20 and 120 DIM was associated with a reduction in hazard of leaving the herd.

Key Words: herd exit, hoof trimming, lameness

Breeding and Genetics Symposium: Fertility—Filling the Gaps

273 Translating the physiology of fertility into improved phenotypes for genetic selection. M. Lucy*, *University of Missouri, Columbia, MO.*

In most dairy systems, the ability to establish pregnancy following insemination is the primary definition of fertility. Highly fertile cows establish pregnancy sooner after calving and require fewer inseminations. Pregnancy is a logical endpoint for defining the fertility phenotype because it is typically recorded on farm and can be verified by using calving dates. Pregnancy is established through a series of individual events that occur in sequence. In postpartum cows, for example, estrous cycles are re-established, estrus is expressed and detected, sperm are deposited in the reproductive tract and capacitate, ovulation occurs and is followed by fertilization, and the corpus luteum forms and produces sufficient progesterone to maintain pregnancy. The oviduct supports early cleavage and the uterus establishes a receptive environment for the developing pregnancy. Each individual event is theoretically heritable and these events collectively contribute to the phenotype of pregnancy after insemination. Genetic selection as it is practiced today does not target individual mechanisms leading to pregnancy because they are difficult to phenotype reliably on a large number of cows. Dairy systems differ with respect to reproductive management applied to cows. In some systems, cows are inseminated after detected estrus with minimal intervention. In these systems, the establishment of pregnancy early postpartum effectively captures the summation of the individual events leading to pregnancy. More intensive systems that use hormonal treatments ($PGF_{2\alpha}$, GnRH, etc.) followed by timed AI do not invalidate the current phenotypes but the individual components that contribute to the phenotype may be more or less important to the establishment of pregnancy. Uterine release of $PGF_{2\alpha}$ and the establishment of adequate circulating estradiol to cause an endogenous GnRH surge and estrus expression, for example, are not necessary to establish pregnancy when timed AI is used. Selecting cows for a functional reproductive system comprised of individual components involved in the establishment of pregnancy may ensure uniform performance of future cows across a variety of reproductive management systems.

Key Words: dairy cow, fertility, phenotype

274 The choice and collection of new relevant phenotypes for fertility selection. A. Fleming[1], C. F. Baes[1], F. Malchiodi[1], L. F. Brito[1], and F. Miglior*[1,2], [1]*CGIL - University of Guelph, Guelph, ON, Canada,* [2]*Canadian Dairy Network, Guelph, ON, Canada.*

The fundamental aspect of dairying is successful reproduction. Impaired fertility represents a major financial loss for the dairy producer and is a prominent reason for involuntary culling. As selection for increased production was realized, deterioration in female fertility was witnessed, and, appropriately, selection indices expanded to include fertility traits, like calving interval, days open, calving to first service, first service to conception and 56d non return rate. The inclusion of fertility traits worldwide has had an impact in reversing the negative trend with regards to calving interval, days open and calving to first service, but little or no gain has been achieved for traits related to conception, such as first service to conception and 56d non-return rate. Fertility traits typically have low heritabilities, and relevant traits closer to the true reproductive biology are difficult to measure, making progress in female fertility challenging and the search for novel phenotypes appealing. Many alternative fertility indicator traits have emerged with the evolution of on- and off-farm technologies, such as milk progesterone and fatty acid profile, energy balance, and body weight and condition. The rise of genomic selection and genotyping breeding candidates becoming more commonplace may be particularly valuable for the improvement of fertility. The identification of novel lethal recessive genes affecting the viability of the pregnancy could aid in future matings. Haplotypes affecting fertility, including AH1 in Ayrshires, have already been described, and genomics may further uncover underlying genetic components influencing fertility for more effective selection. Reproductive technologies have alleviated some problems but perhaps only temporarily concealed the issue at hand. Genetic improvement of fertility of dairy cattle in the future will require more relevant phenotypes to be evaluated that more closely describe fertility, and those, which promote the improvement of fertility without intensive intervention from the producer.

Key Words: fertility, novel phenotype, trait identification

275 Embryonic survival: The other side of fertility—A genomic perspective. H. Khatib*, *Department of Animal Sciences, University of Wisconsin, Madison, WI.*

Currently within in vitro production systems, preimplantation mammalian embryos are selected for transfer to a recipient based on morphological assessment including visual valuation of cell number, fragmentation, compaction, and color for grading the embryo in terms of stage and quality. Though embryos may be of similar morphology, they may not be of similar developmental competence. Transcriptomic analysis performed in our laboratory revealed differential expression of 793 genes between in vitro- and in vivo-derived embryos, with 35 genes uniquely expressed within the in vivo embryos and 10 genes uniquely expressed within the in vitro embryos. A more efficient method of embryo selection would assess the potential reproductive success of both gametes and embryos based on their underlying genetic potential. miRNAs are a class of non-coding RNA that are 18–22 nucleotides in length which act to regulate gene expression of their target mRNAs through a RNA interference mechanism. These miRNAs have been found to be secreted out of cells into the extracellular environment with relatively high stability. Recently, we found that miRNAs are secreted by in vitro produced embryos into their respective culture media, and that these small RNAs are differentially expressed between conditioned media from embryos of differing development competence. We also fund that male and female embryos differentially secrete miRNAs via extracellular vesicles and that these secreted miRNAs serve as signals to the mother. Implications from these studies set the framework for future development of method for non-invasively surveying the developmental potential of embryos based on miRNA biomarkers.

276 Identification of loci associated with pregnancy in Holstein heifers and primiparous cows. H. L. Neibergs*[1], J. N. Kiser[1], E. Clancy[1], E. M. Keuter[1], J. Dalton[2], J. G. N. Moraes[3], C. M. Seabury[4], and T. E. Spencer[3], [1]*Department of Animal Sciences, Washington State University, Pullman, WA,* [2]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [3]*Division of Animal Sciences, University of Missouri, Columbia, MO,* [4]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX.*

Pregnancy per artificial insemination at observed estrus is estimated to be 55–70% for dairy heifers and 35% in lactating dairy cows. Pregnancy rates for females that require more than one breeding are further

reduced. The objective of this study was to investigate if loci associated with pregnancy at first service were shared with loci associated with pregnancy at ≥4 services in Holstein heifers and cows. Nine hundred 2 heifers and 1032 primiparous cows were bred by artificial insemination during observed estrus for up to 5 consecutive estrus cycles for heifers and up to 13 cycles for cows. Pregnancy was determined via palpation on d 35 for heifers and cows not returning to estrus. Heifers and cows were genotyped using the Illumina BovineHD BeadChip (777,962 SNPs) and a genome-wide association study (GWAS) was conducted with a significance threshold of $P < 5 \times 10^{-8}$ to identify individual associations and $P < 1 \times 10^{-5}$ to identify shared associations. The GWAS identified 65 SNPs associated with pregnancy to first service in heifers and 138 SNPs associated with pregnancy to first service in cows. Eleven SNPs associated with pregnancy to first service were shared among heifers and cows. One hundred 64 SNPs were associated with the number of times bred (1, 4 or 5 times) required for pregnancy at d 35 in heifers, 79 SNPs were associated with times bred (1, 4–13 times) in cows and 7 SNPs were shared among Holstein heifers and cows. Two loci were associated with all phenotypes in heifers and cows. These results indicate that although most loci associated with pregnancy per artificial insemination in heifers and primiparous cows are unique, there are shared loci that are important in achieving pregnancy in both groups that could be used for genomic selection. *This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2013-68004-20365 and 2018-67015-27577 from the USDA National Institute of Food and Agriculture.*

Key Words: genome-wide association study (GWAS), dairy heifer, loci

277 Big data genomic investigation of dairy fertility and related traits with imputed sequences of 27K Holstein bulls. J. Jiang[1], P. VanRaden[2], J. Cole[2], Y. Da[3], and L. Ma*[1], [1]*University of Maryland, College Park, MD,* [2]*Animal Genomics and Improvement Laboratory, Beltsville, MD,* [3]*University of Minnesota, St Paul, MN.*

Imputation has been routinely applied to ascertain sequence variants in large genotyped populations based on reference populations of sequenced animals. With the implementation of the 1000 Bull Genomes Project and increasing numbers of animals sequenced, fine-mapping of causal variants is becoming feasible for complex traits in cattle. Using the 1000 Bull Genomes data, we imputed 3 million selected sequence variants to 27,000 Holstein bulls after quality control edits and LD pruning. These bulls were selected to have highly reliable breeding values (PTAs) for 35 production, reproduction, and body conformation traits. We first performed whole-genome single-marker scan for the 35 traits using the mixed-model based association test in MMAP (https://mmap.github.io). The single-trait association statistics were then merged in multi-trait analyses of 3 groups of traits, production, reproduction, and body conformation, respectively. Candidate genomic regions 2 Mb long, were selected based on the multi-trait analyses and used in fine-mapping studies. We implemented a state-of-art fine-mapping procedure with a Bayesian method that can assign a posterior probability of causality to each variant and for each independent association signal generate a minimum set of associated variants whose total posterior probability of causality exceeds a threshold (e.g., 95%). Our fine-mapping identified 36 candidate genes for production traits, 48 for reproduction traits, and 29 for body conformation traits, respectively, including some previously reported causal variants, e.g., Chr6:38027010 in ABCG2 for production traits and Chr7:93244933 in ARRDC3 for reproduction and body conformation traits. The candidate variant list may facilitate follow-up functional validation and expand our understanding of complex traits in dairy cattle. Additionally, our method can be readily applied to other species where large-scale sequence genotypes are available.

Key Words: genomics, reproduction, dairy

278 Genetic cues from fertilization to pregnancy establishment. M. S. Ortega*[1], J. B. Cole[2], T. E. Spencer[1], and P. J. Hansen[3], [1]*University of Missouri, Columbia, MO,* [2]*Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD,* [3]*University of Florida, Gainesville, FL.*

One approach to improve genetic selection for reproductive traits is to identify SNP in genes linked to reproductive processes. Genes in which these SNP reside represent targets for physiological intervention to improve fertility.Sixty-eight SNP previously associated with genetic merit for fertility and production were tested for association with daughter pregnancy rate (DPR) and other fertility traits in an independent population of Holsteins. There were 22 SNP in genes associated with genotypic estimates of fertility in the 2 Holstein populations; moreover, animals carrying allelic variants associated with higher genetic merit for fertility also exhibit more favorable phenotypic measurements of fertility. DPR reflects days open, which entails many physiological events including the ovulation of a competent oocyte, adequate sperm transport in the reproductive tract, successful fertilization in the oviduct, and development and implantation in the uterus. Genes containing SNP repeatedly associated with reproductive traits provides an indication of physiological processes important for variation among cows in reproductive function. Among the genes associated with fertility traits in both populations 14 genes were regulated by steroids, there were also genes involved in processes including oocyte quality (*COQ9*), fertilization (*BSP3*), trophectoderm formation (*WBP1*), and lipid biosynthesis (*ACAT2*, *HSD17B7*, and *HSD17B12*). Future directions should include functional studies involving genome engineering to understand the biological role of genetic variants in the tight regulation of reproductive function in cattle.

Key Words: fertility, embryonic development, pregnancy establishment

279 ADSA®-EAAP PhD Student Travel Award Presentation: Bioactivities of milk proteins evaluated after in vitro digestion and peptidomic/proteomic profile. C. Giromini*[1], I. D. Givens[2], J. A. Lovegrove[3,4], R. Rebucci[1], E. Maffioli[5], G. Tedeschi[5], and A. Baldi[1], [1]*Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy,* [2]*Institute for Food, Nutrition and Health, University of Reading, Reading, United Kingdom,* [3]*Hugh Sinclair Unit of Human Nutrition Department of Food and Nutritional Sciences, University of Reading, Reading, United Kingdom,* [4]*Institute for Cardiovascular and Metabolic Research, University of Reading, Reading, United Kingdom,* [5]*Department of Veterinary Medicine (DiMeVet), University of Milan, Milan, Italy.*

The health-promoting functions of milk peptides are related to their structures, which depend on the parent protein composition. A crucial issue in this field is the demonstration of a cause-effect relationship, from the ingested form to the bioactive form. Thus, the aim our study was to in vitro digest intact whey proteins (WPI) and casein proteins (CP), mimicking in vivo digestion, to investigate their bioactive effects. WPI and CP were digested using a pepsin/pancreatin protocol and ultrafiltered (3kDa cut-off membrane). A permeate (mimicking absorbed fraction) and a retentate (mimicking intestinal fraction) were obtained. Soya protein was included as control (CTR). Antioxidant (AOX) and angiotensin-I-converting enzyme inhibitory activity (ACEI-i) of the WPI and CP fractions were assessed and compared with undigested sample (UND) and CTR. Further, the effects of WPI and CP retentate on proliferation and on mucus production (MUC5AC gene expression and mucin secretion) were assessed in intestinal goblet HT29-MTX cells. Finally, permeate has been characterized by LC-nano ESI MS/MS using a shotgun-peptidomic approach while retentate has been further digested with trypsin and analyzed by mass spectrometry with a shotgun-proteomic approach and the potentially bioactive peptides and polypeptides have been identified. WPI and CP permeate showed a significant ($P < 0.05$) increase in AOX up to 1.58 ± 0.04 and 1.61 ± 0.02 µmol trolox antioxidant equivalents /mg protein (TrAOX equ/mg P), compared with UND (<0.6 µmol TrAOX equ/mg P) and CTR permeate (1.37 ± 0.04% µmol TrAOX equ/mg P). WPI and CP permeate exhibited a higher ($P < 0.05$) ACEI-i (49.20 ± 0.64 and 23.91 ± 0.64%, respectively) compared with UND (<19%) and with CTR permeate (10.40 ± 1.07%). In HT29-MTX cells, WPI and CP retentate, at specific concentrations, were able to modulate ($P < 0.05$) cell proliferation; MUC5AC expression was increased (+20%; $P < 0.05$) by CP retentate and unaltered ($P > 0.1$) by WPI retentate. CP retentate evoked mucin-secretory effect. Our results confirm that milk proteins may be rich sources of bioactive compounds with a greatest beneficial potential of CP at intestinal goblet cell level.

Key Words: milk protein, bioactivity, peptidomic

280 Quantitative difference in proteomic profiles of milk whey protein in Murrah, Nili-Ravi, and Mediterranean water buffalo. S. Li*[1], L. Li[2], J. Liu[1], Y. Yang[3], and D. Ren[1], [1]*Institute of Dairy Science, College of Animal Science, Zhejiang University, Hangzhou, Zhejiang, China,* [2]*Water Buffalo Institute, Chinese Academy of Agricultural Science, Nanning, Guangxi, China,* [3]*Institute of Animal Science and Veterinary Medicine, Anhui Academy of Agricultural Sciences, Hefei, Anhui, China.*

Milk whey proteins play a critical role in immune defense and are beneficial for human nutrition and health. The aim of this study was to characterize the milk whey proteins and their potential activities among different buffalo breeds. In this work, a tandem mass tags (TMT) proteomic approach was used to identify the differences in the proteomic profiles of milk whey proteins in Murrah, Nili-Ravi and Mediterranean water buffalos. Of the 589 identified proteins, there were 64 differentially expressed proteins identified by ANOVA analysis in buffalo milk whey proteomes. The most abundant proteins were polymeric immunoglobulin receptor, α1-antiproteinase, heat shock cognate 71 kDa protein, Acl-CoA-binding protein, pigment epithelium-derived factor, antithrombin-III, and α2-HS-glycoprotein in Mediterranean water buffalo milk, fibroblast growth factor-binding protein 1 in Murrah buffalo milk, while clusterin, actin cytoplasmic 2, peroxiredoxin-2, and sortilin in Nili-Ravi buffalo milk. Gene ontology annotation revealed that molecular function of differentially epressed proteins were protein binding, enzyme regulator activity, and molecular function regulator. Furthermore, pathway analysis indicated that most differentially expressed proteins participated in complement and coagulation cascades pathway, which are strongly related to immune function. In addition to providing insight into the complexity of the buffalo milk whey proteome and their potential physiological functions, our study provided the molecular evidence of nutritive differences among different buffalo breeds. The presence of greater abundance of immune-protection whey protein in Mediterranean water buffalo milk indicated that this high nutritive milk is a high-quality resource for dairy-based functional food exploitation.

Key Words: whey protein, water buffalo, proteomics

281 Testing functional boundaries of dairy ingredients in protein-fortified dairy gel systems. H. Zheng*[1], W. Wang[2], J. Lin[2], and T. Mendes Borges[2], [1]*Dairy Innovation Institute, Animal Science Department, California Polytechnic State University, San Luis Obispo, CA,* [2]*Department of Wine, Food and Molecular Biosciences, Lincoln University, Christchurch, New Zealand.*

The global high protein based food market is strong and it has been increasing since the last decade. According to the latest projection from "technavio," the size of the market for high protein foods (2017–2021) will reach to 91.07 billion USD by 2021. In the current research, Greek-style yogurt (GSY, 6.0% protein content) and Requeijão processed cheese (RPC, 10% protein content) were chosen as high protein food models. Milk protein concentrate (MPC80), micellar casein concentrate (MCC), Ca-caseinate (CaCN), and whey protein concentrate (WPC80) were used as protein fortifiers for increasing the total protein contents of the 2 food models. Using mixture design approach, we studied the functional features of different dairy ingredients in terms of modulating textural attributes and stability of the yogurt and processed cheese systems. The GSY gel was prepared using both fermentation and GDL (glucono delta lactone) induced acidification methods. Principal Component Analysis (PCA) showed that there is no discrimination between fermented GSY and GDL acidified GSY made from different ingredients in terms of textural attributes. Mixture design counter plots (MDCPs) ($P < 0.05$) showed that the GYS syneresis rate was significantly reduced when MPC, WPC, and CaCN mixed at optimum proportion as a protein-fortifier bundle rather than using individual ingredients along indicating the synergistic effect among dairy protein ingredients. The RPC was prepared using direct acidification method, the MDCPs ($P < 0.05$) showed that MPC and MCC had a similar capability in terms of

enhancing the adhesiveness, MCC has unique functionality in increasing firmness and viscosity of the processed cheese comparing with MPC and CaCN. The current research demonstrated the synergistic functionalities of different dairy protein ingredients in protein fortified dairy gel systems, such knowledge may be used for creating "clean-label" or "all dairy" formulations.

Key Words: dairy protein ingredient, Greek style yogurt, processed cheese

282 Micellar casein concentrate: Purity, serum protein removal, and sensory properties. D. M. Barbano*[1] and M.A. Drake[2], [1]*Cornell University, Ithaca, NY,* [2]*North Carolina State University, Raleigh, NC.*

Micellar casein concentrate (MCC) and serum protein (SP) isolate (SPI) can be made from skim milk (SM) using ceramic (C) or spiral wound (SW) polymeric microfiltration (MF) membranes. MCC can be made at different purities (i.e., different SP removal). The purpose of this work was to demonstrate the relationship between values for SP removal and MCC purity. High quality raw SM contains about 3.20% true protein (TP), 2.624% casein, and 0.576% SP and contains about 82% casein as a percent of TP (CN%TP). SP removal (%) with SW and C MF membranes differs, with C membranes having higher SP removal in a 3 stage, 3X MF process at 50°C (72 versus 95% respectively). SP removal is expressed as total Kg SP in permeate removed divided by Kg SP in the original skim multiplied by 100 (measured by Kjeldahl). MCC purity is often expressed as CN%TP. A MF process at 72% versus 95% SP removal in a factory processing 908,000 kg of SM in a day would have a higher yield of 3768 versus 4967 kg of SP per day and a MCC purity of 94.2 and 98.9% CN%TP, respectively. Differences in MCC concentrate purity will have different effects depending on the application (e.g., cheese making versus shelf stable beverages). In retorted or ultrapasteurized beverages, flavor is important. Lower purity of MCC in high-heat-treated beverages may cause more heat induced off flavors due to thermal degradation of milk SP. The lower purity MCC (94.2 versus 98.9%) has a 5 to 6 fold higher SP concentration. The impact of MCC purity is magnified in higher protein beverages as the absolute concentration of the SP increases. Heat induced SP degradation products (e.g., H_2S, methional) have very low sensory thresholds and higher concentrations of residual SP in MCC will be correlated with higher intensities of heat induced off flavors. In addition, the amount of SP in the final MCC will be influenced by thermal denaturation of SP in the pasteurization skim milk before MF, regardless of the type of membrane. Minimum HTST pasteurization will have an apparent increase in CN%TP from 82 to about 83% by Kjeldahl due to SP denaturation. Heat induced covalent binding of SP to casein micelles causes over estimation of MCC purity when measured by classical Kjeldahl methods, so HPLC or SDS-PAGE would provide more correct determination of MCC purity.

Key Words: microfiltration, micellar casein concentrate (MCC), serum protein concentrate

283 Effects of microfluidization on the enzyme coagulation properties of milk. A. J. Bucci[1], D. L. Van Hekken*[1], M. H. Tunick[1,2], and P. M. Tomasula[1], [1]*USDA, ARS, Wyndmoor, PA,* [2]*Drexel University, Philadelphia, PA.*

The chymosin-induced coagulation of microfluidized milk was evaluated to determine its potential in making high-moisture cheese. Raw, thermized (65C, 15s) and HTST pasteurized (75C, 15s) 3.0% (wt/wt) fat milk samples were microfluidized at 4 treatment conditions: 42C/75 MPa, 42C/125 MPa, 54C/125 MPa, and 54C/170 MPa; controls consisted of non-homogenized and homogenized (2-stages at 10/5 MPa) milk. Microfluidized and non-microfluidized milk samples were evaluated for alkaline phosphatase activity and median particle size. After chymosin addition, coagulation time, curd firmness and microstructure were examined. Microfluidization had varied and significant effects ($P < 0.05$) on the size reduction of fat droplets, coagulation time, and curd firmness, while either heat treatment alone did not. Alkaline phosphatase was inactivated in all samples except for the raw and thermized milk non-microfluidized controls and the 75 MPa treatment. Particle size decreased by half during 2-stage homogenization, and 15- to 20-fold after microfluidization yielding median area-weighted values, d(3,2), of 7.9, 4.2, and 0.39 to 0.50 μm, respectively. Milk samples microfluidized at 42C and 75 or 125 MPa were similar to the controls in coagulation times and curd firmness. Compared with the controls, milk microfluidized at 54C and 125 or 170 MPa took 3 to 8 times longer to coagulate and had lower curd firmness, which indicated that protein matrix formation, a critical step in the production of cheese, had been altered. Scanning electron microscopy images of the chymosin curds illustrated that, compared with the controls, the use of nonthermal microfluidization at different pressures resulted in modified casein-lipid structures that reflected the altered interactions of the smaller sized lipid droplets and intact or fractured casein micelles. Use of this technique will help meet consumer demand for novel dairy products such as cheeses with yogurt-like textures, cheese snacks, and desserts.

Key Words: milk, microfluidization, chymosin coagulation

284 Effect of milk protein solution viscosities on electrospun fiber formation. S. Akkurt[1,2], L. M. Bonnaillie[2], and P. M. Tomasula*[2], [1]*Food Science Department, Rutgers, The State University of New Jersey, New Brunswick, NJ,* [2]*Dairy & Functional Foods Research Unit, United States Department of Agriculture, Agricultural Research Unit Service, Eastern Regional Research Center, Wyndmoor, PA.*

Electrospinning has been used to produce edible fibrous mats from micro- or nanosized fibers of sodium caseinate (NaCAS) and calcium caseinate (CaCAS) with pullulan (PUL), a polysaccharide added to facilitate formation of the caseinate fibers. The effects of rheological properties on electrospun fiber morphology are known for many synthetic and water-soluble polymers; however, this information is not available for proteins in aqueous solution such as the milk proteins or in blends with polysaccharides, such as PUL. The objective of this study was to determine the dependence of specific viscosity on aqueous protein concentration, C, for neat NFDM, CaCAS and NaCAS and in blends with PUL, to identify the entanglement concentrations, Ce, the point at which the respective protein chains begin to interact, and to identify the C regions where fibers are formed. In this study, aqueous solutions of NFDM, CaCAS, NaCAS, and PUL were prepared at 20°C at C ranging from 1 to 20 wt% and then 1:1 blends of each protein and PUL solution were prepared with total C also ranging from 1 to 20 wt%. A syringe containing 3 mL of each solution was used to feed an electrospinning unit at flow rate of 3mL/h, and at voltage of 20 kV, with fibers forming a fibrous mat. From plots of specific viscosity as a function of C for the neat and blended protein solutions, 3 regions were identified: the semidilute unentangled ($C < C^*$), a semidilute entangled ($Ce > C > C^*$) and the concentrated entangled ($C > Ce > C^*$). In general, electrospraying (drops) was observed for the blended solutions at $C < C^*$; followed by semidilute entangled region of beaded fibers, and fully formed fibers just after the transition to Ce. Fully formed fibers were not identified at $C > Ce$ for the neat protein solutions but formed powders

since electrospraying behavior predominated over the C range due to the lower solution viscosities. This is the first detailed examination of the dependence of milk protein-based nanofiber morphology on the viscosity and concentration regimens.

Key Words: nanofibers, rheological properties, entanglements

285 Comparison of yogurt gels made from various types of milk proteins. N. Trusler*[1], J. Lucey[1,2], and M. Molitor[1,2], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Center for Dairy Research, Madison, WI.*

We wanted to explore if the concentration of minerals in milk protein products affected the functional properties such as in acid milk gels. Microfiltration (MF) was used to deplete whey proteins and minerals from acidified milk to create soluble casein isolate (SCI), which was compositionally similar to sodium caseinate. Yogurt gels were made from SCI, sodium caseinate, nonfat dry milk, and 4 commercial milk protein concentrates (MPC) that had some level of mineral reduction. All powders were rehydrated and standardized to 5% protein. Yogurt gels were made by inoculating milk with yogurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, and fermented at 40°C until pH 4.6 for the MPC, and to pH 4.3 for sodium caseinate and SCI (both of which were supplemented with peptone to aid fermentation). Rheological properties such as gel stiffness and loss tangent were measured during fermentation, and yield stress was measured upon reaching the target pH. Buffering capacity was measured using acid-base titrations to indicate levels of insoluble calcium phosphate. Soluble calcium levels in rehydrated samples were determined in ultrafiltered permeate by performing inductively coupled plasma spectrometry (ICP) on the permeate. Calcium depleted MPC had a lower buffering capacity than nonfat dry milk, sodium caseinate and SCI had similar (very low) buffering capacities. Calcium depleted MPC also had lower soluble calcium levels than nonfat dry milk, whereas sodium caseinate and SCI had similar but very low levels of soluble calcium. Gels made from MPC had similar gel stiffness to nonfat dry milk, but had higher yield stress, indicating a more cross-linked yogurt gel. Sodium caseinate and SCI had similar (weak) gel stiffness during fermentation, but sodium caseinate gels exhibited higher yield stress values than SCI. Overall, this MF process produced SCI with similar acid gelation characteristics to sodium caseinate.

Key Words: microfiltration, calcium depletion, functionality

286 Performance of dairy and plant proteins in a model high-acid beverage system. H. Jiang* and K. Burrington, *Wisconsin Center for Dairy Research, Madison, WI.*

The protein drink market reached more than $600 million wholesale in 2016 and is expected to continue to grow (Beverage Industry, May 2017). Increases in consumer interest in protein and an insurgence of new protein ingredients has led to the development of new protein based foods and beverages. Each protein ingredient has its unique set of functional and sensory properties, which can contribute to formulation challenges. In this study, the functional properties (heat stability, emulsion, viscosity, and foaming) of 30 commercial dairy- and plant- based protein powders were investigated. Based on heat stability testing at pH3, 7 protein ingredients (whey protein concentrate and isolate, milk-derived whey, potato protein, soy protein, pea protein, and rice protein) were selected for a high acid beverage application. A mango flavored beverage formula with 87% water, 7% sugar and 5% protein was chosen. All the beverages were adjusted to pH 3 with phosphoric acid before heat treatment at 82.2°C for 2min through an HTST process in a pilot plant. The beverages were cold-filled into pre-sanitized bottles and stored at 4°C. One set of beverages was stored in a higher temperature (45°C) incubator for 2 weeks to simulate an accelerated shelf life study. Rice protein beverages showed phase separation immediately after processing. Pea and soy protein beverages phase separated when stored at 45°C. Whey protein and potato protein beverages stayed stable during storage at 45°C. Changes in color (measured by a colorimeter) with storage was observed for all beverages except the rice protein beverage. Viscosity of beverages was measured by a rheometer at one day after processing and after the 45°C storage. No significant difference in viscosity was observed before and after the shelf life test of non-separated beverages. Sensory evaluation showed that the plant protein beverages had more bitter and beany flavors compared with dairy protein. Whey proteins provide better functionalities, and cleaner taste in high acid beverages. Plant proteins may need more modifications to produce improved functional characteristics and sensory properties in high acid beverages.

Key Words: dairy protein, plant protein, beverage

286 See Dairy Foods Processing Symposium (page 376)

Dairy Foods Microbiology Symposium: Whole-Genome Sequencing for Dairy Microbiology

288 Whole-genome sequencing investigations of flavor formation by dairy microbiota. O. McAuliffe*, *Teagasc Food Research Centre, Fermoy, Cork, Ireland.*

Flavor is one of the most important attributes of any fermented dairy product. Dairy consumers are known to be willing to experiment with different flavors and thus, many companies producing fermented dairy products have looked at culture manipulation as a tool for flavor diversification. The development of flavor is a complex process, originating from a combination of microbiological, biochemical and technological aspects. A key driver of flavor is the enzymatic activities of the deliberately inoculated starter cultures, in addition to the environmental or 'non-starter' microbiota. The contribution of microbial metabolism to flavor development in fermented dairy products has been exploited for thousands of years, but the availability of the whole genome sequences of the bacteria and yeasts involved in the fermentation process and the possibilities now offered by NextGen sequencing is stimulating a more 'knowledge-based' approach to the selection of desirable cultures for flavor development. By linking genomic traits to phenotypic outputs, it is now possible to mine the metabolic diversity of starter cultures, analyze the metabolic routes to flavor compound formation, identify those strains with flavor-forming potential and select these strains for possible commercial application. This approach also allows for the identification of species and strains not previously considered as potential flavor-formers, the blending of strains with complimentary metabolic pathways, and also the potential improvement of key technological characteristics in existing strains, strains which are at the core of the dairy industry. An in-depth knowledge of the metabolic pathways of individual strains and their interactions in mixed culture fermentations can allow starter blends to be 'tailor-made' to suit industry needs. Applying this knowledge to starter culture research programs is enabling R&D scientists to develop superior starters, expand flavor profiles and potentially develop new products for future market expansion.

Key Words: flavor, fermentation, whole-genome sequencing

289 Applying whole-genome sequencing to illuminate dairy sporeformers. J. Kovac*[1], R. Miller[2], L. Carroll[2], S. Beno[2,3], and M. Wiedmann[2], [1]*Penn State, University Park, PA,* [2]*Cornell University, Ithaca, NY,* [3]*The University of Alabama at Birmingham, Birmingham, AL.*

Sporeformers are widespread in the farm environment and are commonly transmitted to milk. They comprise a diverse group of microorganisms with a differential ability to grow at low temperatures, tolerate oxygen, and produce toxins. Some of these characteristics vary widely among bacterial strains from 2 common genera isolated from milk – *Bacillus* and *Paenibacillus.* It is critical to identify genetic markers predictive of whether or not these isolates will be able to grow at low temperatures, spoil food or cause the disease. *Bacillus* and *Paenibacillus* isolates are extremely challenging to identify and characterize in this manner using traditional microbiological methods. We have therefore employed whole genome sequencing to characterize isolates from dairy foods and dairy-associated environments. Detailed exploration of the diversity of these dairy sporeformers has led to the discovery of a new psychrotolerant and pathogenic species, *Bacillus wiedmannii*, and several putative new species of *Paenibacillus*. We have shown that using sequence/phylogeny-based approaches for the identification of sporeformers increases accuracy of species identification, and that certain species of *B. cereus* group are more likely to grow at cold temperatures and thereby potentially spoil food. Importantly, genomic analyses coupled with cytotoxicity evaluated in a HeLa cell model revealed that presence of diarrheal toxin genes is not sufficient to cause cytotoxic effects. Some *B. weihenstephanensis* strains carried diarrheal toxin genes and grown at human body temperature (37°C) failed to produce cytotoxic effects on HeLa cells, but were cytotoxic at lower temperatures. Tested *B. thuringiensis,* known for production of insecticidal crystal proteins, were able to produce diarrheal enterotoxin hemolysin BL. Select strains of *B. toyonensis* that have been used as a probiotic feed additive, produced hemolysin BL and were cytotoxic in the tissue culture model. We have integrated this information into a whole genome sequence-based *B. cereus* group characterization tool, BTyper, that identifies microbial species based on the phylogenetic group and identifies known virulence and antimicrobial resistance genes.

Key Words: dairy, sporeformer, genomics

290 Whole-genome sequencing: Applications in dairy veterinary medicine. L. B. Goodman*, *Cornell University, College of Veterinary Medicine, Ithaca, NY.*

With the dramatic reduction in cost for next-generation sequencing, obtaining the complete genomic sequence of small pathogens such as bacteria and viruses is now affordable for many routine veterinary and herd health applications. The primary uses are currently source tracking and virulence typing. Source tracking is helpful in managing persistent issues on a farm such as *Salmonella* contamination on equipment or *Listeria* in bedding. It can also help to inform risks to personnel based on similarities to human strains, such as with rotavirus or *Clostridium perfringens* in calves. Monitoring of influenza D is also an emerging area. Virulence typing has immense potential to provide actionable clinical information based on functional genomics. In addition to serotype, virulence, and antimicrobial resistance prediction, the complete characterization of autogenous bacterins can aid in choosing the most appropriate strains and monitoring responses. Some of the current challenges are interpretation of results to practitioners and protection of confidentiality while contributing to open data initiatives to benefit researchers and public health.

Key Words: *Salmonella*, whole-genome sequencing

291 Whole-genome sequencing for pathogen environmental monitoring: Focus on *Listeria*. M. J. Stasiewicz*, *University of Illinois, Urbana, IL.*

This talk will evaluate whole-genome sequencing as a new tool to identify persistent *Listeria monocytogenes* in food-associated environments. The critical assumptions are two. First—that to eliminate contamination by a persistent strain, we first need to identify that the contamination is, in fact, due to a persistent strain rather than sporadic re-contamination (to apply appropriate interventions). Second—that isolates of a persistent strain will be more closely related than isolates from a sporadic contamination event. Under this paradigm, whole-genome sequencing is emerging as the gold-standard subtyping tool for *Listeria*, and for good reason: analyses of these data provide nearly perfect resolution

of differences at the single nucleotide level. This presents a new reality for pathogen subtyping because we can no longer apply the rule of thumb that isolates which appear indistinguishable represent distinct strains. Instead, we can use phylogenetics and other genomic analyses to identify when closely related isolates are likely to represent persistent strains. These analyses can shed light on several questions: How many SNP count as a close genetic relationship? What are the implications of mobile elements on subtyping? And how might government, academic, and industry even conduct these sophisticated analyses? This talk will address these questions through a survey of recent developments and results from a case study in a related food environment, using whole-genome sequencing of hundreds of *Listeria* from 30 retail delis to identify persist strains.

Key Words: *Listeria*

292 Understanding polysaccharide biosynthesis in lactic acid bacteria: Lessons from whole-genome sequencing and systemic approaches. A. R. Neves*, *Chr. Hansen A/S, Hørsholm, Denmark.*

Production of polysaccharides has long been associated with the technological, functional and health promoting benefits of lactic acid bacteria (LAB). In particular, the capsular polysaccharides and exopolysaccharides (CPS and EPS) have been implicated in modulation of the rheological properties of fermented products. For this reason, screening and selection of exocellular polysaccharide producing LAB has been extensively carried out by academia and industry. To further exploit the ability of LAB to produce polysaccharides, an in-depth understanding of their biochemistry, genetics, biosynthetic pathways, regulation and structure-function relationships is mandatory. Here we will present our efforts comprising a multilevel approach to characterize at the systems level LAB producing polysaccharides. To this end, we combined high throughput experimentation and omics data, genetic features of the clusters and structural characterization of the polysaccharides with computational approaches for data integration. An in-depth understanding of polysaccharide biology in connection to application is expected to facilitate the selection and improvement of LAB strains with superior texturing properties.

Key Words: texture, polysaccharides, lactic acid bacteria

293 PBMC mitochondrial enzyme activity in dairy heifers from birth to pre-breeding. A. Niesen* and H. Rossow, *UC Davis, Davis, CA.*

Mitochondria are central to metabolism, nutrition and health but many factors can influence their efficiency. The objective of this study was to determine if mitochondrial enzyme activity rates of peripheral blood mononuclear cells (PBMC) changed over time and were influenced by shifts in blood composition, average daily gain, or breed. Data from 22 Holstein and 18 Jersey heifer calves was collected from age 4 to 301 d. Blood samples were collected at 1, 2, 9 and 36 wk of age and analyzed using a Hemavet 950FS Hematology Analyzer (Drew Scientific, Miami Lakes, FL) to determine blood composition and health status. Additional blood was used to determine plasma total protein (TP, g/dL) and obtain crude mitochondrial extracts from the PBMC fraction using a mitochondria isolation kit from Abcam (Cambrige, MA). Enzyme activities for citrate synthase (CS), Complex I (CI), Complex IV (CIV) and Complex V (CV) were all determined using kits from Abcam (Cambrige, MA). Activity rates were compared by time point, breed, and blood parameters and were analyzed using the MIXED procedure of SAS (v.9.4) with repeated measures, breed as a fixed effect and time point as a random effect. Time point significantly affected activity in all complexes ($P < 0.01$) but breed was not different ($P > 0.1$). Animals with greater complex I activity showed increased pre-wean ADG ($P < 0.01$) and animals with lower citrate synthase activity tended to have increased 36 wk ADG ($P < 0.1$). CS, CI, and CV showed a reduction in activity from 1 to 36 wk ($P < 0.001$). Activity of CS unexpectedly peaked at 2 wk ($P < 0.001$) plausibly resulting from the increase of scours onset during this time point. These findings suggest that changes in mitochondrial enzyme activity occur as calves age and grow and may be affected by an immune challenge such as scours onset.

Key Words: mitochondria, PBMC, heifer

294 Prenatal choline supplementation improved health and growth of neonatal Holstein calves. M. G. Zenobi*[1], J. M. Bollatti[1], N. A. Artusso[1], A. M. Lopez[1], B. A. Barton[2], J. E. P. Santos[1], and C. R. Staples[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Balchem Corp., New Hampton, NY.*

The objective was to determine if growth, health and IgG absorption of preweaned Holstein heifer calves (n = 59) would be improved by supplementation of ruminally-protected choline (RPC; ReaShure; Balchem Corp., New Hampton, NY) to the dam and or by consuming colostrum (3.8 L within 2 h of birth) produced by dams fed RPC prepartum. The 4 treatments in a 2 by 2 factorial arrangement were dams fed 0 or 12.9 g/d of RPC ions beginning at 21 d before expected calving and colostrum from dams supplemented at 0 or 12.9 g/d of RPC ions. Intake and rectal temperature of heifers were recorded daily. Acute phase proteins and cell types in blood were measured at 0, 7, 14, and 21 d of age. Concentration of IgG, fat, and protein in colostrum fed among treatments did not differ ($P < 0.05$). Heifers fed RPC colostrum, irrespective of dam treatment, had increased apparent efficiency of absorption of IgG compared with heifers fed control colostrum at 1 d of age (28.1 vs 21.8%; $P = 0.01$). During the first 21 d of age, incidence of fever (≥39.5°C) tended to be lower (31 vs. 58%, $P = 0.07$), intake of milk replacer (748 vs. 704 g/d, $P < 0.01$) was greater, and intake of grain mix (54 vs. 40 g/d, $P = 0.08$) tended to greater by heifers born from dams fed RPC, irrespective of colostrum source, compared with heifers born from control dams, respectively. As a result, ADG from birth to 28 d of age tended to be greater by heifers born from RPC-fed dams (531 vs. 462 g, $P = 0.06$), but ADG between birth and 56 d of age did not differ. When heifers were exposed to choline in utero, blood concentrations of RBC tended to be greater whereas those of leucocytes, neutrophils, and lymphocytes were lower ($P \leq 0.10$). Plasma concentrations of fibrinogen tended to be lower ($P = 0.09$) and those of serum amyloid A were lower ($P = 0.05$) in heifers born from dams fed RPC and fed colostrum from those same dams but haptoglobin concentrations were unaffected. Maternal consumption of RPC during late gestation had a positive effect on growth of neonatal heifers during the first 4 wk of life, possibly due to an improved immune system, which may have been further enhanced by feeding colostrum from dams fed RPC.

Key Words: choline, calves, colostrum

295 Alteration in oocyte gene expression due to lactation and progression in milk production in dairy cows. S. Alhojaily*[1], R. Stott[1], S. C. Isom[1], and J. R. Stevens[2], [1]*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT,* [2]*Department of Mathematics and Statistics, Utah State University, Logan, UT.*

In recent decades, fertility in dairy cows has declined as cows have been highly selected for milk production. Dairy cows often experience a state of negative energy balance (NEB) during the first few months of lactation resulting in dramatic fluctuations in circulating levels of a variety of hormones and metabolites. The systemic hormonal and metabolic dysregulation can also have a profound effect on the ovarian follicular micro-environment which can affect the molecular composition and developmental capacity of the oocyte, which is highly responsive to its micro-environment during growth and maturation. The aim of this study was to identify how the stage of lactation and energy status affect gene expression in individual in vivo-matured oocytes. Oocytes were collected by ovum pick-up (OPU) once from each of 8 Holstein heifers at the age of 14 mo, and 3 times from 13 lactating Holstein cows at 47, 75, and 130 d in milk. RNA was extracted from each single oocyte with a total of 19 oocytes from heifer and 119 oocytes from cows for relative transcript abundances for each of 64 genes from a variety of functional categories such as metabolism, apoptosis, redox, heat shock, and epigenetics. Gene expression was evaluated by reverse transcription and quantitative real-time PCR using the powerful BioMark platform for single-cell gene expression analysis from Fluidigm (South San Francisco, CA). Raw qPCR data were normalized and analyzed using the comparative CT method. Out of the 64 genes tested, GLRX, H19, HSPA1A, and NDN were found to be different (adj. $P < 0.05$) between cows and heifers and within lactating cows; ACACA, CAT, EZH2, G6PD, GLRX, GLRX3, and SOD1 were found to be significantly affected by the stage of lactation; and DNMT1, DNMT3B, GLUD1, GSR, HSP90A1, and SUV39H1 were found to be significantly affected by the BHB level within lactating cows. We conclude that lactation and progression in milk production cause a sort of metabolic stress that directly or indirectly alters the molecular constituents of oocytes and thereby potentially impact oocyte developmental competence.

Key Words: oocytes, gene expression, dairy cow

296 Effects of feeding different amounts of milk replacer on growth performance in Holstein calves to 2 months of age using different weaning transition strategies. R. N. Klopp*[1], T. M. Hill[2], F. X. Suarez-Mena[2], R. L. Schlotterbeck[2], and G. J. Lascano[1], [1]*Clemson University, Clemson, SC,* [2]*Nurture Research Center, Provimi, Brookville, OH.*

Many changes occur in the rumen as calves transition from consuming a liquid diet to a completely solid diet. These changes can be greatly affected by diet pre-weaning as well as the rate at which calves are weaned and can influence growth and calf health. A 2x2 factorial design (moderate (MOD) or high (HI) milk replacer (MR) feeding rates and abrupt (AB) or gradual (GR) weaning) was utilized to compare how calf performance pre-weaning is affected. Calves (n = 50, initial BW = 42 ± 1.3 kg) were randomly assigned to 1 of 4 treatments (13 for MOD-AB and MOD-GR, 12 for HI-AB and HI-GR). Data were analyzed as a completely randomized design with repeated measures when applicable by PROC MIXED in SAS. Calves assigned to MOD-AB were fed 0.66 kg (DM basis) MR for first 42 d then 0.33 kg for last 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d. All calves received the same MR (25% CP, 17% fat DM basis) and were given ad libitum access to water and textured starter (42% starch and 20% CP). The initial body weight (BW), hip width (HW), hip height (HH), and body condition score (BCS) did not differ among calves. Starter consumption was greater for MOD calves compared with HI calves ($P < 0.01$) and GR consumed more than AB ($P < 0.01$). Feed efficiency (gain/feed; $P < 0.01$), ADG ($P < 0.01$), final HH ($P = 0.03$), and final BW ($P = 0.02$) were greater for HI calves compared with MOD calves. Final HH was greater for AB compared with GR calves ($P = 0.04$). There were no interactions. These results indicate that HI intake promoted higher BW at a greater rate and more efficient feed conversion before weaning. Whereas, GR weaning lead to more starter consumption and lower structural growth (HH).

Key Words: calves, growth, weaning

297 Effects of feeding different amounts of milk replacer on growth performance in 2- to 4-month-old Holstein calves using different weaning transition strategies. R. N. Klopp*[1], T. M. Hill[2], F. X. Suarez-Mena[2], R. L. Schlotterbeck[2], and G. J. Lascano[1], [1]*Clemson University, Clemson, SC,* [2]*Nurture Research Center, Provimi, Brookville, OH.*

Many changes occur in the rumen as calves transition from consuming a liquid diet to a completely solid diet. These changes can be greatly affected by diet pre-weaning as well as the rate at which calves are weaned and can influence growth and calf health. A 2 × 2 factorial design (moderate (MOD) or high (HI) milk replacer (MR) feeding rates and abrupt (AB) or gradual (GR) weaning) was utilized to study how pre-weaning program affects calf performance from 2 to 4 mo of age. Calves (n = 50) were randomly assigned to one of the 4 pre-weaning treatments. Data were analyzed as a completely randomized design with repeated measures when applicable by PROC MIXED in SAS. Calves assigned to MOD-AB were fed 0.66 kg MR for first 42 d then 0.33 kg for last 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d before the start of the grower phase. For the following 56 d grower portion of the experiment, calves were grouped into 12 pens based on MR program, 4–5 calves per pen. All calves received ad libitum access to water and a textured starter (42% starch and 20% CP) blended with 5% chopped grass hay. Initial body weight (78.9, 74.3 ± 1.36; BW; $P = 0.04$), hip width (HW; $P = 0.03$), and hip height (HH; $P = 0.02$) were greater for HI compared with MOD calves. Only initial HH was greater for AB vs. GR calves ($P = 0.02$), but this was the same at the end of the grower phase for all treatments. There were no other differences between weaning method. There was an interaction where feed efficiency (gain/feed) was reduced with GR weaning in MOD whereas the opposite occurred in the HI group. Final BW, HW, and HH did not differ among treatments. These results indicate that during the grower phase HI calves lost their growth advantage over MOD calves, and weaning strategy can have the opposite effect on feed efficiency for MOD and HI MR.

Key Words: calves, growth, weaning

298 Physiological effects of low rumen pH in calves before, during and after weaning. S. L. Gelsinger*[1] and G. I. Zanton[2], [1]*University of Wisconsin, Madison, WI,* [2]*United States Department of Agriculture Dairy Forage Research Center, Madison, WI.*

The objective of this study was to determine the effects of low rumen pH in dairy calves on performance, rumen passage rate and blood and rumen tissue characteristics. Starter diets were formulated to induce (A) or blunt (B) rumen acidosis in young dairy calves. Diet A was a complete pellet (42% starch, 13% NDF) and B was texturized (31% starch, 22% NDF). Ten (n = 5/diet) Holstein bull calves were assigned to treatments at birth, cannulated at wk 3, and received milk replacer and a starter diet through wk 7, then starter only through wk 16. Rumen contents were sampled and pH recorded −8, −4, 0, 2, 4, 8, 12, and 24 h relative to starter feeding during wk 6, 8, 10, 12, 14, and 16 of age. Cobalt-EDTA was used to estimate liquid passage rate and rumen volume. Blood was drawn once each sampling week to determine complete blood cell count, pH, hematocrit, hemoglobin concentration, and partial pressures of CO_2 and O_2. Calves were weighed weekly; starter intake was recorded daily. Calves were slaughtered and rumen tissue collected at wk 17. Formalin-fixed ruminal tissues were given a lesion score (0 = healthy tissue, 5 = severe degradation and inflammation). Statistical analyses were conducted using mixed models in SAS with $P < 0.05$ considered significant. Mean (min, max) rumen pH was lower for A than B calves: 5.4 (3.3, 7.2) and 5.6 (3.5, 6.8). Lowest mean pH values, 5.0 and 5.5 for A and B diets, were observed in wk 8. Starter consumption and BW were lower in A calves beginning at 3 and 5 wk, respectively. Carcass weights were 68.1 and 82.2 ± 1.8 kg for A and B calves. Rumen liquid volume tended to be greater in B calves ($P = 0.08$) and increased with age. Liquid passage rate was similar between treatments. Blood hemoglobin concentration and hematocrit were reduced in A calves. Blood pH decreased linearly with week of age. No clear trends were observed for other blood parameters. Mean rumen lesion scores of rumen tissue were 3.7 and 2.3 ± 0.2 in A and B calves. Growth and physiological responses were negatively affected in calves receiving A.

Key Words: calf, rumen, acidosis

299 Can processing corn influence growth performance, nutrient digestibility and ruminal and hindgut fermentation in calves fed low or high plane of milk replacer? J. K. van Niekerk*[1], A. J. Fischer[1], L. L. Deikun[2], J. D. Quigley[2], T. M. Hill[2], R. L. Schlotterbeck[2], and M. A. Steele[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Provimi, Brookville, OH.*

The aim of this study was to evaluate how plane of milk replacer (MR; low vs. high) and rate of rumen fermentation of starch in calf starter (CS; slow vs. rapid) can alter growth performance, nutrient digestibility, and rumen and hindgut fermentation in dairy calves. Holstein male calves (n = 48, 2–3 d of age at initiation of the study) were randomly assigned to one of 4 treatments according to 2 × 2 factorial design: low (LO; 0.749 kg/d) or high (HI; 1.498 kg/d) plane of MR, and slow (whole corn; W) or rapid (flaked corn; F) rate of rumen fermentability of starch in CS. Calves were weaned by reducing MR offered by 50% during wk 6. Intake of MR and CS were recorded daily, while BW was measured weekly. Rumen fluid and fecal matter were sampled at wk 5, 6, 7 and 8 for pH, short chain fatty acids, and starch. Nutrient digestibility was measured during wk 5 and 8. Data were analyzed using MIXED procedure of SAS. Calves fed HI-F had greater average daily gain (ADG; 0.71 kg/d) than calves fed LO-F (0.47 kg/d). Calves fed HI had greater ($P \leq 0.001$) ADG than calves fed LO during wk 2, 3, 4 and 5, yet during wk 7 calves fed HI had lower ($P \leq 0.002$) ADG compared with calves fed LO. There was an interaction ($P = 0.03$) between MR plane and type of starch for ADG during the pre-weaning period. Starter intake was greater ($P \leq 0.000$) for calves fed LO compared with HI during wk 4, 5, 6 and 7. Rumen propionate was lower for HI-W compared with LO-W group ($P = 0.013$) at wk 5 and LO-W and HI-F groups ($P = 0.017$) at wk 6. During wk 5 and 8, calves fed LO had increased ($P = 0.001$) ADF and NDF digestibility compared with calves fed HI but had decreased and increased ($P = 0.02$) OM digestibility for wk 5 and 8, respectively, compared with HI fed calves. Treatment did not affect fecal starch, yet calves fed HI-W had lower ($P = 0.001$) fecal pH (6.6 ± 0.06) during wk 8 compared with other groups. Results show that the plane of MR and/or type of starch can affect ADG, fiber digestibility, ruminal SCFA production, and fecal pH.

Key Words: starch, fecal pH, digestibility

300 Effect of solid feed location on feed consumption and growth of dairy calves. S. D. Parsons*[1], K. E. Leslie[2], M. A. Steele[3], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [3]*Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to investigate how the location of solid feed offered to dairy calves affects their intakes and growth during the milk-feeding (d 1–42), weaning (d 43–56), and post-weaning (d 57–70) periods. Sixty Holstein heifer calves were housed in individual pens (152 × 167 cm) and randomly assigned to 1 of 2 treatments: solid feed adjacent to the milk source or solid feed opposite from the milk source within their pen. All calves were offered 12.5L/d of milk replacer (in 2.5-L meals, 5×/d) by an automated rail milk feeder (AMF) until d 43, when they were gradually weaned off milk by d 57. All calves had access to ad libitum water from birth and starter ration of concentrate (95%) mixed with chopped (2.54 cm) wheat straw (5%) from d 5. At d 57, calves had access to only water and starter ration and were monitored to d 70. Feed and water intakes were measured daily and milk intakes were recorded automatically by the AMF. Calf BW was measured 2×/wk. Data were summarized by period and analyzed in repeated measures general linear mixed models. Feed adjacent to the milk source tended to result in greater DMI (0.067 vs 0.056 kg/d; SE = 0.005; $P = 0.09$) during the milk-feeding phase. Feed consumption did not differ between treatments during the weaning (adjacent = 0.81 kg/d, opposite = 0.75 kg/d; SE = 0.056; $P = 0.46$) and post-weaning (adjacent = 2.99 kg/d, opposite = 2.87 kg/d; SE = 0.093; $P = 0.42$) periods. Adjacent treatment calves had greater water intakes during the milk-feeding (0.75 vs 0.55L/d; SE = 0.49; $P = 0.005$) and post-weaning (10.11 vs 9.30L/d; SE = 0.27; $P = 0.04$) periods, and tended to consume more water during weaning (2.55 vs 2.12L/d; SE = 0.16; $P = 0.06$). Greater intakes for the adjacent treatment calves may have contributed to a tendency for greater ADG (1.11 vs 1.05 kg/d; SE = 0.023; $P = 0.08$) during the milk-feeding period. Growth was similar between treatments for the weaning (0.83 vs 0.83 kg/d; SE = 0.04; $P = 0.96$) and post-weaning periods (1.22 vs 1.14 kg/d; SE = 0.06; $P = 0.28$). The results indicate that placement of solid feed close to the milk source encourages calves to increase their starter and water intake during the milk-feeding stage, which may contribute to greater growth during that period.

Key Words: dairy calf, feed location, growth

301 The effects of different feeding practices on heifer growth and reproduction at five California dairies using single-time-point measurements. D. D. Myers* and H. A. Rossow, *University of California, Davis, Davis, CA.*

Feeding for increased growth improves heifer reproductive efficiency and shortens the unproductive period from birth to calving, allowing producers to quickly regain costs associated with raising heifers. The objective of this study was to compare nutrients supplied to growing heifers, heifer weights, and age at first breeding on 5 commercial dairies and form a ranking system for evaluating and improving heifer-raising programs. A rank of 1 indicated the dairy fed DM greater than 10 kg/heifer/d, CP greater than 1.2 kg/heifer/d, and NEg greater than 0.9 Mcal/kg DM, had heifer weights greater than 600 kg at 20–24 mo, and early breeding, i.e., less than 410 d. Each subsequent rank indicated reduced performance from the first in at least one area with the lowest rank being 5. To determine the impact of nutrient supply on heifer growth and reproduction, TMR samples for each heifer diet were collected weekly for 4 wk at each dairy. Heifer weights were estimated using a weigh tape on 10% of the heifers (n = 1747). Reproduction records for the previous year (n = 4809) were collected from DairyComp305 (Valley Ag. Software, Tulare, CA). Least squares means for DM, CP, NEg, weight, and age at first breeding were compared among dairies using the PROC GLM procedure of SAS (v. 9.4, 2014). Dairy E ranked 1 overall due to a NEg of 0.96 Mcal/kg DM ($P \leq 0.05$), weight at 20–24 mo of 602 kg ($P \leq 0.05$), and low SD in age at first breeding (411 ± 16.7 d; $P \leq 0.05$). The other dairies were ranked as follows: dairy D (1.1 Mcal/kg DM, 599 kg, 418 ± 16.9 d, respectively) was ranked second, dairy C (0.84 Mcal/kg DM, 649 kg, 420 ± 27.5 d, respectively) was ranked third, dairy B (0.87 Mcal/kg DM, 589 kg 399 ± 48.3 d, respectively) was ranked fourth, and dairy A (0.82 Mcal/kg DM, 579 kg, 412 ± 17.0 d, respectively) was ranked fifth. Dairies that fed more NEg in the 7 to 12 mo age range had greater heifer weights at parturition and earlier calving compared with the other dairies in this study. These results show that single-time point measurements can be used to create a benchmarking system to evaluate and improve heifer-raising programs in nutrients supplied, growth, and reproduction.

Key Words: heifer, benchmarking, nutrition

302 Effects of supplemental butyrate during the weaning transition on rumen pH in Holstein calves. D. E. McCurdy[1], R. L. Hiltz[1], K. R. Wilkins[1], S. Moreland[2], K. Klanderman[2], and A. H. Laarman*[1], [1]*University of Idaho, Moscow, ID,* [2]*Nutriad Inc., Hampshire, IL.*

The objective of this study was to investigate the role of diet management on rumen pH dynamics during the weaning transition. Thirty-six Holstein bull calves were divided into 4 treatments based on diet and harvest age: calves fed milk and hay only, harvested pre-weaning (PRE-M); milk, hay, and starter, harvested pre-weaning (PRE-S); milk, hay, and starter, harvested post-weaning (POST-S); milk, hay, and starter, harvested post-weaning, with supplemental butyrate fed during the weaning transition at 1% DMI (POST-B). All animals were housed individually and had ad libitum access to water, hay, and calf starter. Calves were fed milk replacer (28% CP, 18% EE) twice daily. Until 6 weeks of age, calves were fed a maximum of 1200 g/d of milk replacer. Then, milk replacer was reduced to 900 g/d for wk 7, 600 g/d for wk 8, then weaned. Calves were harvested at the end of wk 6 (PRE-M, PRE-S), or at the end of wk 9 (POST-S, POST-B). One week before harvest, rumen pH was measured every 2 min for 7 d. Calf starter and hay intake were measured daily, while body weight and blood were sampled weekly. Mean rumen pH was not different among PRE-M, PRE-S, POST-S, and POST-B (6.17 vs. 7.45 vs. 6.40 vs. 5.83 ± 0.66, respectively; $P = 0.27$). The duration where rumen pH was below 5.8 was also not different among PRE-M, PRE-S, POST-S, and POST-B treatments (485 vs. 280 vs. 209 vs. 730 ± 201 min/d; $P = 0.24$). No differences in plasma glucose concentrations were found among the treatments. No differences in plasma BHBA concentrations were detected among treatments before weaning or after weaning. During the weaning transition, plasma BHBA concentrations were higher in the POST-B than the POST-S treatment in wk 7 (1.28 ± 0.10 vs. 0.92 ± 0.10 μM, respectively; $P = 0.04$). After weaning, there was no difference in hay intake, but calf starter intake was greater in POST-B than in POST-S (2698 ± 281 vs. 1839 ± 281 g/d, respectively; $P < 0.01$). These data indicate that rumen pH profile does not appear to shift during the weaning transition and that dietary supplementation with butyrate may improve starter intake post-weaning.

Key Words: calves, weaning, rumen pH

Lactation Biology: Joint ADSA/NMC Session: Advances in Mammary Health and Immunology

303 Immunogenetic control of bovine mammary gland health. H. Atalla, L. Wagter-Lesperance, and B. Mallard*, *University of Guelph, Guelph, ON, Canada.*

The immune system is genetically wired to elicit robust protective responses to infectious organisms and individuals with an appropriately balanced immune response have health advantages. The High Immune Response (HIR) technology classifies cows as High (H), Average (A) and Low (L) immune responders (IR) based on their estimated breeding values (EBV) for antibody-mediated (AMIR) and cell-mediated (CMIR) immune response. Two previous studies by our group; nationwide Canadian study and another a large US dairy, were the first to demonstrate that H responders have a lower occurrence of postpartum related illness and thus require less therapeutic intervention. Notably, several studies show that HIR cows produce colostrum and milk of superior quality; naturally enriched with high concentrations of host-defense related molecules. Specifically, cows classified as H AMIR have greater concentrations of serum antigen or vaccine-induced specific antibodies, total immunoglobulin (Ig), and natural antibodies of both IgM and IgG isotypes, and innate defense molecules in the milk such as β-lactoglobulin. These bioactive molecules provide enhanced passive immunity to newborn calves and improved disease resistance of the mammary gland both during lactation and the dry period. In addition, our group is among the first to show that colostrum and milk exosomes contain unique miRNA profiles. Critical immune-related miRNA are differentially expressed with greater expression observed in colostrum from HIR cows. Exogenous uptake of colostrum and milk exosomes containing miRNA from H responders by human intestinal epithelial cells enhances cell viability and suggests their bioavailability to humans and a potential immunoregulatory role in gut health. Advanced understanding of the interplay between the genome and the epigenome and the environment is expected to open the door for new applications of the HIR technology. Identification of genomic and epigenetic biomarkers for enhanced herd health will add to the growing list of traits that allow the dairy industry to select superior breeding stock. Natural value-added colostrum and milk will benefit mammary gland health, and humans consuming these healthful dairy products.

Key Words: genetic regulation, mamary gland health, healthful dairy products

304 Established approaches and new directions to support innate immunity of the udder. C. D. Nelson*, *University of Florida, Gainesville, FL.*

The bovine udder is equipped with innate defenses to identify and eliminate bacterial pathogens that are ubiquitous in the cow's environment. Those defenses, which include macrophages, neutrophils and a multitude of secreted bactericidal factors, are very effective at protecting the mammary gland from bacterial infection to the point they can eliminate a bacterial challenge without a detectable inflammatory response. Despite the effectiveness of mammary immunity and good hygiene and management practices of dairy farms, dairy cows remain susceptible to mastitis, particularly in the early postpartum period. The cumulative effect of physiological stressors of parturition on the immune system is well known and is arguably a key factor for increased risk of mastitis in postpartum dairy cows. Mitigation of hypocalcemia and negative energy balance, support of antioxidant systems, feeding of prebiotics and use of immunostimulants have been documented to bolster aspects of innate immunity. A major beneficiary of these various nutritional and therapeutic strategies within the immune system is the neutrophil. We know neutrophils are critical in defense of the mammary gland, and that calcium, energy and oxidant status affect function of mature neutrophils in circulation. Yet, the processes guiding neutrophil development are not fully appreciated in dairy cows and undoubtedly are affected by physiological stressors of parturition. Furthermore, although innate immune function often is focused on current status of the cow, programming of innate immunity begins early in life and is modulated over her lifetime. Therefore, efforts to maximize innate defense of the mammary gland should include consideration of developmental programming in addition to the use of nutritional and therapeutic strategies documented to have a positive impact on innate immunity.

Key Words: innate immunity, mammary gland, mastitis

305 Development of vaccines and antibiotics against *Staphylococcus aureus* based on bacterial gene expression during bovine mastitis. F. Malouin*, *Universite de Sherbrooke, Sherbrooke, QC, Canada.*

Staphylococcus aureus is a leading cause of chronic bovine intramammary infections (IMI) resulting in major economic losses for the dairy industry. Most antibiotics used by milk producers to treat *S. aureus* IMI have limited efficacy. To find novel control measures for *S. aureus*, we need to identify new cellular targets. Genes and proteins involved in virulence or that are essential for the pathogen growth in vivo represent a large pool of yet unexploited targets for vaccine and antibiotic discoveries. For such a purpose, we studied the transcriptome of *S. aureus* during the infection process. Mammary gland quarters were experimentally infected by a variety of *S. aureus* strains and milk and bacteria were recovered at each milking over 18 d. The genes expressed in bacteria grown in vivo were identified by hybridization of transcripts to DNA arrays and results were confirmed by qPCR. Several genes were found to be commonly expressed by *S. aureus* strains during infection. Genes of interest were selected for mutagenesis. Attenuation of gene-disrupted *S. aureus* mutants during experimental IMI in cows validated the role of such genes in the infection process. Proteins derived from selected genes were used for immunization of cows before an experimental challenge with *S. aureus*. Vaccinated cows better handled the experimental infection (SCC, bacterial count in milk, milk quality, and milk production). Such a vaccine is now under development. Besides, another in vivo-expressed transcript represented an interesting target for antibiotic action. This novel drug target is a non-coding RNA (guanine riboswitch) which is completely different from protein targets of traditional antibiotics. We subsequently identified small drug-like molecules that mimic the natural riboswitch ligand and prevent expression of an essential gene (*guaA*). We further showed antibiotic activity of one such artificial ligand during treatment of experimental *S. aureus* IMIs in cows. Transcriptomic is a reliable approach for the development of novel treatment and control measures for bovine mastitis.

Key Words: *Staphylococcus aureus*, bovine mastitis, vaccine

306 *Staphylococcus aureus* lineage influences the bovine immune response to intramammary infection. O. M. Keane*[1], D. A. Niedziela[1,2], M. P. Murphy[1], and N. Leonard[2], [1]*Teagasc, Grange, Dunsany, Co. Meath, Ireland,* [2]*University College Dublin, Belfield, Dublin, Ireland.*

Staphylococcus aureus is among the most frequent causes of bovine mastitis worldwide. The strains associated with mastitis belong to bovine adapted lineages, with each lineage encoding a diverse assemblage of regulators and effectors of virulence, which could influence disease presentation and outcome. To investigate the influence of bacterial strain and lineage on the host immune response, 12 bovine adapted *S. aureus* strains, 3 from each of the lineages CC71, CC97, ST136, and CC151, were characterized for their ability to induce a pro-inflammatory immune response from mammary epithelial cells (MEC) and stimulate neutrophil chemotaxis, their propensity to invade MEC and their survival during incubation with granulocytes. Strains from lineage CC97 were strongly pro-inflammatory and induced MEC to produce chemotactic factors that attracted neutrophils. In contrast, strains from CC151 only weakly induced the expression of pro-inflammatory cytokines and did not induce expression of chemotactic factors. There were no differences between strains in ability to survive neutrophil killing while strains belonging to ST136 poorly internalised into MEC. One strain each of CC151 and CC97 were selected for in vivo studies. Healthy first-lactation Holstein-Friesian heifers were infected, 7 with a CC97 strain (Group 1) and 7 with a CC151 strain (Group 2). Clinical signs of infection were monitored for 30 d. Differences in disease presentation between groups were observed, with 2 animals from Group 2 developing clinical mastitis and requiring antibiotic treatment, while one animal from Group 1 did not develop an infection for the duration of the study. Fever was observed in 3 animals from Group 2 and in none from Group 1. Significant differences in SCC and bacterial load between groups were also observed. The results of this study suggest that strains from lineage CC151 may fail to elicit an early immune response allowing them to establish and cause more severe mastitis than strains from lineage CC97. Diversity between strains of *S. aureus* may therefore influence the clinical presentation of mastitis, disease detection and treatment needs.

Key Words: *Staphylococcus aureus*, intramammary infection, immune gene expression

307 Omics in animal science: Promise, potential, and pitfalls. J. D. Lippolis*, *USDA Agriculture Research Service National Animal Disease Center, Ames, IA.*

The first genome took nearly 15 years and approximately a billion dollars to complete, now it can take little over a day and a couple of thousand dollars. Comparing the relative abundance of nearly every messenger RNA transcript from cells in different environments has become so easy that it can take longer to transfer the data than to perform the experiment. DNA detection techniques have become so sensitive that the biggest concern is not false negative but false positive data. Better genome sequencing has led to more complete protein databases and along with more sensitive instrumentation and separation techniques, are bringing us closer to achieving the detection a complete proteome. The promise of these incredible techniques is to lead us to new and unexpected connections between molecular processes and animal health. The new and unexpected observations have the potential to lead to better animal health and production only through subsequent hypotheses-testing research. However, any researcher who wishes to invest the time and resources into an omics experiment should be aware of the common pitfalls and limitations of these techniques. Important questions one should consider are: What is the quality of the databases and how they are annotated? Are the annotations based on experimental results or computational predictions? What assumptions are made by the analysis algorithms and how will this affect the result? Finally, how can the research community use the vast amount of data being generated by omics experiments in ways to achieve the goals of better animal health and production, which is the promise of omics technologies.

Key Words: mastitis, transcriptomics, proteomics

308 Methionine supply during late-gestation triggers offspring sex-specific divergent changes in metabolic and epigenetic signatures in bovine placenta. F. Batistel*[1], R. R. C. Yambao[1], A. S. M. Alharthi[1], Y.-X. Pan[1], C. Parys[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

Our objective was to investigate the impact of methionine supply during late-gestation on metabolism and DNA methylation in bovine placenta from cows carrying male or female calves. Multiparous cows were fed during the last 28 d of pregnancy a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH; 0.09% of DMI) to achieve a 2.8:1 ratio of Lys:Met in the metabolizable protein. Placentome samples were collected from 15 cows/treatment and organized according to diet and offspring sex as follows: Male CTR (n = 7), Male MET (n = 7), Female CTR (n = 8), and Female MET (n = 8). Targeted metabolomics (LC-MS), RT-PCR, Western blotting, and enzyme activity were performed to quantify metabolic activity through the TCA cycle, 1-carbon metabolism, transsulfuration, and global DNA methylation. Compared with cows carrying Male CTR, cows carrying Male MET delivered heavier calves. Compared with placenta from Male CTR, Male MET placenta had greater concentrations of metabolites in the TCA cycle (isocitric acid and NADH) and transsulfuration pathway (cysteinesulfinic acid, glutathione and vitamin B_{12}). Male MET placenta had greater methionine synthase (MTR) activity than Male CTR, while betaine-homocysteine S-methyltransferase (BHMT) and cystathionine-β-synthase (CBS) were not affected. No differences in global DNA methylation or mRNA and protein expression of the DNA methyl transferases were observed between Male CTR and Male MET. Cows carrying Female CTR and Female MET delivered calves with similar body weight at birth. Female MET compared with Female CTR placenta had greater concentrations of metabolites related to 1-carbon metabolism (Met and S-adenosyl-methionine). Enzyme activities in female placenta were not affected by methionine supply. The mRNA and protein expression of DNMT3A and DNMT3B was greater in Female MET than Female CTR, while DNMT1 was not affected by MET supply. Global DNA methylation was lower in Female MET than Female CTR placenta. Overall, our findings suggest that diet affects placental metabolism and DNA methylation and also highlight the importance of studying sex-specific responses to dietary interventions.

Key Words: amino acid, DNA methylation

309 Maternal supply of methionine during late-pregnancy affects hepatic one-carbon metabolism enzyme activity and plasma amino acids during the preweaning period in Holstein calves. A. S. M. Alharthi*[1], F. Batistel[1], C. I. M. Garces[1], C. Parys[2], Y.-X. Pan[1], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

The objectives were to investigate if increasing supply of methionine during late-pregnancy in Holstein cows affects offspring plasma amino acid (AA) concentration and hepatic one-carbon (1C) metabolism. Calves were born to cows fed a control diet (CON) or the control plus rumen-protected methionine (MET) for the last 28 d prepartum (0.09% of DMI), and were fed and managed similarly after birth. Plasma samples and liver biopsies were harvested at 4, 14, 28, and 50 d of age and used for AA, mRNA expression and activity of hepatic 1C metabolism enzymes. Data were analyzed using a Mixed model considering block as random effect and treatment, time and its interaction as fixed effect. At birth, only the concentrations of plasma His and Met differed due to maternal treatment because of lower concentrations in MET calves. After birth, the overall concentrations of Met, Glu, and Leu was greater in MET compared with CON calves. There was an interaction of treatment× day (T×D) for the concentration of Tyr, Phe, Gln, Arg, Thr and Tau due to greater levels early in life in MET compared with CON calves. A T×D was observed for the activity of betaine-homocysteine S-methyltransferase (BHMT), methionine synthase enzyme (MTR), and cystathionine-β-synthase (CBS) in liver; MET calves had greater BHMT activity on d 14 and greater CBS on d 4 and peaked at 28. In contrast, despite a linear increase from d 4 to 28, activity of MTR in MET calves was lower on d 4 and 50. Methionine adenosyltransferase 1A (*MAT1A*) mRNA expression was greater overall in MET compared with CON calves. Greater mRNA expression of betaine-homocysteine S-methyltransferase 2 (*BHMT2*) and *CBS* in MET calves was observed on d 4 and 14. Along with *CBS*, the greater expression of cysteine dioxygenase 1 (*CDO1*) as well as glutathione reductase (*GSR*) and glutathione peroxidase (*GPX1*) on d 4, 14, and 28 in MET calves suggested greater synthesis of glutathione and taurine. Overall, the data indicate that enhanced methionine supply during the last 30 d of gestation not only can benefit calf performance, but increase AA availability and activity of the 1C metabolism pathway to furnish cells with antioxidants.

Key Words: glutathione, taurine

310 RNA sequencing reveals that methionine supply during late-gestation alters neonatal Holstein heifer calf liver transcriptome profiles. A. S. M. Alharthi*[1], F. Batistel[1], V. Palombo[1], C. I. M. Garces[1], C. Parys[2], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

The objectives were to investigate if increasing supply of methionine during late-pregnancy in Holstein cows affects hepatic transcriptome profiles in female offspring. Heifer calves were born to cows fed a control diet (CON) or the control plus ethyl-cellulose rumen-protected methionine (MET) for the last 28 d prepartum (0.09% of dry matter intake), and were fed and managed similarly after birth. Liver biopsies were harvested at 4 d of age from 8 calves per treatment. Extracted total RNA was sequenced using the Illumina platform, and mapped to the *Bos taurus* genome assembly (UMD_3.1.1). Statistical analysis was conducted using the Bioconductor edgeR package, with treatment as fixed effect and animal as random effect. Dynamic Impact Approach (DIA) analysis was performed to uncover the most-impacted cellular pathways. A total of 622 differentially expressed genes (False Discovery Rate $P < 0.25$, uncorrected $P < 0.01$) were detected. The 25 most-impacted pathways from the DIA analysis indicated that genetic information processing pathways related with transcription and translation (e.g., RNA polymerase, purine metabolism, protein processing in endoplasmic reticulum, ribosome and spliceosome) were upregulated in MET than CON calves. In contrast, critical signaling pathways related to cell cycle, cellular metabolism and immunity (e.g., protein digestion and absorption, p53 signaling pathway, PI3K-Akt signaling pathway, Jak-STAT signaling pathway, calcium signaling pathway, cytokine-cytokine receptor interaction, cellular growth and dead, cellular senescence, endocytosis and leukocyte transendothelial migration) were biologically more important and highly-upregulated in CON than MET

calves. Overall, preliminary analysis indicated that molecular changes observed early after birth in response of maternal methionine supply during late-gestation are beneficial to overall hepatic function, especially in terms of metabolism and inflammatory status.

Key Words: fetal programming, liver, transcriptomics

311 Maternal supply of methionine during late-pregnancy alters the fecal microbiome in neonatal Holstein heifer calves during the preweaning period. A. Elolimy*[1], M. Zeineldin[2], A. Alharthi[1], F. Batistel[1], A. Helmbrecht[3], C. Parys[3], and J. Loor[1,4], [1]*Mammalian NutriPhysioGenomics, Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL,* [3]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany,* [4]*Division of Nutritional Sciences, Illinois Informatics Institute, University of Illinois, Urbana, IL.*

The objective of the current study was to investigate the impact of dietary methionine supply during late-pregnancy in dairy cows on gut microbiome and metabolome composition and their association with growth performance in neonatal calves from birth to weaning. Twenty-six Holstein heifer calves (n = 13/treatment) born to cows receiving a control diet (CON) or CON plus ethylcellulose rumen-protected methionine (MET; Mepron®, Evonik Industries AG, Germany) during the last 4-weeks of pregnancy. Calves received 3.8 L of first-milking colostrum from the respective dam within 8 h after birth. Calves were housed in individual outdoor hutches bedded with straw, fed twice daily with a milk replacer and had ad libitum access to a starter grain mix throughout the study. Fecal samples were collected at d 0 (i.e., at birth before colostrum feeding), 14, 28, and 42 (before weaning). Genomic DNA from fecal samples was used for amplification and sequencing of the V3-V4 hypervariable region of the 16S rRNA gene using Illumina MiSeq. Sequencing data were processed and analyzed with QIIME 2 software using the DADA2 pipeline. Calves from MET-fed cows had greater dry matter intake and average daily gain from birth to weaning. Chao1 and OTU index analyses indicated that MET compared with CON heifers had a more diverse microbiota. Compared with CON, MET heifers had greater relative abundance of *Fusobacterium*, *Ruminococcus*, and *Akkermansia*, and lower abundance of *Fecalibacterium*, *Blautia*, *Arthrobacter*, and *Clostridium*. No differences were detected between groups in the abundance of *Dorea*, *Bifidobacterium*, *Oscillospira*, *Succinivibrio*, *Butyricicoccus*, and *Brachybacterium*. Overall, results indicate that maternal supply of methionine altered the diversity and structure of gut microbiota in neonatal heifers. The mechanistic link between maternal methionine supply, performance, and gut metabolome remains to be determined.

Key Words: calves, microbiota, methionine

312 Maternal late-gestation metabolic stress is associated with changes in immune and metabolic responses of dairy calves. T. Ling[2], M. Hernandez-Jover[2,3], L. M. Sordillo[1], and A. Abuelo*[1,3], [1]*Michigan State University, Department of Large Animal Clinical Sciences, East Lansing, MI,* [2]*Charles Sturt University, School of Animal and Veterinary Sciences, Wagga Wagga, NSW, Australia,* [3]*Graham Centre for Agricultural Innovation (Charles Sturt University and NSW Department of Primary Industries), Wagga Wagga, NSW, Australia.*

This study aimed to investigate if metabolic stress in late gestation dairy cows is associated with changes in the metabolic and immune responses of their offspring during the first month of life. Holstein-Friesian cows (n = 12) were blood sampled at 28 and 15 d before expected calving. The average between these 2 sampling points in the concentrations of nonesterified fatty acids (NEFA), haptoglobin (Hp), and oxidative stress index (OSi) were calculated as indicators of lipid mobilization, inflammation, and oxidative stress (OS), respectively. Calves received 4 L of colostrum from their respective dams within 12 h of life and were classified into groups (n = 6 each) according to their dams' high or low degree of lipid mobilization, inflammation, and OS. The metabolic responses of calves in each of these groups were compared weekly up to 1 mo by assessing serum concentration of NEFA, Hp, and OSi. Additionally, whole blood at each sampling was subjected to a LPS-stimulated TNFα production assay to assess cell-mediated innate immunity against induced inflammatory responses. Mixed models with repeated measures were used. Calves born to cows with higher NEFA or OSi showed significantly lower birthweights, whereas no association between maternal metabolic stress groups and ADG was identified. Calves exposed to high maternal OS had higher concentrations of Hp ($P = 0.013$) and TNFα ($P = 0.031$), indicating greater basal inflammatory responses. In contrast, LPS-induced inflammatory responses were less robust in calves exposed to higher maternal Hp and OSi (Table 1), suggesting compromised immune responses. Collectively, these data suggest that prenatal exposure to maternal metabolic stress may adversely impact some metabolic and inflammatory responses of the offspring that could influence disease susceptibility.

Table 1 (Abstr. 312). TNFα release according to the degree of maternal OSi[1]

	10 ng/mL LPS stimulation		5 µg/mL LPS stimulation	
Week	Low OSi	High OSi	Low OSi	High OSi
Wk 1	136.1±12.53	51.8±8.01*	243.7±20.65	95.4±13.27**
Wk 2	117.2±11.47	24.3±4.55**	313.9±24.67	155.3±13.49**
Wk 3	87.19±5.99	39.7±6.89*	362.2±22.00	217.4±15.59**
Wk 4	72.9±2.92	32.0±5.62*	445.3±14.07	274.9±21.19**

[1]Results expressed in percentage increase from control concentration (mean±SE).

Key Words: calf health, metabolic stress

313 Evaluation of the biochemical and hematological profile of Holstein calves submitted to LPS challenge and pegbovigrastim injection. F. Kegles, O. Madruga, L. F. Bragança, U. S. Londero, H. Jessica, M. N. Corrêa, F. A. B. Del Pino, E. Schmitt, and R. C. B. Grazziotin*, *Federal University of Pelotas (UFPel), RS, Brazil; Center of Research, Teaching and Extension in Animal Science (NUPEEC), Pelotas, Rio Grande do Sul, Brazil.*

The hypothesis of this study was that the exacerbated response of the innate immunity stimulated by pegbovigrastim when associated with a lipopolysaccharide challenge could negatively affect the liver, renal and protein metabolism of the dairy calves. Metabolic and hematological parameters were analyzed in dairy calves that recieved a dose of 0.25 µg/kg BW of *E. coli* lipopolysaccharide (LPS) associated with a dose of 25 µg/kg BW pegbovigrastim 24 h later. Twenty Holstein calves (D60 ± 15) were randomly distribued into 4 groups: LPS (n = 5) that received a single intravenous (IV) application of LPS (D0); PEG (n = 5), received a SC application of pegbovigrastim on d 1 (D1); PEG + LPS (n = 5), received a LPS injection D0 and pegbovigrastim at D1; and CTR group (n = 5), received a dose of 0.9% sodium chloride by IV at D0 and another SC dose at D1. For analysis of biochemical and hematological parameters, blood samples were collected on d −1, 0, 1, 2, 3, 4, 8, 14

and 21. The biochemical parameters analyzed were albumin, aspartate aminotransferase, creatinine, gamma glutamyl transferase, PPT, urea, C-reactive protein, haptoglobin and activity of the paraoxonase 1 (PON1). Total leukocyte counts and other hematological variables were also analyzed at the same sampling points. Outcomes were analyzed using a repeated-measures ANOVA (Proc MIXED, SAS Studio). The LPS, PEG, and LPS + PEG groups showed an increase in the number of total leukocytes ($P < 0.0001$) in relation to the CTR, and the PEG and LPS + PEG groups remained with the highest number of cells from d 2 to d 21. The concentration of PON1 was lower in LPS+PEG in comparison to PEG ($P = 0.02$), but not different to the other groups. Moreover, the LPS + PEG group had higher GGT ($P = 0.0042$) e lower AST ($P < 0.0001$) and urea ($P = 0.02$) concetration that the other groups. Althought some hepatic, renal and protein markers had been diferents in the LPS+ PEG these were maintained in physiological concentrations. The results demonstred that PEG injection even in LPS association increased the leukocytes for 21 d without compromising hepatic renal and protein metabolism.

Key Words: pegbovigrastim, immunity, paraoxonase (PON)

315 Embryonic development, luteal size and blood flow area, and metabolite of $PGF_{2\alpha}$ concentrations in dairy cows fed palm or sunflower oil supplement. C. Navanukraw*[1,2], A. Kraisoon[1,3], J. Kaokejon[1], W. Inthamonee[1], and S. Navanukraw[1], [1]*Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand,* [2]*Agricultural Biotechnology Research Center for Sustainable Economy (ABRCSE), Khon Kaen University, Khon Kaen, Thailand,* [3]*Center of Excellence on Agricultural Biotechnology, Bangkok, Thailand.*

Benefits of dietary lipid supplementation during transition period have been shown to improve reproductive performance in dairy cows (Mattos et al., 2004; Thatcher et al., 2006). However, effect of different oil sources on reproduction has not been consistent (Funston, 2004). Thus, the objective was to examine effects of sunflower (SO) and palm oil (PO) supplements in diet on early embryonic development, luteal size and blood flow area, $PGF_{2\alpha}$ metabolite (PGFM), and progesterone (P4) concentrations. Prepartum cows (n = 42) were randomly assigned into one of 3 dietary treatments (control, 4% PO, and 4% SO supplements). Animals were fed diets individually from d 28 prepartum to d 111 postpartum. Cows were synchronized estrus with 2 injections of $PGF_{2\alpha}$ given 11 d apart. Blood samples were taken from d 5 until d 35 postpartum and throughout the estrous cycle (d 3, 5, 7, 9, 11, 13, 15, 17 and 19) for PGFM and P4 analyses, respectively. Luteal size and blood flow area were determined throughout the estrous cycle by Doppler ultrasonography. Oocytes were collected in 3 ovum pick-up sessions at 2-wk intervals for the in vitro embryo production starting on d 83 postpartum. Total DMI and milk yield were greater ($P < 0.05$) in cows fed plant oil supplements than control group during 35 d postpartum. Oocyte characteristics and embryonic development were not affected by dietary treatments. Cows fed 4% SO had greater ($P < 0.05$) concentration of PGFM from d 15 to d 35 postpartum than those cows fed 4% PO and control group. On the mid-luteal phase (d 11 of the estrous cycle), serum P4 concentrations (6.0 ± 0.7, 5.7 ± 0.5, and 4.7 ± 0.6 ng/mL), luteal size (7.0 ± 0.2, 6.5 ± 0.2, and 5.3 ± 0.1 cm^2) and luteal blood flow area (1.3 ± 0.2, 1.2 ± 0.1, and 0.9 ± 0.1 cm^2) were greater ($P < 0.05$) in cows fed 4% SO and 4% PO than control group, respectively. Thus, plant oil supplements in diet affected luteal size and serum P4 and PGFM concentrations, but not early embryonic development. Such changes in secretion of $PGF_{2\alpha}$ and P4 indicate that plant oil supplements during pre- and postpartum may alter uterine and luteal functions.

Key Words: corpus luteum, uterus, PGFM

316 Resynchronization treatments in dairy cows at non-pregnancy diagnosis based on corpus luteum status. J. A. Sauls*[1], B. E. Voelz[1], K. C. Dhuyvetter[2], and J. S. Stevenson[1], [1]*Kansas State University, Manhattan, KS,* [2]*Elanco, Greenfield, IN.*

We tested (1) a shortened version of Ovsynch (OVS: GnRH1–7 d-$PGF_{2\alpha}$-24 h- $PGF_{2\alpha}$-32 h-GnRH2–16 h-AI) that excluded GnRH1 for resynchronization in cows bearing a corpus luteum (CL) at non-pregnancy diagnosis (NPD); (2) the value of including progesterone (P4) + OVS in absence of a CL compared with presence of a CL + OVS; and (3) the accuracy of detecting a functional CL by transrectal ultrasonography. Lactating Holsteins (n = 1,589) in 3 herds were enrolled in 3 treatments at NPD (32 ± 3 d after AI). Cows bearing a visually detected CL were assigned randomly to OVS or Short Synch (SS; $PGF_{2\alpha}$-24 h-$PGF_{2\alpha}$-32 h-GnRH2–16 h-AI), whereas cows with no CL were assigned to OVS + CIDR insert (CIDR). Blood collected at NPD (d 0) determined accuracy of treatment assignment based on P4 (functional CL cut point >1 ng/mL). In 1 herd, ovaries of 108 SS cows were scanned at d 0, 2 d after $PGF_{2\alpha}$, and 6 d after AI and on d 0, 7, 9, and 16 in OVS (n = 97) and CIDR (n = 68) cows to determine follicle diameter and ovulation risk. Treatment contrasts were made: OVS vs. CIDR and OVS vs SS. Ovulation risk after GnRH1 was greater ($P = 0.04$) for CIDR (40.3%) than OVS (27.1%) cows. Dominant follicle diameter before $PGF_{2\alpha}$ was greater ($P = 0.05$) for SS than OVS cows and P4 was less ($P < 0.01$) in CIDR compared with OVS cows. No differences were detected for luteolysis after $PGF_{2\alpha}$ (>96.6%) and ovulation risk after GnRH2 was 94.2, 91.7, and 85.2% for SS, OVS, and CIDR, respectively. Accuracy of treatment assignment was 59.6, 79.5, and 82.4% for CIDR, OVS, and SS cows, respectively. Technicians were more ($P < 0.01$) accurate in detecting a functional than non-functional CL. Pregnancy per AI (P/AI) in all cows was greater ($P = 0.03$) when P4 was ≥1 ng/mL at d 0. With herd as a random effect, P/AI was greater ($P = 0.02$) for OVS than SS but did not differ from CIDR at 32 d (29.6% [n = 644], 21.5% [n = 676], and 25.9% [n = 269]), respectively. When cows within treatment were retrospectively categorized based on P4 cut point, P/AI did not differ among treatments (30.2% [OVS; n = 511], 27.4% [SS; n = 562], and 25.3% [CIDR; n = 164]). Short synch is a viable option when CL status can be accurately detected.

Key Words: artificial insemination, fertility, $PGF_{2\alpha}$

318 Hypothalamic metabolomics profiling in cattle with divergent residual feed intake. A. Elolimy*[1,2], Z. Zhou[3], D. Shike[2], and J. Loor[1,2], [1]*Mammalian NutriPhysioGenomics, Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [3]*Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC.*

The objective of the current study was to apply untargeted metabolomics profiling to determine potential hypothalamic metabolite signatures unique to the most and the least feed-efficient animals based on residual feed intake (RFI) classification. One hundred forty-nine Red Angus cattle were allocated to 3 groups according to herd origin. Animals were fed a finishing diet for 78 d to determine the RFI category for each. Within each contemporary group, the 2 most-efficient (n = 6; RFI coefficient = −2.69 ± 0.58 kg dry matter/d) and least-efficient animals (n = 6; RFI coefficient = 3.08 ± 0.55 kg dry matter/d) were selected. Hypothalamic

tissue was collected immediately after slaughter for metabolomics using a high-resolution mass spectrometry-based untargeted approach. Metabolites were analyzed using Q-Exactive MS system after LC separation. Data analysis was performed using the MetaboAnalyst 4.0 program. Metabolites with 'importance in projection (VIP)' scores >1.0 and a 2-fold difference between groups were considered significantly different. The top 15 metabolites with highest VIP score were identified by molecular weight (mass error ppm <5) for comparison between groups. There were 47 distinct metabolite features identified and a clear discrimination between groups revealed through multivariate analysis (PLS-DA and OPLS-DA). Among the top 15 metabolites identified by the VIP analysis, succinic anhydride (VIP = 3.4), tris(butoxyethyl) phosphate (VIP = 2.9), and etioporphyrin III (VIP = 2.7) were greater in the most-efficient compared with the least-efficient animals. Because of the well-established physiologic role of hypothalamus on feed intake control, the data indicate that untargeted metabolomics profiling could be helpful for identifying RFI-specific biomarkers that may play a role in determining feed efficiency in cattle.

Key Words: residual feed intake (RFI), hypothalamus, metabolomics

319 The potential role of choline to alter histone methylation status revealed through a fluorescent protein system in bovine mammary epithelial cells. F. Rosa* and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Dietary choline can be a source of methyl groups for histone methylation (HM), which can affect gene expression and consequently milk biosynthesis. Therefore, we evaluated the effect of choline on histone methylation in bovine mammary epithelial alveolar cells (MacT). Prior to transfection, cells were cultivated in high glucose Dulbecco modified Eagle's medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and Fungizone antimycotic. The plasmids used in this study were the pcDNA3-K9 and pcDNA3-K27 (Addgene) for analysis of HM through fluorescence resonance energy transfer (FRET) technology. Cells were seeded 24h before transfection at 30,000 cells/well in a 96-well plate. Cells were transfected with Lipofectamine 3000 at 0.3 uL/well and at 50 ng/well of plasmid in a reduced serum medium (OptiMEM) deprived of FBS. Transfected cells were treated for 24h in triplicates with 0, 200, 400, and 800 ug/mL of choline. An inverted fluorescent microscope for live imagining (EVOS FL Auto) equipped with a motorized scanning stage, and an environment-controlled chamber at 37°C and 5.0% of CO_2 was used to take 4 pictures/well at 4x magnification 0, 12, and 24h post-treatment. Transfection efficiency, viability, and quantification of HM were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS and significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.15$. Overall HM tend to decrease in K9 ($P = 0.13$) and increased in K27 ($P \leq 0.01$) during the 24h treatment. In K9 transfected cells, the 400 and 800 choline treatments maintain a HM status, whereas HM decreased ($P < 0.01$) over time in the control and 200 choline. In K27 transfected cells, the 200 choline treatment produced the greatest ($P < 0.01$) HM by 24h post-treatment. To expand on these effects, global DNA methylation and gene expression analysis will be performed. Our results indicate that choline can affect the HM status of histone tail residues (K9 and K27) differently, and this may be reflected in transcriptional changes and consequently in milk biosynthesis.

Key Words: choline, histone methylation, fluorescent proteins

320 Development of an integrated dairy farm decision support system to facilitate dairy management–I. Data integration and warehousing. S. R. Wangen*[1], H. D. Rodriguez[2], D. Liang[2], A. Christensen[1], M. Ferris[1], and V. E. Cabrera[2], [1]*The Wisconsin Institution for Discovery, University of Wisconsin-Madison, Madison, WI,* [2]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

The Virtual Dairy Farm Brain project proposes innovative ways of transforming the way farmers collect, use, and apply data on their farms. In this session, we describe Part 1 of the project: Data integration and warehousing. Disparate data streams describe many of the activities that take place on a large dairy farm (i.e., feeding, milking, management decisions, genetic testing, DHI test, milk composition, and economics). Independently these data streams are informative, but through integration it is possible to build a much richer picture of farm operations. The project aims to utilize the data richness to develop a decision support system (DSS) that can provide insights to the farm in near real-time to help dairy farmers optimize decisions. Records are extracted from farm management, feed monitoring and milking parlor software and are transferred from the farm to centralized server on a daily basis. Genetic, milk composition, and DHI test data, collected from the third-party services, are transferred on a monthly basis. All of these data are subjected to a normalizing and cleaning processes to create a flexible data warehouse structure that can be used to develop farm-level performance dashboards, perform fundamental research by aggregating farms to create large data sets for predictive model development, and provide enhanced analytic tools for the farm by applying those research models to farm-specific data. A proof of concept web interface will also be shown that illustrates the ability of the system to aggregate multiple sources of information to investigate the impacts of health events on daily milk production and feed efficiency at the herd and cow-level. In the future we hope to integrate a suite of analytical tools that will allow users to navigate, visualize, and analyze their own farm data across multiple sources in a single interface. In addition to data visualization and descriptive analysis, the DSS will allow farmers to investigate the tradeoffs of different management scenarios to help find optimal farm management strategies.

Key Words: data integration, decision support systems, farm management

321 Development of an integrated dairy farm decision support system to facilitate dairy management–II. Analysis from integrated data. A. Chrsitensen*[1], D. Liang[2], H. D. Rodriguez[2], S. R. Wangen[1], M. Ferris[1], and V. E. Cabrera[2], [1]*The Wisconsin Institution for Discovery, University of Wisconsin-Madison, Madison, WI,* [2]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

The Virtual Dairy Farm Brain project proposes innovative ways of transforming the way farmers collect, use, and apply data on their farms. In this session, we will build on Part 1 and describe Part 2 of the project—performing analyses from integrated data. The single data source that aggregates multiple data streams from multiple farms described in Part 1 provides a uniquely broad and comprehensive datasource of dairy operations to use in analysis, providing the opportunity to study any number of fundamental research questions ranging from prediction and early detection of health issues, to treatment effectiveness, to expression of genetically inherited traits. In this second part, we describe some of the analyses we are performing which leverage this datasource. Specifically, we will focus on describing how we create individualized lifespan models for each cow that provide an accounting of costs and revenues generating over the lifetime of an individual. The result of this model can be thought of as a cow-specific net present value (NPV) analysis. NPV analyses have previously been developed that accept data on milk production, and reproductive likelihoods, but the data inputs have traditionally been developed from average cows rather than taking a cow-specific approach. This work utilizes near real-time dynamic updates to the data set to continuously update cow-specific milk production functions using observed milk weights, observed cow life-stage information, and cow-specific reproductive data from available genomic analyses. When performed across all individuals within a herd, these NPVs become a powerful analytical tool to aid in optimizing herd composition, evaluating herd expansions/culling, or even understanding the return on investment for large capital improvements.

Key Words: net present value, Markov chain model, dynamic value forecasting

322 Association between measures of seasonality in milk yield, somatic cell count, and herd size across the United States. F. C. Ferreira*[1], J. S. Clay[2], and A. De Vries[1], [1]*University of Florida, Gainesville, FL,* [2]*Dairy Records Management Systems, Raleigh, NC.*

Quantification of seasonal variation in herd performance contributes to management planning and decision-making. Our objectives were to study the association between measures of seasonality of milk yield (MILK), somatic cell count (SCC), and herd size (SIZE) across the US in 2016. The regions analyzed (herds) were Southeast (SE, 183), Northeast (NE, 2,332), and Midwest (MW, 2,240). We used linear regression models with sine and cosine functions to describe seasonality per herd per lactation (LACT) to find the annual low (minimum) and peak (maximum) for each variable. Annual low-to-peak ratios were measures of maximum seasonality, and they were not adjusted (LP) or adjusted (LPADJ) for days in milk to correct for seasonal calving. Traditional summer-to-winter (SWr) ratios of unadjusted observations were used for comparison. We excluded cows with fewer than 5 test-day observations, herds with fewer than 15 cows per LACT group, and farms that were expanding or shrinking. Overall, the correlation between SWr and LP were 26% for MILK, but −26% SCC. The correlation between seasonality in MILK and SCC was significant but not high across herds (10% for both LP and LPADJ). The correlations between SIZE and seasonality of MILK were 13% (LP) and 12% (LPADJ), and 25% for SCC (for both LP and LPADJ). An increase of 100 cows decreases seasonality (LP) in MILK by 0.003 in all regions, a percentage of 2.9% (SE), 1.8% (NE), and 1.9% (MW) and in SCC by 0.008 (0.6%, SE), 0.015 (2.7%, NE), and 0.014 (2.5%, MW). The difference between LP and LPADJ decreased by 0.0006 (0.3%) for every increase in 100 cows in herd size for MILK, and by 0.0001 (6%) for SCC. In conclusion, LP were better measures of seasonality in MILK and SCC than SWr. Large farms were less seasonal than small farms for both MILK and SCC in all regions. Small farms had the greatest reduction in seasonality when adjusted for calving pattern.Our work highlights the importance of using

appropriate measures to avoid under- or over-estimation of true seasonality. Calving pattern should be taken into consideration by the industry when implementing seasonal milking price and milk quality plans.

Key Words: seasonality, calving pattern, sine

323 Effect of calving pattern on seasonality of milk yield and somatic cell count across the US. F. C. Ferreira*[1], J. S. Clay[2], and A. De Vries[1], [1]*University of Florida, Gainesville, FL,* [2]*Dairy Records Management Systems, Raleigh, NC.*

Seasonality in milk yield and SCC in the US can be partially attributed to a seasonal calving pattern, but detailed information of the magnitude is limited. The aim of this study was to describe the seasonality with or without adjustment for calving pattern in milk yield/cow/d (MILK), and SCC of dairy herds across the US in 2016. The regions analyzed (herds) were Southeast (SE, 183), Northeast (NE, 2,332), and Midwest (MW, 2,240). We excluded farms that were expanding or shrinking, lactations with less than 5 test-day observations, and herds with fewer than 15 cows per lactation group (LACT). Sine and cosine functions were used to describe seasonality per herd per LACT to find the annual low (minimum) and peak (maximum) of MILK and SCC. Annual low-to-peak ratios were measures of maximum seasonality, and were not adjusted (LP) or adjusted (LPADJ) for cows' days in milk to correct for seasonal calving. Traditional summer-to-winter (SWr) ratios of unadjusted observations were used for comparison. The median seasonality (SWr, LP, LPADJ) for MILK was 0.88, 0.80, 0.83 (SE); 0.99, 0.87, 0.89 (NE); and 0.98, 0.87, 0.88 (MW). For SCC, the values were 1.15, 0.46, 0.47 (SE); 1.14, 0.46, 0.47 (NE); and 1.17, 0.47, 0.47 (MW). Adjusting for calving pattern resulted in the greatest decrease in seasonality for MILK in the SE, by ((1–0.80)-(1–0.83))/(1–0.80) = 15%, but not for SCC. Overall, LPADJ reduced seasonality for at least one of the LACT in 87% of the SE herds for MILK (mean decrease of 29%), and in 76% of the SE herds for SCC (decrease of 8%). Overall, LACT 1 cows were less seasonal than older cows. For MILK, the median dates (month/day) of the low and peak for LP were 09/15, 03/21 (SE); 09/16, 05/05 (NE); 09/15, 05/06 (MW). For SCC, LP low and peak dates were 04/10, 09/15 (SE); 04/19, 08/31 (NE); 04/18, 08/24 (MW). In conclusion, LP ratios were more accurate measures of seasonality than SWr. LPADJ models were able to adjust for the effects of calving pattern on seasonality.

Key Words: seasonality, calving pattern, sine

324 Culling to achieve reduced somatic cell counts: An economic analysis. D. T. Nolan*[1], T. B. Mark[2], and R. M. Dwyer[1], [1]*Department of Animal Science, University of Kentucky, Lexington, KY,* [2]*Department of Agriculture Economics, University of Kentucky, Lexington, KY.*

The objective of this analysis was to quantify the economic impact of culling high SCC cows to decrease bulk tank SCC. An average US Holstein herd was simulated using @Risk (Palisade Corporation, Ithaca, NY) from Dairy Metrics data provided by Dairy Records Management Systems (Raleigh, NC). The herd consisted of 204 cows with an average SCC of 221,000 cells/mL and an average cull rate of 5.8% for milk quality. A β distribution was utilized to model herd SCC, culling rate, and milk price to make the variables stochastic. A 10,000-iteration simulation of the herd was run culling 12 cows (5.8%) for milk quality per year. Each cow culled represented the cow contributing the most to the bulk tank SCC, which prevented the herd from reaching a monthly SCC threshold of 200,000 cells/mL and a $0.01/kg milk quality premium. Cow contributions to the bulk tank SCC ranged from 5% to 23%, depending on milk production yield and SCC. Benefits of culling the high SCC cows consisted of the premium for milk sold throughout the year by the average farm. Costs included lost milk production from the replacement of the culled cow with a primiparous animal and the retention payoff value of the culled cow. Retention payoff was calculated by taking the sum of the potential future income less operational cost of the cow in question minus the potential future income less operational cost of her replacement. Average ± standard deviation (SD) milk price was $0.41 ± $0.05/kg, and retention payoff of the 12 cull cows was $501.33 ± 238.59 per cow at culling. The average ± SD benefit of culling the 12 high SCC cows was $6,048.02 ± $119.02 per cow-year. Results were highly dependent on the DIM of the culled cow, as milk yield and retention payoff are highly dependent on DIM. Though culling was a profitable option in 100% of iterations in the baseline scenario, this may not be suitable for all herds. Future analysis should include differing herd sizes because as herd size increases, cows contribute a smaller proportion of cells to the bulk tank SCC, herds will have to cull more cows to reach SCC premium levels.

Key Words: premium, retention pay off, milk quality

325 Evaluation of differential SCC as a rapid and affordable tool to improve detection of subclinical mastitis in regular DHI samples. D. E. Santschi*[1], D. Haine[2], A.-M. Christen[1], D. Schwarz[3], J. Durocher[1], S. Dufour[2], and D. M. Lefebvre[1], [1]*Valacta, Ste-Anne-de-Bellevue, QC, Canada,* [2]*Université de Montréal, St-Hyacinthe, QC, Canada,* [3]*Foss Analyticals, Hillerød, Denmark.*

Mastitis is a frequent and expensive disease in the dairy industry, particularly in its subclinical form. Regular SCC testing and bacterial culture are the tools used to monitor subclinical mastitis. However, SCC is usually only analyzed on a monthly basis as part of DHI testing, and bacterial culture is time consuming and rarely done. The composition of somatic cells changes significantly over the course of an inframammary infection. Milk from healthy udders contains mainly macrophages, whereas milk from actively infected glands has a high proportion of polymorphonuclear neutrophils (PMN). Differential SCC (DSCC) offers the possibility to refine the regular SCC analysis by providing the combined proportion of PMN and lymphocytes. 969 cows from 11 farms were involved in the study, from July to October 2017. Once a month and for 2 consecutive milkings, composite aseptic samples (n = 5052) were collected (hand-strip) before milking, and metered samples (n = 7780) were collected throughout milking (plus one 24h composite metered sample). Aseptic samples were cultured and analyzed by Maldi-ToF for bacterial identification. Metered individual and composite samples were analyzed for regular composition, SCC and DSCC. Average DSCC results did not differ between the 4 mo of sample collection, nor between AM, PM and 24h samples. Major pathogens were found in 2507 samples, minor pathogens were in 467 sanples, and 1296 samples had no growth or healthy bacteria. Mean DSCC values were higher for samples where major pathogens were identified (77.7 ± 13.1%) than samples with minor (62.2 ± 17.5%) or no pathogens (58.2 ± 12.0%). Sensitivity and specificity of SCC and DSCC alone or in combination to detect an intramammary infection (any pathogen) were respectively 69.0% and 77.6% for SCC alone; 71.8% and 75.8% for DSCC alone; and 71.7% and 75.4% when SCC and DSCC were used in combination ($P = 0.50$). These results suggest DSCC is highly correlated with SCC but may not increase the ability to detect subclinical mastitis during lactation compared with the precision of SCC alone. Further data analysis is required and will be focused on detection of specific individual pathogens rather than non-specific infection.

Key Words: SCC, differential somatic cell count, DHI

326 The lifetime impact of a clinical mastitis case during the first 100 lactation days in first lactation. H. Delgado*, D. Liang, and V. Cabrera, *University of Wisconsin-Madison, Madison, WI.*

The objectives were to identify lifetime differences in reproduction parameters, genetic traits, and production variables between cows affected by clinical mastitis during the first 100 lactation d in first lactation (1st100dCM) and cows not affected (control). A data warehouse was developed with data extracted from production, health, and genetic software currently in use in 3 herds in Wisconsin. After verifying the availability of complete records for production, reproduction, and health from cows removed of their herds between 2012 and 2017, data from 7,885 cows that included 482 cases of 1st100dCM were used for the analysis. There were no significant differences for age at first calving and number of breedings between animals affected with 1st100dCM and the control group. From the selected animals, 2,021 had records for Genetic Net Merit (GNM) and 217 of these animals were cases of 1st100dCM. There were no significant differences in GNM between cows affected with 1st100dCM and the control group. Using Proc Lifetest (SAS 9.4) for survival analysis, it was found that animals affected with 1st100dCM lived shorter lives (1,413 d ± 25) than the control group (1,608 d ± 17). To test if there were significant variations in production, a mixed model was used including the random effects of the herd and of the year of culling. Least squares means for Lifetime milk and fat were 20,161 kg ± 889 and 820 kg ± 33, respectively, for cows affected by 1st100dCM, whereas these values were 28,780 kg ± 256 and 1065 kg ± 21, for cows not affected (control group). These results indicate significant differences of 6,803 ± 924 ($P < 0.0001$) for milk and 172 ± 40 ($P < 0.0003$) for fat between the 1st100dCM and the control group in benefit of the control. These findings are useful for the construction of benchmarks and decision support systems to help farmers and stakeholders identify the long-term impact of 1st100dCM. With the inclusion of new herds and data sources it is expected in the future to identify factors that have an effect on first lactation heifers to present 1st100dCM.

Key Words: data integration, lifetime management, decison making

327 Predicting clinical mastitis at 30 to 60 DIM using an integrated real-time data warehouse. D. Liang*[1], A. Golechha[2], V. Cabrera[1], and J. Patel[2,1], [1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI,* [2]*Department of Computer Science, University of Wisconsin-Madison, Madison, WI.*

This project aims to permanently predict the onset of cow-level clinical mastitis (CM) using daily milk production (MY) and health records from an integrated data warehouse in the University of Wisconsin-Madison. Milking data and the management records are being transferred from farm computers to our server on a daily-basis since May 2017. With 976,921 daily cow-level MY and 35,169 health-related management records, 125 CM cases between 30 to 60 DIM (30–60 CM) and 4,626 MY records before the diagnosis were found from 118 cows. In the same data set, 21,214 daily MY records between 30 and 60 DIM were found for 3,265 cows (5,844 lactations) that were diagnosed 30–60 CM (NCM). Daily MY was adjusted to the difference from the average MY between 20 and 30 DIM to minimize the MY variation among cows. Whether the cow had CM in the previous lactation (for multiparous) or during the first 30 DIM were included in the model. First month DHI test somatic cell score (SCS) was also included in the model. Mastitis diagnosis DIM and NCM cow DIM were broken into 10-d intervals and only the 10-d MY prior diagnosis was used. R (3.4.1) and Python (2.7, with the scikit-learn package) were used for data analysis and fitting a logistic regression model. Due to a highly-skewed class distribution, a cost-sensitive classification approach was used with different values of misclassification costs. Five CM prediction windows (1 to 5d before diagnosis) were compared. History of CM in the previous lactation and during the first 30 DIM of the current lactation were significantly associated with 30–60 CM ($P < 0.01$). First DHI SCS was significantly higher in the 30–60 CM cows than the NCM cows (2.56 ± 2.17 vs. 1.79 ± 1.60). The logistic regression model was tested on a held-out test set, which contained 40% of the original data. Precision values (actual sick cows over all predicted sick cows) at a sensitivity level of 0.7 ± 0.01 showed an increasing trend from 0.60 to 0.76 when shortening the predicting window from 5d to 1d before diagnosis. Multiple-step prediction with different algorithms will be tested in the future to improve the accuracy of the model.

Key Words: logistic regression, machine learning, early lactation

328 Thermal and electrical energy and water consumption in a Midwest dairy parlor. K. T. Sharpe*, B. J. Heins, E. S. Buchanan, M. H. Reese, J. E. Tallaksen, and L. J. Johnston, *University of Minnesota West Central Research and Outreach Center, Morris, MN.*

The objective of this study was to measure natural gas for heating, electricity, and water use to determine specific areas of high consumption in a seasonal calving Midwest dairy parlor. Data were collected in a Swing-9 parabone milking parlor at the West Central Research and Outreach Center, Morris, Minnesota, pasture-based dairy. Natural gas, electricity, water use, and milk production were monitored from 2014 to 2016. Average herd size across the study period was 212 cows milked. Multiple electric loads were monitored on the barn side of the electric utility meter to reveal areas of highest usage. Consumption of natural gas, electricity, and water were recorded and expressed per kilogram of milk produced (Table 1). Total natural gas and electricity use ranged from 0.29 to 0.46 MJ and 0.27 to 0.33 MJ, respectively. Water use ranged from 1.29 to 1.49 L/kg of milk produced. Natural gas used for heating the milking parlor used the largest proportion of total energy in all 3 years of the study. As expected, natural gas use remained low during warmer months (average = 0.2 MJ/kg milk/d from May to September) and increased during colder months (average = 0.6 MJ/kg milk/d from November to March). Seasonal consumption trends of electricity use mirrored the seasonal milk production curve. Total electricity usage increased from May to August as well as during November to February when milk production peaked compared with other months. Improving the efficiency of thermal and electrical components and reducing water consumption could provide opportunities to improve the carbon footprint and sustainability of dairy production systems.

Table 1 (Abstr. 328). Natural gas, electricity, and water used by the dairy parlor to harvest milk

	Year		
Usage	2014	2015	2016
Natural gas, MJ/kg milk	0.46	0.37	0.29
Electricity, MJ/kg milk	0.33	0.27	0.27
Total MJ/kg milk	0.89	0.74	0.27
Water, L/kg milk	1.49	1.38	1.29
Energy use,[1] % of total MJ			
Parlor heat	35.0	43.0	34.0
Water heating	28.0	21.0	24.0
Milk cooling	8.0	10.0	11.0
Miscellaneous	14.0	7.0	6.0

[1]Only includes the 4 highest consuming electrical loads.

Key Words: dairy, energy, water

329 Using inductive learning methods as a tool to facilitate culling decisions in first lactation dairy cows. M. Lopez-Suarez*[1], L. Castillejos[1], E. Armengol[2], and S. Calsamiglia[1], [1]*Animal Nutrition and Welfare Service, Department of Animal and Food Sciences, Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*IIIA-Artificial Intelligence Research Institute, CSIC-Spanish Council for Scientific Research, Bellaterra, Barcelona, Spain.*

Dairy data analysis may facilitate the selection of which cows should be culled to maintain or increase herd productivity. The objective of this study was to explore the usefulness of inductive learning methods (Decision Tree, DT) to identify cows with low productive expectations susceptible to voluntary culling. A database of 97,987 Holstein-Frisian cows provided by the Confederación Nacional de la Raza Frisona Española was used. The attribute Prod/dLife (average milk production/day of productive life, from first calving until culling) was selected to split the main root of the DT. The model input attributes, obtained from first lactation available information, were: peak milk data (kg milk/d, %fat, %protein and SCC from mo 2 and 3), reproductive indexes (calving-first AI interval and Days Open), cow's birth season, age and season of first calving, genetic indexes of production (KL) and total merit (ICO), and morphological qualification. Numerical attributes were classified in 4 quartile classes: VL (very low), L (low), H (high) and VH (very high). Each DT branch represented a pattern met for those animals with the same classification for Prod/dLife and for other attributes. Patterns were considered significant when more than 10% of cows met the criteria. The most significant patterns were obtained with the attribute KgMilkPeak (average peak production of first lactation). From the 27,179 cows belonging to the quartile KgMilkPeak = VL, 64% were classified in the class Prod/dLife = VL and 22% in Prod/dLife = L. Therefore, 86% of the cows with a VL production at the first lactation peak had a VL or L average production in their productive life. This pattern could be used to classify cows according to milk yield of first lactation peak and to identify those with the lowest expected lifetime production. These results suggest that cows with poorly productive life could be detected in early stages of their first lactation and, thus, be classified as early culling candidates.

Key Words: inductive learning methods, milk production, culling

330 Predicting pregnancy status from mid-infrared spectroscopy in dairy cow milk using deep learning. W. Brand*, A, T. Moran, and M. Coffey, *SRUC, Edinburgh, United Kingdom.*

Accurately identifying pregnancy status is imperative for a profitable dairy enterprise. Mid-infrared (MIR) spectroscopy is routinely used to determine fat and protein concentrations in milk samples. MIR has also been used to predict fatty acid content, mineral content, body energy status, lactoferrin and methane emissions. Machine learning has been used in a variety of fields to find patterns in vast quantities of data. This study aimed to use deep learning to establish pregnancy status from MIR data. Milk spectral data were from 2 sources: the extensively phenotyped Langhill research herd and National Milk Records (NMR) where there is a large volume of data. The spectral data were standardised and spanned 3–50 μm. First, predictions were trained on the Langhill herd and then used to predict on a subset of Lanhill data. Subsequently, a prediction model was trained on NMR data and predicted on Langhill data. Three neural networks were tested. First was with no hidden layers (ANN) for linear classification and as benchmark, second was a fully connected dense network (DNN) and third was a convolutional, backpropagation network (CNN). Training accuracy, validation accuracy was highest with the CNN and also had the lowest loss value. The CNN fully trained model (training accuracy 0.8951 and loss 0.2967) predicted pregnancy status on 5,371 spectral records consisting of 45 lactations where the animal lost a calf during that lactation. Each record consisted of 225 spectral points. The model successfully predicted 82.61% onsets of pregnancy with an average of 16.233 d after insemination. The model could also identify 73.33% of cases where a cow lost a calf during gestation. Predictions after 350 d in milk became less accurate with most cows having a at least one low probability for pregnancy. Results showed that MIR data contains features relating to pregnancy status and the underlying reproductive hormonal changes in dairy cows and can be identified by means of deep learning. Prediction equations from trained models can be used to alert farmers of nonviable pregnancies as well as verify conception dates.

Key Words: deep learning, pregnancy status, mid-infrared spectroscopy

331 Knockdown of transcripts for prostate androgen-regulated mucin-like protein 1 (*PARM1*) decreases trophectoderm formation and alters gene expression in the pre-implantation bovine embryo. A. Zolini*, V. Negron, and P. Hansen, *University of Florida, Gainesville, FL.*

Prostate androgen-regulated mucin-like protein 1 (PARM1) is an anti-apoptotic glycoprotein involved in the endoplasmic reticulum (ER) stress response. A single nucleotide polymorphism in the coding region of *PARM1* has been associated with competence of bovine embryos to develop to the blastocyst stage. The importance of PARM1 for development was tested by evaluating consequences of reducing *PARM1* mRNA abundance on embryonic development and differentiation, gene expression and resistance to heat shock. Embryos produced in vitro were treated with 5 µM GapmeR antisense oligonucleotide targeting *PARM1* (knockdown; KD), 5 µM scrambled GapmeR (scrambled; SC), or vehicle (VEH) at 22 h after insemination. All experiments were performed in 4 replicates. In experiment 1, percent of putative zygotes that cleaved or became blastocysts was not affected by treatment; however, KD *PARM1* decreased number of trophectoderm cells ($P < 0.04$) and increased number of inner cell mass cells ($P = 0.09$). In experiment 2, compact morulae and blastocysts were collected on Days 5 and 7 of culture, respectively, to evaluate expression of 100 genes related to embryonic differentiation, chemokine signaling, epigenetic modification, cell polarity, and ER stress modulation. KD decreased *PARM1* expression for morulae ($P = 0.05$) and blastocysts ($P < 0.0007$). In addition, KD increased ($P \leq 0.05$) transcript abundance for *DAB2, INDL, KLF4, STAT3* and reduced ($P = 0.03$) transcript abundance for *CCR2* in morulae. In blastocysts, KD increased ($P \leq 0.05$) transcript abundance for *PECAM* and *TEAD4* and decreased ($P = 0.03$) abundance for *CCR7*. In experiment 3, embryos from each treatment were subject at d 5 of culture to either heat shock of 41°C or to control temperature of 38.5°C for 24 h; blastocyst formation was assessed on d 7 of culture. Heat shock decreased ($P = 0.07$) blastocyst formation but there was no effect of *PARM1* KD or interaction with heat shock. Results indicate that *PARM1* participates in formation of TE and is involved in regulation of expression of genes important for embryonic development. *Research supported by USDA-NRI AFRI 2013-68004-20365 and BARD US-4719-14.*

Key Words: transcript, prostate androgen-regulated mucin-like protein 1 (PARM1), embryo

332 Generation of an animal model of clinical endometritis to study infertility in dairy cows. R. L. Piersanti*[1], R. Zimpel[1], Z. Ma[1], K. C. Jeong[1], J. E. P. Santos[1], I. M. Sheldon[2], and J. J. Bromfield[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Swansea University Medical School, Swansea, United Kingdom.*

Bacterial infection of the uterus occurs after parturition in approximately 40% of dairy cows and reduces fertility after the resolution of disease. To understand the mechanisms of uterine infection associated infertility, we induced uterine disease in virgin Holstein heifers. Heifers received either bacterial infusion (n = 4) or control vehicle infusion (n = 6) and were monitored for 95 d. All heifers were free from disease before experimentation. Estrous cycles were synchronized and uterine inoculation was performed on d 2 of the estrous cycle. Endometrial scarification was implemented before inoculation of vehicle or the bacteria, which were isolated from clinical cases of postpartum uterine disease in dairy cows (*Escherichia coli* MS499 + *Trueperella pyogenes* MS249). Animal health and disease development was evaluated by rectal temperature, vaginal discharge, visualization of uterine content by transrectal ultrasonography, plasma concentrations of inflammatory mediators, and hematology until reproductive tracts were collected at d 95. Data were analyzed by mixed models using the MIXED procedure of SAS. Bacterial infusion increased vaginal discharge above grade 2 at d 4, whereas controls remained below grade 1. Vaginal discharge scores remained greater in bacterial infusion heifers compared with controls until d 10. Ultrasound revealed the presence of echogenic pus in the uterus following bacterial infusion. Bacterial infusion increased ($P = 0.02$) circulating lymphocytes on d 14 and reduced ($P = 0.05$) red blood cells on d 18 compared with control. Rectal temperature, general health, and appetite did not differ between treatments. No differences were observed in plasma IL-6, TNFα or haptoglobin concentrations between treatments. As indicated by increased vaginal discharge after bacterial infusion, this model successfully induces clinical endometritis in virgin heifers. This model isolates the negative effects of uterine infection on fertility from confounding factors that can occur during the postpartum period in dairy cows. *Supported by the Eunice Kennedy Shriver NICHD R01HD084316.*

Key Words: induced clinical endometritis, inflammation

333 Automated system is better than visual observation for detection of estrus in Holstein Friesian cows. M. Ahmed[1], A. Husnain*[1], A. A. Channa[1], M. Z. Tahir[1], H. ul Rahman[2], and N. Ahmad[1], [1]*Department of Theriogenology, Faculty of Veterinary Science University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan,* [2]*Dairy Animal Training and Research Center, Ravi Campus, University of Veterinary and Animal Sciences, Pattoki, Punjab, Pakistan.*

The present study was aimed to compare automated heat watch system (AHWS) with visual observation (VO) for detection of estrus in Holstein Friesian cows. In this study, total 76 estrus and 1520 non-estrus days of 38 cows were used over period of 42 d. The frequency of VO method was 6 times in a day with 3 h interval. The duration of observation, at each time, was an hour. Heat was counted in VO on the basis of estrus behavior scoring. Within AHWS (Nedap livestock management, Groenlo, the Netherlands), cows had either smart leg (SL = 12) or neck tags (NT = 21). Cows underwent trans-rectal ultrasonography thrice weekly for ovarian status and during estrus every 3 h, to estimate the time of ovulation. A true estrus period was defined as 1) presence of large follicle, absence of corpus luteum on ovary 2) uterine tone. When detected heat, from either method, was coincided with a true estrus period defined as true positive (TP) otherwise false positive (FP). When no estrus was detected on true non-estrus days declared as true negative (TN) otherwise false negative (FN). Sensitivity was calculated as TP/ (TP + FN) *100%. PPV was calculated as TP/ (TP + FP) *100% and specificity was calculated as TN/ (TN + FP) *100%. Estrus detection efficiency was defined as number of true estrus/ ((total cows in breeding herd x days in period)/21) * 100. Data for efficiency of estrus detection, sensitivity, specificity, positive and negative predictive value for both methods, was analyzed by using Chi-squared (SPSS, IBM version) and compared. Results revealed that the efficiency of estrus detection was significantly higher ($P < 0.05$) in VO (x4) than in VO (x2), (73% (56/76) vs. 50% (38/76) respectively. Furthermore, the efficiency of estrus detection in NT (93%, 36/39) or SL (89%, 21/24) was significant ($P < 0.05$) higher than VO (≤x3; 63%,

48/76). The sensitivity and negative predictive value was significantly higher ($P < 0.05$) in AHWS than VO. It is concluded that detection of estrus by AHWS is better than VO. This is promising because of labor cost involved in VO method.

Key Words: automated heat watch system, visual observation, Holstein Friesian cow

334 Economics of replacement dairy heifers managed with reproductive management programs that favor insemination at detected estrus or timed AI. M. Masello*[1], M. M. Perez[1], G. E. Granados[1], M. L. Stangaferro[1], B. Ceglowski[2], M. J. Thomas[2], and J. O. Giordano[1], [1]*Cornell University, Ithaca, NY,* [2]*Dairy Health and Management Services, Lowville, NY.*

Our objective was to evaluate the economics of dairy heifers managed for first AI service with programs that relied primarily on insemination at detected estrus (AIE) or timed AI (TAI). After a voluntary waiting period (VWP) of 12 mo, Holstein heifers from 2 farms received first AI after: 1) PG+AIE (n = 246): AIE after PGF2α (PGF) treatments 14 d apart (up to 3) starting at the end of the VWP. Heifers not AIE within 9 d of the third PGF were enrolled in a 5d-Cosynch protocol (5dCP = CIDR+GnRH-5d-CIDR-out+PGF-3d-GnRH^{+}TAI), 2) TAI (n = 235): TAI after a 5dCP protocol, and 3) PG+TAI (n = 250): AIE after the second of 2 PGF treatments 14 d apart. Heifers not AIE within 9 d after the second PGF were enrolled in the 5dCP. Individual heifer costs and revenues were collected for 15 mo after their VWP [all rearing period (RP) and part of the first lactation (FL)]. Total cash flow (CF) for 15 mo was the aggregation of RP reproductive cost (RC), RP feed cost (FDC), RP replacement cost (RPLC), RP fixed cost (FXC), calf value (CFV), FL income over feed cost (IOFC), FL RPLC, and FL FXC. All results are in Table 1. Time to pregnancy was only reduced for TAI vs. PG+AIE. During the RP, the RC was greater for TAI but no differences ($P > 0.10$) were observed for FDC, RPLC, and FXC. During FL, all parameters evaluated were similar ($P > 0.10$). We conclude that despite some differences in reproductive performance and some factors that affect overall cash flow, economics were similar for heifers managed with reproductive programs that relied primarily on AIE or TAI. *Supported by NYFVI Project AOR2015-020.*

Key Words: profitability, timed AI, dairy heifer

335 Luteal blood flow measured by Doppler ultrasonography during the first three weeks after artificial insemination in pregnant and non-pregnant *Bos indicus* dairy cows. N. Ahmad*[1], M. Hassan[1], U. Arshad[1], M. Bilal[1], M. Avais[1], A. Sattar[1], and H. Bollwein[1,2], [1]*University of Veterinary and Animal Sciences, Lahore, Pakistan,* [2]*University of Zurich, Winterthurerstrasse* 260*, Zurich, Switzerland.*

The objective of this study was to determine the relationships between luteal size (LS), progesterone (P4), and luteal blood flow (LBF) during the first 3 wk after insemination in pregnant and non-pregnant *Bos indicus* dairy cows. Lactating cows (n = 13) of mixed parity that were exhibiting normal estrous cyclicity were enrolled in the study. Estrus detection was carried out twice daily to determine the onset of standing heat (d 0). All cows were artificially inseminated and were classified as pregnant (n = 8) or non-pregnant (n = 5). To compare the LS and LBF after AI, B-mode and color Doppler sonography of ovaries were performed on d 4, 5, 6, 7 (luteal growth phase; LGP), 8, 10, 12, 14, 16, (luteal static phase; LSP), in pregnant and non-pregnant cows. In addition, non-pregnant cows were also examined on d 17, 18, 19, 20, 21 (luteal regression phase; LRP). The corpus luteum (CL) size was measured by taking 3 cross sectional images with maximum areas of the CL. Blood samples from the jugular vein were collected after each sonographic examination for measurement of P4. The mean values and relationships between LS, concentrations of P4, and LBF were subjected to ANOVA of repeated measurements using the PROC GLIMMIX procedure and Pearson correlation coefficient, respectively. Results revealed that the mean LS, concentrations of P4, and LBF increased significantly ($P < 0.05$) every day during the LGP. Mean LS, P4, and LBF showed highest values ($P < 0.05$) during the LSP and then declined ($P < 0.05$) abruptly in the LRP in non-pregnant cows. In pregnant cows, the mean LS, P4, and LBF increased also linearly ($P < 0.05$) during the LGP and LSP that remained elevated thereafter. A positive correlation (r = 0.95; $P < 0.05$) existed between LS and concentrations of P4; and equally strong correlation (r = 0.96; $P < 0.05$) was observed between LBF and P4 during d 4 to 21 in pregnant cows. In conclusion, the present study has provided new information about relationships between LS, P4, and LBF during the first 3 wk after AI in pregnant and non-pregnant *Bos indicus* dairy cows.

Key Words: luteal blood flow, pregnancy, *Bos indicus*

Table 1 (Abstr. 334). Reproductive performance and economics

Item	PG + AIE	TAI	PG + TAI	*P*-value
Time to pregnancy, HR (95%CI)[1]	Referent	1.20 (1.02–1.42)	1.07 (0.91–1.26)	0.08
Rearing, $/heifer				
RC	56.0 ± 1.9[a]	73.1 ± 2.6[b]	59.8 ± 1.9[a]	<0.01
FDC	395 ± 4.0	390 ± 4.5	387 ± 3.8	0.41
RPLC	3.31 ± 3.1	1.33 ± 1.5	0.40 ± 0.8	0.59
FXC	266 ± 2.6	264 ± 3.1	261 ± 2.4	0.43
First lactation				
CFV	126 ± 3.9	125 ± 4.0	132 ± 3.5	0.33
IOFC	1,034 ± 26	1,057 ± 31	1,083 ± 25	0.48
RPLC	99.1 ± 21.5	76.0 ± 18.9	67.2 ± 17.3	0.50
FXC	349 ± 7.7	354 ± 9.1	365 ± 7.0	0.43
CF 15 mo	−23.3 ± 36.4	3.1 ± 38.0	61.6 ± 32.6	0.25

[1]HR TAI vs PG+TAI 1.13 (0.96–1.32).

336 Effects of dietary undigested and physically effective neutral detergent fiber on dry matter intake, milk yield and composition, and chewing behavior of lactating dairy cows. W. A. Smith*[1], K. Ishida[2], J. W. Darrah[1], H. M. Dann[1], C. S. Ballard[1], M. D. Miller[1], and R. J. Grant[1], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*ZEN-NOH National Federation of Agricultural Cooperative Associations, Tokyo, Japan.*

The objective of this study was to evaluate the effect of feeding different dietary concentrations of 240-h undigested neutral detergent fiber (uNDF240) and physically effective neutral detergent fiber (peNDF) on dry matter intake (DMI), milk production and composition, and chewing behavior. Sixteen Holstein cows averaging 123 (SD = 9) days in milk (DIM) were used in a replicated 4 × 4 Latin square design with 4-wk periods. Cows were fed diets formulated to be isoenergetic and isonitrogenous but varying in uNDF240 and peNDF by changing forage to concentrate ratio and particle length of hay. Treatments were (1) 8.6% uNDF240 and 18.4% peNDF (LULP), (2) 8.6% uNDF240 and 19.9% peNDF (LUHP), (3) 10.5% uNDF240 and 16.2% peNDF (HULP), and (4) 10.5% uNDF240 and 19.0% peNDF (HUHP). Cows were housed in individual tie stalls, fed TMR once daily, and milked 3×/d. Feed intake and milk yield were collected daily from d 20 to 26. Milk composition was collected for 6 consecutive milkings on d 25 and 26 and body weight was collected on d 28 of each period. Behavior was collected for 72 h on d 23 to 25. Data were summarized by period and analyzed as a replicated Latin square design with model effects for diet, period, and replicate using the MIXED procedure of SAS (version 9.4). Cow within replicate was a random effect. Greater forage particle size with low uNDF240 diets resulted in DMI, ECM, and chewing response similar to higher uNDF240 diets with smaller particle size (Table 1).

Key Words: digestibility, particle size, undigested fiber

337 Estimating lactation residual energy intake in Holstein cows: When and how long? A. Fischer*[1,2] and P. Faverdin[2], [1]*Institut de l'élevage, Paris, France,* [2]*PEGASE, Institut National de la Recherche Agronomique, Agrocampus-Ouest, Rennes, France.*

Improving feed efficiency is expected to reduce resource use while maintaining production. This achievement depends on the ability to identify the most and least efficient animals. The objective of the current study was to analyze the impact of measurement period length and start point during lactation on the ability to identify the most and the least efficient dairy cows. Individual daily feed intake, milk net energy, metabolic BW, and BCS were monitored frequently during the first 224 DIM for 117 Holstein cows fed a same total mixed ration. Different period durations and start points were defined: duration went from 14 to 196 consecutive days and start points went from DIM 1 to 210 with 14-d increments. Feed efficiency was estimated with the residual energy intake (REI) as the residuals of a multiple linear regression estimating average net energy intake from average milk net energy, average metabolic BW, and body condition score loss and gain. This model was adjusted over the 224 d, the reference period in this study, and within each short period. Ability to identify the most and least efficient cows was estimated with the positive predicted value (PPV) and the sensitivity. PPV is the percentage of most and least efficient cows identified within a short period, which are also identified as such over the reference period. Sensitivity is the percentage of most and least efficient cows over the reference period, which are also identified as such within a short period. The most and least efficient cows were the cows outside the range of REI average plus and minus one standard deviation. Measurement start point had a positive linear effect on PPV ($P < 0.01$), but not on sensitivity ($P = 0.12$). Period duration had a positive linear effect ($P < 0.01$) and a negative quadratic effect ($P < 0.01$) on both PPV and sensitivity, highlighting that PVV and sensitivity reached a maximum. A minimum of 112 d were necessary to achieve a sensitivity and a PPV, which were not significantly different ($P > 0.06$) from their respective maximum: 86% and 79%. To decrease the risk of identifying efficient cows as inefficient or inefficient cows as efficient, REI should be estimated over at least 112 d to identify a maximum of the efficient and inefficient cows.

Key Words: feed efficiency, repeatability, cattle

Table 1 (Abstr. 336).

Item	LULP	LUHP	HULP	HUHP	SE	*P*-value
DMI, kg/d	27.5[a]	27.3[a]	27.4[a]	24.9[b]	0.6	<0.001
DMI, % of BW	4.02[a]	4.04[a]	3.99[a]	3.73[b]	0.10	0.003
Milk, kg/d	46.1[a]	44.9[ab]	44.0[bc]	42.6[c]	0.9	<0.001
Milk fat, %	3.68[b]	3.66[b]	3.93[a]	3.92[a]	0.10	<0.001
De novo fatty acids (FA), g/100 g FA	0.87	0.85	0.90	0.88	0.03	0.176
Mixed origin FA, g/100 g FA	1.41[b]	1.40[b]	1.51[a]	1.51[a]	0.04	<0.001
ECM, kg/d	47.0[a]	45.7[ab]	46.4[ab]	44.6[b]	0.9	0.031
ECM/DMI, kg/kg	1.71[ab]	1.68[b]	1.70[ab]	1.79[a]	0.04	0.023
Eating, min/d	255.4[b]	262.5[b]	279.1[ab]	300.3[a]	12.4	<0.001
Rumination, min/d	523.2	526.5	531.8	544.5	16.4	0.357
Eating time per DMI, min/kg	9.1[c]	9.6[bc]	10.1[b]	11.9[a]	0.5	<0.001
Rumination time per DMI, min/kg	18.6[b]	19.3[b]	19.3[b]	21.7[a]	0.8	<0.001

[abc]Within a row, different superscripts differ ($P \leq 0.05$).

338 Nutrient allocation between the pellet and PMR can affect eating behavior of mid-lactation dairy cows. J. L. Haisan* and M. Oba, *University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to determine the effects of nutrient allocation between the partial mixed ration (PMR) and pellet on feeding behavior and blood metabolites of mid-lactation dairy cows. A portion of an experimental diet was offered as a high-fiber (F; 7.7% starch, 41.2% NDF) or high-starch (S; 59.5% starch, 14.7% NDF) pellet fed at 1 or 3 kg twice per day. Four types of PMR were formulated for each pellet treatment to provide the same overall diet (PMR + pellet) among all treatments assuming DMI of 25.0 kg/d. Eight ruminally cannulated cows were used in a 4 × 4 Latin square design study with 14-d periods. Cows were fed PMR once daily at 1200 h, and pellet twice daily at 0600 and 1800 h. Disappearance of PMR was measured on d 11–13 of each period, as the amount of feed consumed every 3 h following PMR delivery, and blood was collected every 90 min on d 14. Treatment did not affect milk yield (42.7 kg/d), milk fat content (3.75%), or total dry matter intake (27.7 kg/d). In addition, there was no change in daily minimum, mean or maximum rumen pH, or evidence of PMR sorting among treatments. However PMR intake was reduced when more pellet was fed (22.9 vs. 25.3 kg/d; $P < 0.01$). When cows were fed the S pellet (with high-fiber PMR), PMR intake immediately after feed delivery was reduced as compared with the F pellet (28.5 vs. 33.5% of PMR intake; $P = 0.04$), and tended to be increased during other periods of the day. In addition, feeding the F pellet (with high-starch PMR) increased daily mean plasma concentrations of glucose (70.0 vs. 66.0 mg/dL; $P < 0.01$) and insulin (2.24 vs. 1.90 ng/mL ; $P = 0.02$), indicating that nutrient content of the PMR appeared to affect feeding behavior and blood metabolites to a greater extent than the pellet in the current study. These results suggest that nutrient allocation between PMR and pellet can affect actual PMR intake, feeding behavior, and plasma metabolites, and this should be considered when formulating diets consisting of PMR and pellet.

Key Words: partial mixed ration, feeding behavior, blood metabolites

339 Evaluation of a dynamic mechanistic dairy cow model: feed intake predictions. V. Ambriz-Vilchis*[1,2], M. Webster[2], J. Flockhart[1], and J. Rooke[1], [1]*SRUC Future Farming Systems Group, Edinburgh, United Kingdom,* [2]*BioSimetrics Ltd., Edinburgh, United Kingdom.*

Mathematical modeling allows the simulation of feeding regimens and consequent performance of livestock. To achieve this, accurate prediction of feed intake is paramount. Therefore the objective of this study was to evaluate the accuracy of a whole animal dynamic mechanistic model to predict intake of dairy cows fed 2 contrasting total mixed ration (TMR).

A trial was carried out at SRUC's Dairy Research Centre, Scotland UK. Forty Holstein Friesian dairy cows were offered two contrasting diets: 1) forage based TMR (Grass silage, Maize silage, Crimped wheat, Beans and Minerals) or 2) concentrate/by products based TMR (Wholecrop alkalage, Megalac, Whey, Water, Minerals and concentrate). Using electronic feeders (HOKO, Insentec, The Netherlands) individual feed intakes (FI, kg fresh matter (FM) /cow/day) were recorded daily. Recorded data was used to evaluate the accuracy of intake predictions of a mechanistic dairy cow model (BSM-Dairy, BioSimetrics Ltd., Scotland UK). Details of the animals (body weight, condition score, milk (yield and characteristics) and lactation number) and the TMR (composition, chemical and fermentation characteristics) consumed were recorded weekly and were used as inputs for BSM-Dairy. The intake predictions obtained with BSM-Dairy for each animal were compared to those obtained on-farm. To evaluate the predictions a linear mixed effect model (LMEM), a modified version for repeated measures of the limits of agreement (LoA) method and the concordance correlation coefficient (CCC) were used. All statistical analyses were carried out using R. Observed and predicted values were in good agreement. When evaluated with the LoA method the relationship between feed intake observed and predicted by BSM-Dairy showed no tendency of the model to over or under predict intake, the lower limit of agreement was −8.62 kg/FM/ day and the upper limit of agreement at 9.58 kg/FM/ day. Overall, the model gave acceptable intake predictions compared to consumption observed on-farm (mean 0.48 kg/FM/ day). The equation obtained with the LMEM had an intercept = 1.69 and residual = 0.97 and the CCC = 0.88. In conclusion, given an adequate description of the animal and the diets consumed, BSM-Dairy successfully predicts intake of dairy cows consuming TMR.

Key Words: modelling, intake, dairy cow

340 Modeling feed intake and dairy performance with different grass ley harvesting strategies. D. Pang*[1], S. J. Krizsan[1], A. Sairanen[2], and P. Huhtanen[1], [1]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden,* [2]*LUKE-Agrifood Research Finland, Animal Production Research, Jokioinen, Finland.*

A meta-analysis of animal feeding trials fed grass silages was conducted to study the effects of different forage harvests and maturity stages on feed intake and dairy performance in dairy cows. Data were collected from studies in Europe from 1981 to 2017. They were divided into 2 subsets: 69 diets from 11 studies were used for comparision of silages made from primary growth and regrowth of grass (harvest), and another 157 diets from 28 studies were used for comparision of digestibility influenced by the maturity of grass ensiled (*D*-value). The minimum prerequisite for an experiment to be included in the data set was that milk production (milk and ECM yields, concentration and yield of milk component), animal intake (DM, MP and ME), live weight (LW) and silage parameter (DM, CP, NDF, total acids, soluble N and *D*-value) were reported or could be estimated according to Finnish feed table. Additionally the harvest subset should involve a comparison of different cuts within the same experiment. Both subsets were analyzed using mixed model procedure of SAS. When comparing different harvests, the best fit model for milk protein concentration was: Milk protein concentration (g/kg) = 27.0 – 1.82 × a + (0.17 + 0.10 × a) × DMI (kg/d) + 0.004 × *D*-value (g/kg DM) (a = proportion of first cut silage in silage DM (0 or 1), Residual SE = 0.13, and AIC = 214). The best fit model for DMI was: DMI (kg/d) = – 11.3 + 1.43 × a – 0.005 × Silage soluble N (g/kg total N) + 0.057 × LW (kg) (a = proportion of first cut silage in silage DM (0 or 1), Residual SE = 0.16, and AIC = 186). When comparing the different maturity stages identified by *D*-value, the best fit model for ECM yield was: ECM yield (kg/d) = 16.0 + 0.105 × MEI (MJ/d) – 0.014 × *D*-value (g/kg DM) + 0.018 × silage CP (g/kg DM) – 0.031 × silage total acids (g/kg DM) – 0.007 × silage DM (g/kg DM) (RMSE = 0.84, $R^2 = 0.95$ and AIC = 462). The best fit model for DMI was: DMI (kg/d) = 8.81 + 0.03 × *D*-value (g/kg DM) – 0.01 × silage total acids (g/kg DM) (RMSE = 0.28, $R^2 = 0.89$ and AIC = 426). The prediction models from the meta-analysis could be used to improve the current ration formulation systems, or as prerequisites for economic models optimizing milk production in various farming systems.

Key Words: dairy cow, grass silage, harvesting strategy

341 Grass silage chop length when fed alone, or with corn silage, affects performance and milk quality of dairy cows. U. Tayyab*[1], R. G. Wilkinson[1], C. K. Reynolds[2], and L. A. Sinclair[1], [1]*Harper Adams University, Newport, United Kingdom,* [2]*University of Reading, Reading, United Kingdom.*

The particle size (PS) of a diet along with its fiber content is important to maintain optimum rumen function and animal performance. Most studies have focused on the PS of alfalfa and corn silage (CS) with relatively little work on grass silage (GS) PS despite its predominance in the diets of cows in Northern Europe. The objective of our study was to evaluate the effect of GS chop length and diet GS:CS ratio on cow performance and milk composition. Grass was harvested at 2 theoretical chop lengths (short; 10 and long; 44 mm) and mixed with corn silage (GS:CS) at 100:0 or 40:60 (dry matter [DM] basis) of DM in isonitrogenous total mixed rations. Sixteen multiparous Holstein-Friesian cows were used in a 4 × 4 Latin square design with 4 periods each of 28-d duration. Dry matter intake (DMI), milk yield, and milk composition were measured during final week of each period. Composite milk samples from 4 consecutive milkings were extracted and methylated before fatty acid (FA) analysis by gas chromatography. Data were analyzed as a 4 × 4 Latin Square and 2 × 2 factorial design using GenStat 18. A short chop length GS increased ($P < 0.04$) DMI by 0.9 kg DM/d compared with the long chop length. There was an interaction ($P < 0.02$) between chop length and GS:CS ratio with a short chop length GS increasing milk yield in cows fed the 100:0 but not the 40:60 GS:CS based diets. There was a tendency ($P = 0.09$) for a higher milk fat yield (1.2 g/kg) for the long chop length GS. Cows fed the 40:60 GS:CS based diets had higher milk fat concentrations of C18:0 ($P < 0.01$), C18:2_{n6} ($P < 0.01$) and total polyunsaturated fatty acids ($P < 0.02$) compared with the 100:0 GS diets. A short chop length GS increased ($P < 0.01$) milk C18:3_{n3} concentration compared with the long chop length. Cows fed the 40:60 GS:CS diets had a 1.5 g/kg higher ($P < 0.01$) milk protein content. In conclusion, a longer GS chop length reduced DMI and milk yield, but had little effect on milk fat yield and FA content. Including CS in the diet increased DMI and milk performance irrespective of the chop length of the GS.

Key Words: chop length, milk fatty acids

342 Using carbon emissions and oxygen consumption to estimate energetics parameters of cattle consuming forages. S. A. Gunter*[1], C. Burrus[2], C. A. Moffet[1], and P. Gregorini[3], [1]*USDA, Agricultural Research Service, Woodward, OK,* [2]*Southern Arkansas, Magnolia, AR,* [3]*Lincoln University, Christchurch, New Zealand.*

To evaluate a newer indirect calorimetry system (ICS; GreenFeed, C-Lock, Inc., Rapid City, SD) able to measure carbon dioxide (CO_2), methane (CH_4), and oxygen (O_2) fluxes, 8 cross-bred beef steers (initial BW = 241 ± 4.10 kg) were used in a 77-d experiment to examine the energetic parameters calculated with the gas fluxes. Steers were individually fed in a pen equipped with Calan gates (hay) and they visited the head box of the ICS 4 times/d while consuming their supplement. After a 2 wk training period, steers were randomly assigned to 1 of 3 treatments in multiples of maintenance ME intake (MEx, 7.43 Mcal/d): 1.1 (n = 3), 1.4 (n = 2), and 1.8 (n = 3) (GE intake = 17.4, 23.1, and 31.4 Mcal/d, respectively). Diets were long-stemmed wheat hay (*Triticum aestivum* L.; 15% CP, 64% TDN) plus a daily supplement of 1.03 kg of alfalfa-based pellets (*Medicago sativa* L.; 14% CP, 69% TDN). On d 1 and 77, BW was recorded after a 17-h fast to access BW gain. Heat production (HP) was calculated from gas fluxes and predicted urinary N excretion [0.62 × N intake (g/d) - 3.72 × DMI (kg/d) - 3.93] using the Brouwer (1965) equation (HP (Mcal/d) = [3.866 × O_2 (L/d) + 1.200 × CO_2 (L/d) – 1.44 × N excretion (g/d) – 0.518 × CH_4 (L/d)]/1,000). Dependent variables were analyzed with the MIXED procedure (SAS Inst., Inc.; Cary, NC) and least squares means were separated with linear and quadratic contrasts. Both BW gain and HP linearly increased ($P < 0.01$) with MEx (9.2, 22.3, and 50.1 kg, and 11.4, 11.8, and 13.3 Mcal/d, respectively). Energy losses from CH_4 emissions (2.15, 2.24, and 2.24 Mcal/d) did not differ ($P > 0.62$) among MEx. The percentages of energy losses from CH_4 decreased linearly ($P < 0.02$) with increasing MEx (12.1, 11.1, and 8.9% of GE intake, respectively). Percentage of HP decreased linearly ($P < 0.01$; 66.1, 50.8, and 42.1% of GE intake, respectively) with increased MEx. The percentages of CH_4 and urinary energy loss, and HP relative to GE intake are similar to other reports for steer consuming forages (Kurihara et al., 1999; Br. J. Nutr. 81:227–234). These results indicate that measurements of gas fluxes with this indirect calorimetry system produce estimates that are similar to researchers using respiration chamber.

Key Words: indirect calorimetry, energetic efficiency, maintenance

343 Effect of grass silage chop length when fed alone or with corn silage, on eating behavior and diet selection in dairy cows. U. Tayyab*[1], E. L. Forrest[1], G. L. Charlton[1], R. G. Wilkinson[1], C. K. Reynolds[2], and L. A. Sinclair[1], [1]*Harper Adams University, Newport, United Kingdom,* [2]*University of Reading, Reading, United Kingdom.*

Adequate forage chop length (CL) along with a sufficient dietary fiber content can benefit the rumen environment and cow performance. Numerous studies have investigated the CL of alfalfa and corn silage (CS), but there is less research on ryegrass silage (GS). Our aim was to evaluate the effects of GS CL and GS:CS ratio on the eating behavior and sorting activity of dairy cows. Grass silage was chopped at harvest at 2 mean CL (short or long) and either fed alone or mixed with CS at a GS:CS ratio of 40:60 (DM basis). All diets were supplemented with concentrates at a 54:46 (DM basis) and fed as a total mixed ration. Sixteen multiparous Holstein-Friesian cows were used in a 4 × 4 Latin square design with 4 periods of 28-d duration in a 2 × 2 factorial arrangement. Jaw activity was visually recorded through instantaneous scan sampling at 5-min intervals for 48h. Diet particle distribution was measured at 0 and 24h post-feeding for 5-d using a modified Penn State Particle Separator. Sorting was calculated as the actual intake of each fraction expressed as a % of the predicted intake of each fraction. Values < 100% indicate selective refusals, > 100% is preferential consumption, and = 100% is no sorting. Data were analyzed using a mixed model procedure. Cows when fed GS alone spent 1.1 h/d longer ($P < 0.01$) eating compared with those fed the GS:CS mix. Eating time was 2.84 min/kg DMI higher for the longer CL GS compared with the short CL. There was an interaction ($P < 0.03$) for ruminating time (RT) and RT (min/kg DMI), where a longer CL increased RT when fed the GS:CS mix but had little effect when fed GS alone. An interaction ($P < 0.01$) was also observed for sorting of > 19 mm fraction, where a shorter CL resulted in a refusals when fed GS alone but there was no sorting when fed the GS:CS mix. In contrast, cows when fed GS alone had a greater preferential consumption ($P < 0.03$) for the 4–8 mm fraction. In conclusion, a longer GS CL increased eating time but had no effect on ruminating time. Cows when fed GS as the sole forage source spent a longer time eating and ruminating that might benefit rumen function, but also resulted in more diet sorting.

Key Words: ryegrass, chop length, behavior

344 Development of a wet sieving method for measuring corn silage processing score (CSPS). R. Ward*[1] and D. R. Mertens[2], [1]*Cumberland Valley Analytical Services Inc., Waynesboro, PA,* [2]*Mertens Innovation & Research LLC, Belleville, WI.*

Current CSPS is the percentage of starch passing through a 4.75-mm sieve with vigorous vertical shaking of a dried corn silage (CS). Starch in immature, extensively processed, or fermented CS may adhere to large particles when drying causing CSPS to be underestimated. Assuming that CS DM is a measure of immaturity, 5500 samples were used to assess the relationship between current CSPS and CS DM. The R^2 was < 0.0001 indicating no relationship. The objective was to develop a wet sieving method that could remove soft starch that might stick to large particles. In the wet CSPS (WCSPS) method, a 150 g sample of undried CS is placed in a tall Tyler sieve of 30.5 cm diameter with 4.75-mm openings. At the top of the stroke, the sample is outside of the water in a tub and at the bottom of the stroke the sieve is immersed in water to a depth of about 10 cm. Samples are sieved for 90 s at 60 cycles/min. The residue retained is rinsed with a spray nozzle, dried, and analyzed for starch, and WCSPS was calculated as 100*[(total - retained starch)/ total starch]. Dry and wet CSPS was determined on 189 CS. Ordinary least squares (OLS) regression is not appropriate when X and Y variables have similar variation. Geometric regression has the same correlation as OLS; however, the geometric equation fits Y on X and X on Y. Three replicate measures of CSPS and 10 replicates of WCSPS had standard deviations of 2.71 and 2.34, respectively. For the 189 CS, average CSPS and WCSPS was 70.5 and 72.9, respectively. The geometric regression was: CSPS = 21.78 + 0.675*WCSPS; R^2 = 0.43. Low R^2 suggests that the methods may not measure the same characteristic. Both methods also measure the DM retained (DMR) on the 4.75-mm sieve which obtained the geometric regression: DMR = −6.37 + 0.983*(Wet DMR); R^2 = 0.42. When sorted into groups averaging 28, 33, 37, and 44% DM, the WCSPS averages were 76, 74, 72 and 60; and the CSPS were 68, 71, 72 and 69, respectively. WCSPS were higher than CSPS for the wetter silages, but correlations between DM and WCSPS or CSPS were <0.2. The WCSPS may address some of the problems with current CSPS, but low correlations suggest additional study.

Key Words: kernel processing

345 Effects of starch source and particle size on ruminal fermentation, starch digestibility, and milk production of dairy cows. M. N. T. Shipandeni*[1,2], E. M. Paula[3], A. P. Faciola[3], and E. Raffrenato[1], [1]*Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa,* [2]*Department of Animal Science, University of Namibia, Windhoek, Namibia,* [3]*Department of Animal Sciences, Gainesville, FL.*

The aim of the study was to intentionally shift site of digestion for starch. Specific objectives were to evaluate the effects of starch sources varying in particle sizes on digesta flow, starch digestibility, ruminal fermentation, and performance of dairy cows. Four ruminally-cannulated multiparous Holstein cows (718 ± 59 kg of BW; 230 ± 57 DIM, 25.29 ± 6.80 milk/d) were used in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. Treatments were the combinations of 2 starch sources (corn or sorghum, C or S) either finely or coarsely ground (using a 1- or 4-mm screen sieve, F or C) and fed within a total mixed ration. Diets were formulated to contain similar starch concentration. Digesta flow was quantified using reticular sampling technique, applying the triple-marker method. Data were analyzed using a mixed model with cow as a random factor. The geometrical mean particle size of corn and sorghum milled at 1 or 4 mm were 255.6, 250.6, 550.1, and 728.5 µm, for CF, SF, CC, and SC, respectively. While milk yield and composition were not affected by dietary treatments, intake tended to be greater (P = 0.09) for cows fed the coarser grains. Fine corn tended to result in greater milk yield (P = 0.06). Sorghum C resulted in greater rumen and reticulum pH (P < 0.05) when compared with other treatments. Propionate concentration was greater for both corn diets (33.21 vs. 29.87 mM; P < 0.05) and ruminal ammonia N was similar across treatments. Organic matter ruminal digestibility tended to be greater for the coarse grain (P = 0.07) mainly for greater NDF digestion. Starch from the coarser grains tended to be less ruminally digested and had a significant greater flow to the abomasum when compared with the fine particles (0.90 vs. 1.22 kg/d). The difference was greater for corn. Despite the different ruminal digestions and flows, apparent total-tract digestibility were similar. Only SC resulted in lower starch digestibility (81%, P = 0.03) compared with the other treatments. Coarse corn tended therefore to be more digested post-ruminally.

Key Words: corn, sorghum, starch flow

346 Antioxidant capacity of dairy cows after supplementation with dietary probiotic *Bacillus subtilis* during the transition period. W. Choonkham*[1] and W. Suriyasathaporn[2], [1]*Graduate/PhD Degree Program in Veterinary Science, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand,* [2]*Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand.*

Dairy cows undergo an increase in metabolic processes during the transition period, leading them to be more susceptible to metabolic and oxidative stresses that are implicated in many periparturient diseases. Therefore, the neutralizing capacity of antioxidants plays an important role in reducing oxidative stress, particularly during the transition period. Accordingly, the objective of this study was to evaluate the effect of oral supplementation of dietary probiotic *Bacillus subtilis* (PROBS) on the overall antioxidant capacity in periparturient dairy cows. Twelve cows from a smallholder dairy farm were randomly assigned into 4 groups: a control group (CON), a group with a single intramuscular administration of 1,000 mg of α - tocopherol acetate (vitamin E) and 10 mg of selenium selenite on d 21 prepartum (ES), a group with daily oral supplementation with PROBS at 0.5×10^{11} (BS0.5), and a 1.0×10^{11} (BS1.0) colony - forming unit (cfu) during d 21 prepartum to d 14 postpartum. Blood samples were collected on days −14, 0, and 14 relative to the expected calving date for the serum total antioxidant status (TAS) measures of overall antioxidant capacity during the transition period. Repeated measure analysis was conducted to determine the effect of PROBS on TAS. Overall means and standard errors of the mean (SEM) of TAS on days −14, 0, and 14 were 1.77 ± 0.094, 1.72 ± 0.131, and 1.77 ± 0.107 mmol/L, respectively. Cows in BS0.5 (2.01 mmol/L) were the only group that had a trend for higher TAS than the CON (1.40 mmol/L). In conclusion, supplementation of dietary probiotic *B. subtilis* at 0.5×10^{11} cfu/cow per day during 3 weeks prepartum to 2 weeks postpartum could be utilized as a beneficial feed additive with improved antioxidant capacity in dairy cows during the transition period.

Key Words: *Bacillus subtilis*, total antioxidant status, dairy cow

347 Effects of a recombinant bacterial expansin and an exogenous fibrolytic enzyme on preingestive fiber hydrolysis, fermentation and digestibility of corn silage. A. A. Pech-Cervantes*[1], Y. Jiang[1], F. X. Amaro[1], D. Kim[1], K. Arriola[1], M. Flores-Tensos[1], C. F. Gonzalez[2], L. F. Ferraretto[1], N. Dilorenzo[3], D. Vyas[1], and A. Adesogan[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL,* [3]*Department of Animal Sciences, University of Florida, North Florida Education Center, Marianna, FL.*

The objective was to examine individual and synergistic effects of recombinant bacterial expansin proteins and an exogenous cellulolytic and xylanolytic enzyme (EFE) on preingestive hydrolysis, in vitro ruminal fermentation and digestion, and sugar profile of whole-plant corn silage. In Experiment 1 (E-1), the EFE (0, 2.33 mg/g) and expansin (0, 304, 616, 888 μg/g) were incubated with dried ground corn silage (0.50 g; 1 mm) in buffered rumen fluid in quadruplicate for 24 h at 39°C in 3 independent runs. Gas production was measured after 0, 3, 6, 12 and 24 h. In Experiment 2 (E-2), a similar approach was used to examine simulated preingestive effects by incubating the corn silage with deionized water containing 0.02% sodium azide in quadruplicate for 24 h at 25°C in 2 independent runs. Treatments were arranged in a 2 × 4 factorial. Data were analyzed using the NMLE package of R for a randomized block design and run was the blocking factor. In E-1, EFE alone increased gas production at 3 h and 6 h compared with the control (15.3 vs 17.3 and 16.1 vs 17.8 mL/ g DM, $P < 0.01$) and adding the highest dose of expansin tended to increase gas production (22.2 vs 21.6 mL/ g DM $P = 0.09$). Adding EFE alone increased NDF digestibility (34.0 vs 32.9% $P = 0.04$) but expansin did not ($P = 0.45$). Total CH_4 production and VFA profile were not affected by EFE ($P > 0.05$) or expansin ($P > 0.05$) addition. In E-2, EFE application increased ($P < 0.05$) DM, NDF and ADF disappearance as well as ($P < 0.01$) concentrations of cellobiose, glucose, xylose, and arabinose. Whereas adding expansins alone increased only arabinose concentration (2.1 vs 1.4 mg/ ml $P < 0.01$). In conclusion, EFE application alone increased fiber digestibility and sugar release and the highest expansin dose increased gas production, however no synergistic effects of applying both additives on rumen fermentation parameters or fiber digestibility were detected.

Key Words: digestibility, expansins, fibrolytic enzymes

348 Synergistic effects of a recombinant bacterial expansin and a fibrolytic enzyme on digestibility, gas production and sugar release from bermudagrass silage. A. A. Pech-Cervantes*[1], Y. Jiang[1], F. X. Amaro[1], D. Kim[1], K. Arriola[1], M. Flores-Tensos[1], C. F. Gonzalez[2], L. F. Ferraretto[1], N. Dilorenzo[3], D. Vyas[1], and A. Adesogan[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL,* [3]*Department of Animal Sciences, University of Florida, North Florida Education Center, Marianna, FL.*

This study examined the effects of recombinant bacterial expansin and exogenous xylanase and cellulase enzyme preparation (EFE) on preingestive hydrolysis, in vitro ruminal fermentation, and sugar profile of bermudagrass silage. In Experiment 1, the effects of 2 levels of EFE (0, 2.33 mg/g) and 4 levels of expansins (0, 304, 616, 888 μg/g) were evaluated using an in vitro batch culture of buffered-rumen fluid. Dried ground bermudagrass silage (0.50 g; 1 mm) was used as substrate. Treatments were arranged in a 2 × 4 factorial using a randomized block design. Samples were incubated for 24 h at 39°C in quadruplicate per run in 3 independent runs. Gas production was measured at 0, 3, 6, 12 and 24 h. In Experiment 2, preingestive effects of EFE and expansins were evaluated by incubating the substrate in deionized water containing 0.02% (vol/vol) sodium azide. Samples were incubated for 24 h at room temperature (25°C) in quadruplicate in 2 independent runs. Data from both experiments were analyzed using NLME package of R. Model included the effects of EFE, Dose and run was used as a blocking factor, repeated statement was used to estimate gas production. In Experiment 1, EFE and high dose of expansins synergistically increased gas production at 3, 6, 12, and 24 h compared with EFE only (56.2 vs 54.7 mL/ g OM $P < 0.01$). Similarly, synergistic increases in digestibility of DM (47.5 vs 43.9% $P = 0.04$), NDF (33.9 vs 30.7% $P = 0.08$) and ADF (27.3 vs 23.8% $P = 0.09$) were detected. Adding EFE alone increased total VFA concentration (71.4 vs 63.2 mM $P < 0.01$) and decreased total CH_4 production while adding expansin alone decreased acetate-to-propionate ratio (2.62 vs 2.67 $P < 0.02$). In Experiment 2, EFE and expansins synergistically increased % losses of DM, and NDF, regardless of the expansin dose ($P < 0.05$). Similarly, compared with EFE alone, synergistic increases in release of total reducing sugar (42.5

vs 35.3 mg/ g $P = 0.04$) and cellobiose (1.74 vs 1.29 mg/mL $P = 0.05$) were detected. In conclusion, the combination of EFE and expansins synergistically increased NDF hydrolysis and DM and digestibility of bermudagrass silage.

Key Words: digestibility, fibrolytic enzymes, expansins

349 The effect of fibrolytic enzymes on lactation performance, feeding behavior, and digestibility in high-producing dairy cows fed a barley silage-based diet. B. Refat*[1], D. A. Christensen[1], J. J. McKinnon[1], A. D. Beattie[2], T. McAllister[3], W. Yang[3], O. AlZahal[4], and P. Yu[1], [1]*Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada,* [2]*Crop Development Center, Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada,* [3]*Lethbridge Research and Development Centre, Lethbridge, AB, Canada,* [4]*AB Vista, Marlborough, United Kingdom.*

The objectives of this study were to evaluate the effects of pre-treating dairy cow rations with a fermentation extract derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase; AB Vista, UK) on lactation performance, digestibility, and feeding behavior of dairy cows fed a barley silage-based diet. Before starting the in vivo experiment, in vitro incubations of barley silage (using a batch culture technique) were conducted to determine whether the addition of FETR would have a response on these animal performance characteristics when applied to a barley silage-based diet for dairy cows. The in vivo experiment was performed using 8 Holstein dairy cows. Cows were assigned randomly to one of 4 treatments: 0, 0.5, 0.75, and 1 mL of FETR/kg DM of diet in a replicated Latin square design. The pre-treatment was applied to the complete diet during the mixing process. The experimental period lasted for 22 d with each experimental period comprising 16-d adaptation period and 6-d sampling period. Feeding time and daily feed intake of each individual cow was monitored using Insentec feed bins. Feeding behavior characteristics were assessed in this study in the last 6 d of each experimental period using feed bin attendance data. Data were analyzed using the mixed procedure of SAS. The application of FETR linearly ($P = 0.02$) increased in vitro DM digestibility and tended to improve in vitro NDF digestibility in barley silage. The diet supplemented with an intermediate dosage level of FETR (0.75 mL FETR/ kg of TMR) has exhibited a higher milk fat (1.2 vs. 1.4 kg/cow/day), FCM (38.9 vs. 36.4 kg/cow/day) and ECM (38.9 vs. 36.8 kg/ cow/day) compared with control. The milk protein percentage linearly ($P < 0.05$) improved in response to FETR. Increasing FETR levels resulted in a quadratic effect ($P < 0.05$) on feed efficiency. There was no effect ($P > 0.10$) of FETR level on feeding behavior. In conclusion, pre-treating dairy cow barley silage-based diet with FETR increased the feed efficiency without affecting DMI. The positive effect of adding FETR could benefit the dairy industry in Western Canada where barley silage-based diets are common.

Key Words: feeding efficiency, fiber digestibility, fibrolytic enzyme

350 Feeding a *Saccharomyces cerevisiae* fermentation product during the periparturient period may decrease inflammation of dairy cows. C. E. Knoblock*[1], W. Shi[1], I. Yoon[2], and M. Oba[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Diamond V, Cedar Rapids, IA.*

The objective of this study was to measure indicators of oxidative stress and serum concentrations of acute phase proteins of dairy cows (n = 38) fed a *Saccharomyces cerevisiae* fermentation product (SCFP, NutriTek, Diamond V, Cedar Rapids, IA) during the calving transition period. Four weeks before calving cows were fed a common close up diet consisting of 13% starch with or without SCFP. For 3 weeks after calving, cows were fed high or low starch (HS vs. LS; 27 and 21%, respectively) diets with (SCFP) or without SCFP (CON). Animals were assigned to one of 4 treatments (CON+HS, CON+LS, SCFP+HS, SCFP+LS) balanced for BCS and parity in a randomized block design. Data were analyzed with the Fit Model procedure of JMP. There was no interaction between starch content and SCFP treatments. The dietary starch content did not affect serum concentrations of haptoglobin (Hp) and serum-amyloid A (SAA), or plasma concentrations of total-antioxidant capacity (TAOC), malondialdehyde (MDA), and reactive oxygen metabolites, but SCFP feeding decreased serum Hp concentration on d 7 after calving compared with CON (0.26 vs. 0.62 mg/mL; $P = 0.03$) indicating reduced post-calving inflammation. Serum-amyloid A did not differ between treatments although there was a positive relationship between Hp and SAA concentrations (r = 0.46; $P < 0.01$). Serum Hp concentration was positively correlated with plasma concentrations of free fatty acids (r = 0.14; $P = 0.05$) and β-hydroxybutyrate (r = 0.23; $P < 0.01$), but not to glucose (r = 0.02). Responses to SCFP in plasma concentrations of TAOC and MDA on d 21 after calving were affected by parity and the dietary starch content; feeding SCFP increased plasma MDA concentrations for primiparous cows fed the HS diets (20.8 vs. 15.2 μ*M*; $P = 0.03$), and decreased plasma TAOC concentration for multiparous cows fed the LS diets (0.57 vs. 1.05 m*M*; $P = 0.03$). These data suggest that supplementation of a SCFP during the periparturient period may reduce inflammation after calving, but may not alleviate oxidative stress of dairy cows.

Key Words: acute phase proteins, *Saccharomyces cerevisiae* fermentation product, inflammation

351 The effect of supplementation type and vitamin E level on milk production, milk composition and rumen health parameters of grazing dairy cows in late lactation. Z. C. McKay*, M. B. Lynch, F. J. Mulligan, G. Rajauria, S. Kirwan, and K. M. Pierce, *University College Dublin, Lyons Research Farm, Celbridge, Naas, Ireland.*

Grass based dairy production systems use concentrate supplementation primarily when there is a scarcity of grass.There is however, a paucity of information regarding the influence of supplement type on production and DMI, particularly in late lactation. Oat based supplements have shown improvements in milk yield and composition in early lactation, while Vitamin E (VE) plays a role in cell membrane structure and has potential to prevent deterioration of mammary cells. Therefore, the objective of this research was to investigate the effect of concentrate type and VE supplementation on milk yield, milk composition and rumen fermentation in late lactation dairy cows. Forty 8 Holstein Friesian dairy cows were blocked on days in milk (+185DIM) and balanced for parity, pre-experimental milk yield, milk composition and BCS. Cows were assigned to one of 4 dietary treatments in a randomized complete block design (n = 12). The dietary treatments (T) were: grass only (T1); grass + 2.6kg DM barley based concentrate + 350IU VE/kg (T2); grass + 2.6kg DM oat based concentrate + 350IU VE/kg (T3) and grass + 2.6kg DM oat + 1050IU VE/kg based concentrate (T4). The diets were fed for a 14-d acclimatization period and then for a further 49d. Concentrate supplementation increased milk yield and fat and protein kg (T2 20.74kg, 1.74kg/d; T3 20.56kg, 1.81kg/d; and T4 20.56kg, 1.77kg/d respectively; $P < 0.0001$) in comparison to pasture only (T1 17.78kg, 1.60kg/d). Cows offered T2 had lower milk fat (4.60%) concentration than T1 (5.00%,

$P < 0.0001$) and T3 (4.90%, $P = 0.001$). Rumen pH of cows offered T1 (6.70) was higher than T2 (6.55, $P < 0.0001$), T3 (6.50, $P < 0.0001$) and T4 (6.58, $P = 0.001$). T4 reduced rumen ammonia (5.28mmol/L, $P = 0.002$) in comparison to T1 (5.83mmol/L). In conclusion, concentrate supplementation increased milk yield and kgs of fat and protein. The oat based concentrate had a higher milk fat concentration in comparison to barley. Increasing the level of VE reduced rumen ammonia concentration but did not alter milk yield or milk composition parameters for cows fed oat based supplements.

Key Words: late lactation, oats, vitamin E

352 Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on liver and plasma biomarkers of metabolic function in transition dairy cattle. K. E. Olagaray*[1], S. E. Sivinski[1], L. K. Mamedova[1], B. A. Saylor[1], C. Koehn[1], J. A. Sauls[1], I. Yoon[2], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*Diamond V, Cedar Rapids, IA.*

Biomarkers of carbohydrate and lipid metabolism were evaluated in transition cows fed control (CON; n = 30) or SCFP (NutriTek, Diamond V, Cedar Rapids, IA; n = 34) TMR (45% NDF, 14% starch prepartum; 32% NDF, 19% starch postpartum) fed from −29 ± 5 to 42 d relative to calving. Blood samples were collected at the following time points relative to calving: d −28 to −24 (wk −4), d −14 to −10 (wk −2), d 3 to 7 (wk +1), d 12 to 16 (wk +2), and d 31 to 35 (wk +5). Plasma samples were analyzed for free fatty acids (FA), β-hydroxybutyrate (BHB), glucose, and insulin. Liver biopsies were taken once between d −19 and d −12 (wk −3) and at 14 DIM for analysis of liver triglyceride (TG) and cholesterol content and relative abundance of metabolic transcripts (*FGF21*, *CPT1a*, *cPEPCK*). Results were analyzed with repeated measures in a mixed model. Treatment did not affect plasma metabolites (FA, BHB, glucose, and insulin; all $P > 0.35$); mean FA concentrations (peak of 538 ± 42 μEq/L, wk 2) indicated a modest lipolytic rate. Relative mRNA abundance of *CPT1a* did not differ by treatment ($P = 0.19$); however, both *cPEPCK* and *FGF21* tended to be greater for SCFP ($P = 0.06$ and 0.08, respectively). Liver TG increased from wk −3 to +2 ($P < 0.001$) but did not differ by treatment ($P = 0.41$). Liver cholesterol, in contrast, decreased from wk −3 to +2 ($P < 0.001$) and wk +2 liver TG and cholesterol concentrations were negatively correlated ($R^2 = 0.18$; $P < 0.01$). Liver cholesterol content tended to be lesser in SCFP than CON ($P < 0.10$). Despite a lack of treatment effect for plasma metabolites and no differences in BCS and BW, subclinical ketosis incidence (38% vs. 12%, diagnosed by daily urine ketone analysis) and days of glucogenic treatment (1.7 vs. 0.4 ± 0.3 d) were greater in SCFP than CON (both $P \leq 0.02$). In conclusion, SCFP supplementation during the transition period did not alter plasma metabolites and only slightly affected cholesterol metabolism, but resulted in greater incidence of subclinical ketosis. Bioactive components of SCFP may have promoted ketogenesis in the absence of elevated NEFA supply.

Key Words: transition cow, lipid metabolism

354 The effects of adding exogenous amylases and proteases on ruminal *in vitro* dry matter and starch digestibility of dent corn grain. F. X. Amaro*, K. G. Arriola, D. Kim, T. Fernandes, M. C. N. Agarussi, V. P. Silva, A. P. Cervantes, Y. Jiang, L. F. Ferraretto, D. Vyas, and A. T. Adesogan, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective was to evaluate the effects of exogenous amylases and proteases on ruminal in vitro dry matter (IVDMD) and starch (IVSD) digestibility of dent corn grain. Two separate experiments were performed to evaluate 10 exogenous amylases (Experiment 1), and 3 exogenous proteases (Experiment 2) using an in vitro batch culture of buffered-rumen fluid. Dried ground (4-mm) dent corn was used as substrate (0.50 g per F57 bag). For Experiment 1, treatments were Control (no enzyme) and 10 different amylases (1LAT, 2AK, 3AC, 4Cs4, 5Trga, 6Afuga, 7Fvga, 8Star, 9Syn, and 10Tg). For Experiment 2, treatments were Control (no enzyme) and 3 different proteases (11P14L, 12P7L, and 13P30L). In each experiment, each enzyme was applied at 0.25, 0.50, 0.75, and 1.0 mg of enzyme/g of substrate DM. Samples were incubated for 7 h in quadruplicate per run in 3 independent runs. Data were analyzed for both experiments using the GLIMMIX procedure of SAS. Dose was used as fixed effect in the model while run was considered a random factor. In Experiment 1, enzymes 2AK, 3AC and 10Tg did not increase ($P > 0.10$) IVDMD and IVSD, whereas the lowest dose of enzymes 1LAT, 5Trga and 8Star increased ($P < 0.01$) IVDMD by 23, 47 and 62% and IVSD by 35, 41 and 58%, respectively, compared with the control. Enzymes 4CsC, 6Afuga, 7Fvga and 9Syn increased IVDMD and IVSD in a dose-dependent manner ($P < 0.01$) and some of the greatest increases in IVDMD (82.9%) and IVSD (85.9%) resulted from applying the highest dose of enzyme 6Afuga instead of the Control. In Experiment 2, the lowest dose of exogenous proteases, 11P14L and 12P7L increased ($P < 0.01$) IVDMD by 98 and 87% and IVSD by 57 and 64%, respectively, whereas the highest dose of 13P30L increased ($P = 0.02$) IVDMD by 44.8% and IVSD by 30%, relative to the control. Starch and DM disappearance were increased by exogenous amylase and protease addition. Future in vivo studies are required to validate these findings before these enzyme additives can be recommended for improving the digestibility of ruminant feeds.

Key Words: enzyme, starch digestibility

355 Effects of administering an oral probiotic drench containing *Megasphaera elsdenii* NCIMB 41125 to dairy cows fed a high starch diet in early lactation. M. R. O'Neil*[1], E. H. Branstad[1], C. S. McCarthy[1], B. C. Dooley[1], D. C. Beitz[1], A. J. Hund[1], and G. A. Ducharme[1], [1]*Iowa State University, Ames, IA,* [2]*MS Biotec, Wamego, KS.*

The objectives of this experiment were to evaluate the effect of a single oral drench of *Megasphaera elsdenii* NCIMB 41125 (Lactipro Advance, MS Biotec, Wamego KS) on milk production and feed intake of dairy cows upon an abrupt change to a high-starch diet immediately post-calving. Thirty-six multiparous Holstein cows were blocked by their predicted 305ME and assigned randomly to one of 2 treatments: (1) 100 mL drench of viable *M. elsdenii* (LACT; 2×10^8 cfu/mL) 72 h postpartum, and (2) a drench of 100 mL of autoclaved product as control (CON) administered at the same time as LACT. Both treatment groups were offered a fresh-cow diet formulated to contain 29.6% starch and 46.2% NFC (DM basis) via individual feeding gates. Daily milk production and feed intake as well as weekly BW, BCS, and milk components were recorded through 90 DIM. Data were analyzed using repeated measures in the MIXED procedure of SAS. On average, cows drenched with LACT consumed 6% less DM ($P = 0.04$) compared with control cows (23.5 vs 25.1 ± 0.51 kg/d, respectively). This decreased feed intake was not associated with differences in average milk production between LACT and CON (49.27 vs 48.26 ± 1.31 kg/d, respectively; $P = 0.57$). Consequently, cows drenched with LACT tended to produce milk with greater efficiency ($P = 0.07$); BW and BCS were similar for both treatments ($P \geq 0.36$; 668 ± 13 kg and 3.29 ± 0.04, respectively). Control cows tended to have increased ($P = 0.10$) concentration of milk fat relative to LACT cows. This trend resulted in similar ECM and FCM

production efficiency for both treatments ($P \geq 0.29$). Yield of milk fat and protein were similar ($P \geq 0.24$) for both treatments averaging 1.90 ± 0.06 kg/d and 1.47 ± 0.04 kg/d, respectively. These results demonstrate that a single, postpartum administration of a drench containing *Megasphaera elsdenii* NCIMB 41125 decreased DMI and tended to improve milk production efficiency in early-lactation cows while maintaining yield of milk and milk components.

Key Words: cow, probiotic, direct-fed microbial

Ruminant Nutrition Symposium: Interface of Environment and Nutrition—Targeted Nutrition to Overcome Heat Stress

356 Heat stress: Hypophagia and hypogalactia. Y. Xiao and B. J. Renquist*, *University of Arizona, School of Animal and Comparative Biomedical Sciences, Tucson, AZ.*

Heat decreases production and profitability of US animal agriculture by billions of dollars each year. The heat-induced decrease in feed intake (hypophagia) fully explains the depressed growth in heat exposed animals. Yet, it only explains 40% of the acute depression in milk production (hypogalactia). We hypothesized that a mouse model of heat exposure (35°C; 50% humidity) would recapitulate the heat-induced hypophagia, acute hypogalactia, and depressed mammary gland development observed in the dairy cow. We first established that heat robustly depressed feed intake relative to mice at 22°C (>50% reduction; $P < 0.05$). We next showed that heat exposure from d 5–11 of lactation (21-d lactation) depressed dam weight, litter mass gain, and milk production ($P < 0.05$). By pair feeding (equivalent to the *ad libitum* feed intake of heat stressed animals) during that same period, we showed that approximately 40% of the decrease in cumulative milk production and litter mass gain was independent of feed intake. Thus, the relative role of hypophagia in acute heat induced hypogalactia is similar in lactating mice and cows. In the cow, heat exposure during the dry period suppresses mammary gland development, decreasing mammary gland size and milk production throughout the entire lactation. We tested the effect of heat exposure on mouse mammary gland development, by heat exposing throughout the final 5 d of pregnancy (last 1/4 of parturition). Heat stress during this time decreased cumulative dam weight gain (21%; $P < 0.05$), feed intake (28%; $P < 0.05$), pup weight at parturition (14%; $P < 0.05$), and total mammary gland weight at parturition (21.5%; $P < 0.05$). Pair-feeding decreased cumulative dam weight gain (30%), pup weight at parturition (10%) and mammary gland weight (21.5%) relative to thermoneutral control mice ($P < 0.05$). Thus, the heat-induced decrease in mammary gland development is completely attributed to hypophagia. Together these studies propose that the mouse may serve as an ideal model of heat stress hypophagia and hypogalactia. Using the genetic tools unique to the mouse will expand our mechanistic understanding and encourage informed treatments to alleviate the production losses resulting from heat exposure.

Key Words: heat stress, feed intake, lactation

357 Nutritional strategies to overcome physiological adaptations to heat stress. A. G. Rius*, *University of Tennessee, Knoxville, TN.*

Heat stress triggers physiological and metabolic adaptations in lactating dairy cows reducing gut absorption and net portal flow of amino acids. Heat stress also impairs mammary blood flow, a local mechanism affecting milk yield, and milk protein synthesis. Limited flow of amino acids to the mammary gland partially accounts for changes in milk protein synthesis. Mounting evidence indicates that mammary clearance of amino acids increases to sustain milk protein synthesis as a local mechanism to adapt to reductions in plasma amino acid concentrations. Heat-stressed cows also elicit metabolic adaptations to support milk protein synthesis when metabolizable protein supply fails to meet requirements. Recent studies also demonstrate that milk protein synthesis in heat-stressed cows is maintained despite noticeable reductions in dietary rumen degradable and undegradable protein contents. Our research indicated that a 690 g reduction of metabolizable protein supply led to a 60 g reduction in milk protein yield; however, energy-corrected milk yield was not affected. Plasma analysis revealed that insulin concentrations declined by 45% and nonesterified fatty acids increased by 40% in association with lowering metabolizable protein supply in heat-stressed cows. Insulin responds to physiological state and nutritional status, and inhibits mobilization of fatty acids reducing fuel availability for oxidation and precursors for milk fat synthesis. Thus, reduction of insulin may partly mediate improvements in the utilization of amino acids and nonesterified fatty acids and provide substrate for milk synthesis in the heat-stressed cow. The insulin-signaling pathway may also be affected by changes in circulating insulin during heat-stress. The signaling pathway mammalian target of rapamycin is a promising candidate to study because it integrates physiological and nutritional signals to affect protein synthesis and nutrient utilization in the mammary cell. Future studies testing nutritional interventions on physiological adaptation to heat stress will continue to improve our understanding and will provide feeding strategies to improve cow productivity.

Key Words: heat stress, metabolizable protein, milk protein

358 The effects of progressive heat stress on muscle dysfunction. J. T. Selsby*[1], S. Ganesan[1], A. J. Brownstein[1], O. Volodina[1], S. Pearce[1], N. K. Gabler[1], R. P. Rhoads[2], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Heat stress negatively affects agricultural productivity, including milk production, growth, and reproduction. It also jeopardizes human and animal health, regionalizes food production, and compromises food security. Despite the broad, negative consequences of heat stress, interventions are largely limited to cooling, which fails to address underlying cellular dysfunctions. Our investigations have focused on heat stress-mediated changes to skeletal muscle in swine as this is an important agricultural tissue and because of its mass and energetic demands, it contributes greatly to regulation of systemic metabolism. We discovered oxidative stress and apoptotic signaling after only 2 h of heat stress, however, this appeared to be transient as it decreased linearly through 6 h despite persistent stress; a scenario suggesting activation of a corrective mechanism. Of interest, this transient dysfunction was associated with markers of increased autophagy and mitophagy, which would facilitate removal of damaged, pro-oxidant mitochondria. As heating continued through 12 and 24 h there was a reemergence of oxidative stress that corresponded with autophagic dysfunction and accumulation of autophagosomes. Indeed, following 24 h of heat stress we discovered increased markers of mitochondrial abundance, independent of biogenic signaling, which supports a failure of adequate removal. Similar changes were noted through 7 d of heat stress. Given these findings, mitochondrial injury and autophagic dysregulation appear to be key mediators of hyperthermic muscle dysfunction. Our future work will address the role of mito-protection and stimulation of autophagy during heat stress.

Key Words: mitochondria, autophagy, hyperthermia

359 Practical considerations for feeding cows under heat stress. D. Diaz*, *University of Arizona, Tucson, AZ.*

Heat stress is a major cause of production losses in the dairy industry with some estimates indicating that it could be costing dairy producers globally into the billions of dollars. In commercial operations, prevention of physiological responses to heat stress is far more effective than mitigation post exposure. Because these responses are a result of a confluence of factors, the approach to mitigation is often complex in nature. Dietary adjustments can play an important role in heat stress management. Their primary role is to first prevent, delay or minimize intake reduction and second to compensate for higher nutrient needs during these periods of high demands (e.g., mineral losses, oxidative stress). Lastly, maintaining intestinal health during high THI periods can also play an important role in prevention or delaying of heat stress related production losses. Therefore, nutritional adjustments can be utilized strategically during these periods to reduce the impact of heat stress. Supplementation with feed additives that target affected system could offer beneficial returns on investments for dairymen and nutritionist. The following review will attempt to discuss the field tools utilized by dairy producers and their nutritionist to cope with the complex responses associated with heat stress. We will discuss the link between management tools and nutrition and explore feed additives and their potential roles in heat stress management at the farm. Lastly, we will discuss the limitation of some heat stress experimental models and discuss why some field research observations could be beneficial in the interpretation of effectiveness intervention strategies.

360 Nutritional and metabolic strategies to improve reproductive performance during heat stress. M. L. Rhoads*, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Impaired fertility during periods of heat stress is the culmination of several physiological responses to heat stress, ranging from reduced estrus expression and altered follicular function to early embryonic death. Furthermore, heat-stressed dairy cattle exhibit a unique metabolic status that likely contributes to the observed reduction in fertility. An understanding of this unique metabolic status can be used as a basis for improving cow management and feeding strategies, thereby reducing the negative effects of heat stress on reproduction. One potential opportunity for improved management during heat stress requires investigation of altered insulin and glucose dynamics and their effects on fertility. During heat stress, adipose responsiveness to an adrenergic stimulus is blunted, indicating that cows experiencing a thermal load reprioritize fuel selection in favor of glucose. Thus, heat-stressed dairy cattle have higher circulating concentrations of insulin while circulating glucose is lower (compared with pair-fed counterparts). Despite some reported positive effects on aspects of reproductive performance, insulin damages oocyte quality, even in the absence of heat stress. Altered mitochondrial function, organelle distribution and cytoskeletal components have been described in oocytes exposed to elevated insulin and purportedly induce metabolic stress at the molecular level. These changes are particularly concerning because if fertilization does occur, the resulting embryo is reliant on the organelles and cytoplasmic machinery contributed by the oocyte. For these reasons, it is likely that insulin-mediated damage to oocyte quality is manifested in both the immediate reduction in fertility and the characteristic delay in return to full fertility following a heat-stress event. Previous studies indicate that dietary interventions could alleviate insulin-mediated damage to the oocyte, thereby improving the fertility of dairy cattle during and after a heat stress event.

Key Words: oocyte, insulin, heat stress

ADSA Southern Branch Symposium: Sustaining the Southern Dairy Industry: University Research, Teaching, and Extension Outlook

361 Dairy extension programs in the southern region: Finding novel ways to meet the needs of our producers. J. K. Bernard*, *University of Georgia, Tifton, GA.*

The dairy industry in the southern United States continues to change resulting in fewer, but larger, more intensely managed herds. Today most producers employ consultants (e.g., agronomic, nutrition, reproduction and health, farm business, facilities design) to help management and improve production and return on investment. Also, agribusinesses that serve the dairy industry provide technical support to large producers as they compete with other companies to maintain market share. Most universities have undergone budget cuts necessitating changing how dairy extension programs within each state are operated. Today most university dairy extension specialists have split appointments with research and/or teaching responsibilities, which limits their time for traditional farm visits or county meetings. Specialist must continually work to identify county agent and producer needs that are not being met and develop relative programs and resources. While traditional activities such as conferences, field days, and youth activities are still viable in many areas, specialist must continue to look for new ways to communicate information to producers who are becoming more technologically savvy. In addition to publishing information in newsletters, popular press publications are constantly looking for timely information. The use of blogs and up-to date websites to provide information has increased, but these must be easy to access and utilize so producers are comfortable using them. Decision tools for evaluating different technologies, management practices, etc. are needed as producers work to maintain profitability during periods of low milk prices. Basic training materials and videos for producers to use training new or existing employees are a viable tool. While not a part of most traditional dairy extension programs, it is essential to partner with industry and dairy organization to provide basic information for consumers who have questions about milk production. These activities should be coordinated among the various universities to take advantage of specialist expertise and avoid duplication of effort. As the industry continues to change, dairy extension programing must evolve to meet the needs within the industry as well as those of the consumer.

Key Words: extension, southern region

362 Dairy teaching programs in the southern region: Keeping dairy science students relevant for tomorrow's industry. C. C. Williams*, *Louisiana State University, Baton Rouge, LA.*

The number of dairy science degree programs in the United States has decreased in recent years. Of the 9 universities with dairy science degree programs, only 2 are from the southern United States. While many animal science programs offer courses in dairy science, these courses are not always enough to provide the level of education and experience needed to prepare students for careers in the dairy industry. The dairy teaching programs of today must continue to evolve with the changing demographics of the student population. More students are entering college with little to no dairy experience, yet these talented individuals are passionate about agriculture and possible careers in the dairy industry. Students should be encouraged to seek opportunities beyond the traditional classroom experience. Participation in undergraduate research, internships, national student exchanges, and international programs will allow students to expand their knowledge base and experience beyond the limitations of the traditional university degree. The North American Intercollegiate Dairy Challenge and American Dairy Science Association annual meetings will also provide educational experiences as well as opportunities for networking with potential employers and graduate schools. As dairy science education continues to evolve, non-traditional educational opportunities will better prepare students for careers in the dairy industry and become the industry leaders of the future.

Key Words: education

363 Dairy research programs in the southern region: Importance of multi-institution and industry collaboration in moving the dairy industry forward. M. A. Ballou*, *Texas Tech University, Lubbock, TX.*

Dairy science research will be required to address complex issues of producing more milk and milk products with fewer resources while maintaining the stewardship of the natural resources. Unfortunately, research funding for agriculture are far less when compared with other federal research programs and are not keeping pace with investments made by emerging countries. The expectation is that the agricultural private sector will make up the difference in research funding; however, advancements made by the industry depend significantly on government investments in research. Further, the grand challenges facing the dairy industry will require a trans-disciplinary approach, which will require many partners and bringing scientists together from diverse backgrounds. The USDA has a unique but effective system of funding agricultural research, and the funding streams broadly classify into: (1) USDA Agricultural Research Service, (2) capacity-building funds through land-grant institutions, (3) and competitive grants programs administered by the National Institute of Food and Agriculture. A recent report evaluated the effectiveness of capacity-building funding through USDA and one of the main advantages identified was the financial leveraging of government funds, and that much of the leveraging was at the local level. A program that was authorized in the 2014 Farm Bill, the Foundation for Food and Agriculture Research, relies on leveraging financial support from non-federal sources, and maybe a model for future funding. Although, the US dairy industry competes in a global market many of the challenges faced by dairy producers are regional. Some repetition across dairy research programs is needed, but with contracted funding and improved technology in telecommunications, programs need to invest their resources more strategically. Institutions could prioritize financial support to develop core research areas and rely more on collaborations. Investing in both foundational and innovative dairy science research is essential to keep the American dairy industry competitive in this global economy.

Key Words: collaboration, funding, research

364 Understanding the association between hock and knee injuries on lameness in dairy cattle. A. M. Armstrong*, T. F. Duffield, D. B. Haley, and D. F. Kelton, *Department of Population Medicine, Guelph, ON, Canada.*

Injuries and lameness are both prevalent across the dairy industry, however, there is little research addressing the potential link between them, and whether one predisposes the other. The majority of lameness studies have focused on hoof lesions and attributed lameness to these lesions. Few studies have examined the proportion of lameness that can be attributed to injuries. The objective of this study is to determine the association between mild, moderate and severe hock and knee injuries with lameness in dairy cattle. This research is being conducted at the Livestock Research and Innovation Centre (LRIC), Elora, Canada from November 2017–2018. Working with the hoof trimmer at the LRIC, cows between 120 and 160 DIM, cows entering the lactating herd, and cows that are being examined as a follow-up to a previous lameness intervention, are being trimmed and scored for the presence of hoof lesions. Currently 136 cows have been enrolled in the study: 114 lactating cows, 5 dry cows, and 17 heifers. All cows at the LRIC are also being assessed weekly for lameness to identify and characterize hock and knee injuries. Hock injuries are being scored on a 4-point scale, 0–3; with 0 indicating no swelling and no hair loss; and 3 indicating major swelling (>2.5 cm), a bald area greater than the size of a 25 cent piece and a lesion on the bald area. Of the sampled cows, hock injury scores of 2 or greater were distributed as follows: 44% on right lateral, 15% on right point, 32% on left lateral, and 8% on left point. Lameness is being scored on a 5-point scale, with 1 being sound and 5 being severely lame. Twenty-six percent of cows had a lameness score of 3, 4% a score of 4, and 22% of cows have been treated for lameness. Lesions included digital dermatitis (3%), white line disease (2%), sole ulcers (1%), the remaining 16% were listed as treated with unknown causes. Based on preliminary analysis (Fisher's exact test), cows with hock injury scores of 2 or 3 were 3.82 (95% CI: 1.55–10.1) times more likely to be lame than cows with a hock injury score of 0 and 1. The projected implications are to provide the industry with information on the association between hock injuries and lameness and what can be done to decrease their prevalence.

Key Words: lameness, hock injury, welfare

365 Automatic classification of dairy cattle skin injury type and severity using machine-learning techniques. A. A. Boatswain Jacques[1], R. S. Knight[1], M. Leduc*[2,3], V. I. Adamchuk[1], and E. Vasseur[2], [1]*Bioresource Engineering Department, McGill University, Montreal, PQ, Canada,* [2]*Animal Science Department, McGill University, Montreal, PQ, Canada,* [3]*Valacta, Sainte-Anne-de-Bellevue, PQ, Canada.*

Dairy cattle raised in indoor housing are exposed to potential sources of skin injuries that include swelling, wounds and other visually detectable lesions. Unfortunately, current diagnosis relies on qualitative visual evaluation and requires a trained observer to conduct the assessment. Computer vision assessment can be adapted to screen and potential cattle injuries, allowing any evaluator with minimum injury detection experience to obtain rapid assessment of the severity of cow injury almost instantly. To implement and test such a system, a machine-learning tool was developed using a data set of 2,364 images of both injured and non-injured cows. Skin injuries corresponded to either broken hair (BH), complete hair loss (CHL), white or dry scabs (WDS), red and wet scabs (RWS) and open wounds (WO) and were present at 3 injury locations (knee, lateral calcanei, and lateral tarsal). The data set was split into 3 separate subsets each corresponding to one of the injury locations. Regions of interest (ROIs) were first extracted systematically from each image using a pattern recognition module and were then resized and normalized. ROIs were then passed to a convolutional neural network (CNN) classifier constructed for each injury type in each location. These image subsets were then split into a training set, a validation set, and a test set to allow CNN optimization and evaluation of system performance. The classification accuracy rates of the CNN show an average accuracy of 93.9, 68.1, 76.4, 74.6 and 98.5% accurately detected injuries for BH, CHL, WDS, RWS and WO respectively. A ZeroR classifier predicting the majority category class was used as a baseline, and average accuracy values for this model were 90.3, 61.4, 77.0, 72.2, and 98.7% for BH, CHL, WDS, RWS and WO respectively. The CNN classifier for BH, CHL, and RWS outperformed the ZeroR model and a model performing random class assignment. According to test results, this system can be used as a rapid cow injury screening tool. A more extensive data set is being processed to further increase CNN accuracy.

Key Words: skin injury, welfare assessment, machine learning

366 The impact of tiestall facilities on dairy welfare and the broader dairy industry. K. M. Morrill*[1], E. Yeiser-Stepp[5], J. Jonker[5], N. B. Cook[3], A. De Vries[2], J. E. Lombard[4], K. F. Gingrich[7], and S. Nolt[6], [1]*Cornell University, Ithaca, NY,* [2]*University of Florida, Gainsville, FL,* [3]*University of Wisconsin-Madison, Madison, WI,* [4]*APHIS-USDA, Fort Collins, CO,* [5]*National Milk Producers Federation, Arlington, VA,* [6]*Elizabethtown College, Elizabethtown, PA,* [7]*American Association of Bovine Practitioners, Ashland, OH.*

The roster of standard operating procedures and recommended practices on dairy farms is constantly evolving, shaped by new technology, science and practical experience. This evolution is increasingly driven by measurable animal welfare outcomes and societal pressures about what is acceptable by consumers. Consumer concerns and questions about management practices need to be addressed using science and ethics. Our objective is to discuss the concern of permanent tethering of lactating cattle, the demographic of dairy farms that utilize tie stall systems, best management practices (BMP) for tie stalls, how tie stalls compare with other housing options based upon animal welfare indicators as well as the economic and societal impact of transitioning away from tie stall facilities. In 2016, the dairy industry was presented with customer concern around tie stalls for lactating cattle and how they potentially limit freedom of movement. The National Dairy FARM Animal Care Program, administered by the National Milk Producers Federation, developed a task force to address the customer concern, as well as to develop BMP for tie stalls. A literature review was conducted to evaluate current tie stall design recommendations and reported rates of welfare indicators. Based on research available, tie stall facilities that implement BMP provide equal opportunities for sound welfare for lactating dairy cattle in comparison to those housed in other types of facilities. Reported disease incidence, mortality, BCS, hygiene and locomotion score of 1 are similar in tie stall and freestall systems. Tie stalls tend to have a greater percentage of cows with a hock and knee score (7.2 vs. 2.1%) and hygiene score of 3 as (17.3 vs 10%) compared with freestall facilities. More research is needed to evaluate the type and

age of facility, environmental control, lameness rates, disease risk, udder health, and welfare of cows that are housed in tie stalls and compare these values to those obtained in other housing systems. Results of this literature review will be used to enhance BMP in the next revision of the animal care standards for the FARM Program, which are scheduled to be implemented on dairy farms in the United States starting in 2020.

Key Words: welfare, tiestall, facilities

367 The effect of tie-rail placements on neck injuries and lying and rising ability of tiestall-housed dairy cows. J. St John*[1], J. Rushen[2], S. Adam[3], and E. Vasseur[1], [1]*McGill University, Animal Science, Montréal, QC, Canada,* [2]*University of British Columbia, Dairy Research and Education Center, Agassiz, BC, Canada,* [3]*Valacta, Sainte-Anne-de-Bellevue, QC, Canada.*

Many dairy cows are housed in tiestalls, but few experimental studies have investigated ways to improve cow comfort through tiestall design. Studies on commercial farms show that tie-rail placement affects lying and may be linked to neck injuries. Our objective was to develop new recommendations for tie-rail placement combining both vertical and horizontal positions. Four treatments were tested: 2 new tie-rail positions that follow the neck line of cows (N1, N2), current recommendation (CR), and the tie-rail position commonly found on farm (CF). Forty-eight cows blocked by number and stage of lactation, were divided between 2 start dates and randomly allocated to a treatment for 10 wk. Live injury scoring was performed weekly using a 4-point system; 0 being no injury and 4 being severely injured. Cows were recorded 1 d/wk by overhead cameras and 6 lying and rising events were evaluated per recording. Differences over time were analyzed using a mixed model with a Scheffé adjustment for multiple comparisons and a Dunnett adjustment to compare N1, N2, and CF to CR. CR (difference from wk 0: +0.9 ± 0.16) had an increase in proximal neck injuries compared with N2 (+0.1 ± 0.15; $P \leq 0.05$). N2 (+0.8 ± 0.16) and N1 (+0.5 ± 0.16) had an increase in medial neck injuries compared with the CR (−0.1 ± 0.18; $P \leq 0.05$). All treatments showed a decrease over time in average lying intention time (−5.9 s/event; $P \leq 0.05$), lying-down time (−1.1 s/event; $P \leq 0.05$), contact with stall (−32.5%; $P \leq 0.05$) and slipping (−9.4%; $P \leq 0.05$) during lying. All treatments showed a decrease over time in average backward movement on knees (−10.8%; $P \leq 0.05$) and contact with tie-rail (−14.3%; $P \leq 0.05$) during rising, and overall abnormal rising (−15.7%; $P \leq 0.05$). Results suggest that the injury location on the neck shifted based on tie-rail placement. For all tie-rail placements lying and rising ability improved over time, suggesting that cows adapted to their housing. All placements resulted in neck injury, leading to questions of whether the metal bar is the issue and alternatives such as a flexible bar or chain should be investigated further.

Key Words: tie-rail, comfort

368 Impact of hoof health on Holstein cow behavior. A. L. Adams Progar*, L. R. Dearmin, and A. R. Allen, *Washington State University, Pullman, WA.*

Digital dermatitis is linked to approximately 50% of lameness cases in dairy cattle. These hoof lesions may alter cow feeding and estrus behavior. This study investigated how different hoof lesion sizes and the healing status affect cow activity, rumination, and feeding behavior. Over 2 yr, the rear feet of Holstein cows (n = 219) were observed for digital dermatitis lesions during milking once a month. Each lesion was classified as either small (<0.6 cm), medium (0.6 > 1.27 cm), or large (>1.27 cm). Additionally, each lesion was categorized as active (A) or digressing (D), cows with swollen feet were noted. Cow ear temperature, activity, rumination, and eating behaviors were recorded hourly using CowManager. Only behavior data from the day of each hoof evaluation was used for data analysis. Lesion and behavior data were analyzed to compare differences between cows using PROC MIXED in SAS. The percent of time cows were inactive was not affected by hoof lesion size (30 ± 5%; $P = 0.58$) or healing status (30 ± 1%; $P = 0.77$). An interaction between ear temperature and hoof ailment was detected, in which, cows with lesions and high ear temperatures spent the least time (34 ± 2%) ruminating, and cows with at least one swollen rear foot and low ear temperatures spent the most time ruminating (44 ± 4% of time; $P = 0.03$). Hoof lesion size did not influence cow eating behavior (8 ± 1% of time; $P = 0.73$), but cows with at least one active lesion tended to spend less time eating (7 ± 1% of time) than cows without a lesion (9 ± 0.3%; $P = 0.10$). High activity was observed more frequently (D: 14 ± 0.6% vs. A: 11 ± 0.9%; $P = 0.008$) and ear temperature was higher (D: 23.9 ± 0.6°C vs. A: 20.8 ± 0.8°C; $P = 0.005$) in cows with digressing lesions than cows with active lesions. These results indicate that the size of hoof lesions may not impact cow behavior, but the healing status of lesions may influence cow eating and high activity behaviors. The relationship between ear temperature and hoof lesions should be further investigated. This information implies that heat detection systems, such as CowManager, provide behavioral data that can be used for the early detection of hoof ailments.

Key Words: cow behavior, digital dermatitis, hoof health

369 Effect of prepartum exercise on calving behavior and cortisol concentrations. R. A. Black*[1,2] and P. D. Krawczel[2], [1]*University of California, Cooperative Extension, Santa Rosa, CA,* [2]*University of Tennessee, Knoxville, TN.*

The objective was to assess the effect of exercise on behavior and cortisol concentrations around calving in dairy cows. Twenty-nine primiparous and 31 multiparous, pregnant, nonlactating Holstein (n = 58) and Jersey-Holstein crossbred (n = 2) dairy cows were assigned to control (n = 20), exercise (n = 20), or pasture (n = 20) treatments at dry-off using rolling enrollment. Cows were housed in a deep-bedded sand freestall barn at the University of Tennessee's Little River Animal and Environmental Unit (Walland, TN). Exercise cows walked indoors 5×/wk for 1.4 ± 0.1 h at 1.88 ± 0.58 km/h Pasture cows were moved to an outdoor paddock 5×/wk for 1.8 ± 0.3 h/d. Control cows remained in the pen. Cows moved to maternity pens on the day of projected calving or when cows displayed signs of calving (i.e., holding of tail, swollen vulva) and treatments were discontinued. Video data of calving was reviewed by one observer with continuous sampling for 3 visually observable periods: initial observation of amniotic sac to amniotic sac rupture (P1); amniotic sac rupture to initial observation of calf's feet (P2); initial observation of calf's feet to calf expulsion (P3). Assisted calvings were excluded. Blood samples were collected on d 0 and 3, relative to calving, to assess cortisol concentrations. Accelerometers were attached to the rear fetlock of cows 3 d before dry-off, removed 14 d postpartum, and summarized by day for the 7 d before and after calving into lying time (h/d), lying bout frequency (bouts/d), lying bout duration (min/bout), and steps (n/d). Data were analyzed using mixed linear models (SAS v9.4). P2 (40.1 ± 5.9 min) lasted longer than P1 (4.4 ± 7.1 min) and P3 (24.0 ± 5.6 min; $P < 0.01$), regardless of treatment. Cortisol concentrations were greater on the day of calving than d 3 (6.23 ± 0.47 vs. 2.68 ± 0.49 ng/mL, respectively; $P < 0.01$), but did not differ among treatments ($P = 0.86$). Control cows had shorter, more frequent lying bouts compared with pasture and exercise cows ($P \leq 0.04$), but lying time and steps did not differ ($P \geq 0.21$). Exercise during late

gestation may offset discomfort during calving without altering labor times or cortisol concentrations.

Key Words: calving, behavior, cortisol

370 Effects of oral administration of acetylsalicylic acid on physiological parameters and biomarkers of inflammation, pain and stress in organic dairy cows that experienced vulvar lesion at calving. A. A. Barragan*[1], S. Bas[2], L. M. Bauman[2], J. Lakritz[3], J. Velez[4], J. D. Rozo Gonzalez[4], G. M. Schuenemann[2], and R. J. Van Saun[1], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [3]*Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH,* [4]*Aurora Organic Farms, Boulder, CO.*

Calving is a natural process in dairy farms that allows cows to initiate lactation and provide the future replacements for the herd. During calving, cows can experience damage of the birth canal soft tissues, such as the vulva, which may impair cow performance and well-being. The objective of this study was to assess the effects of treatment with acetylsalicylic acid on physiological parameters and biomarkers of inflammation, pain and stress in dairy cows that experienced vulvar lesions after calving. Cows from 3 organic dairy herds (n = 563) were included in this study. Cows were blocked by parity and calving ease, and randomly assigned to 2 groups: 1) ASP (n = 278): within ~12 h after parturition cows received 4 treatments with acetylsalicylic acid (100 mg/kg; 2 boluses) every 12 h; or 2) PLC (n = 285): within ~12 h after parturition cows received 4 consecutive treatments with gelatin capsules containing water every 12 h. Vulvar lesion scores (VLS; NO = no lesion; MILD = swollen or vulvar laceration > 1 cm; SEVERE = swollen and vulvar laceration > 1 cm) was assessed at enrollment. Blood samples were collected (ASP = 76; PLC = 76) immediately before each treatment administration for assessment of haptoglobin (HP), substance P (SP) and cortisol. Similarly, rectal and vulvar temperatures were measured after blood collection. The data were analyzed using MIXED procedure of SAS as a randomized block design with repeated measures. A greater ($P < 0.05$) proportion of cows with SEVERE VLS (28.81%) experienced dystocia compared with cows with MILD (13.16%) and NO VLS (9.33%). The vulvar temperature was higher ($P < 0.0001$) in cows with SEVERE (36.96 ± 0.19°C) and MILD (36.08 ± 0.09°C) VLS compared with cows with NO VLS (35.72 ± 0.10°C). Overall, cows treated with ASP had lower ($P = 0.001$) concentration of HP. Furthermore, ASP cows with MILD VLS had lower concentration of SP compared with PLC cows with MILD VLS (ASP = 52.18 ± 1.58; PLC = 57.62 ± 1.66; $P = 0.018$). There was no difference in cortisol concentration between VLS groups. Results of this study suggest that treatment with aspirin may decrease inflammation and pain in cows with vulvar lesions.

Key Words: dairy cattle, vulvar lesion, acetylsalicylic acid

371 Physiological and behavior response of dairy calves disbudded with oral administration of an herbal tincture as a method to reduce pain and stress. H. N. Phillips* and B. J. Heins, *University of Minnesota West Central Research and Outreach Center, Morris, MN.*

The objective of this study was to investigate the effects of Dull It, an herbal tincture made with white willow bark, St. John's wort, chamomile, arnica, and fennel, on blood cortisol levels and calf behaviors during and after hot iron disbudding. Holstein and crossbred heifer calves that were 5 to 8 wk of age (n = 54) were housed in 6 pens of 9 calves. Calves were randomly assigned to 1 of 3 treatment groups: 1) Dull It (2 mL) under the tongue before disbudding with a second dose immediately following (D; n = 18), 2) Lidocaine (5 mL/ bud) cornual nerve block before disbudding (L; n = 19), or 3) Sham disbudded with a cold hot iron (S; n = 17). Calves were acclimated to handling and restraint 7 d before disbudding. Blood samples for serum cortisol analysis were taken 10 m before and 1, 30, 90, 210, and 450 min following disbudding. Calves were video recorded for frequencies of ears flicks, head jerks, and tail flicks during disbudding and to 6 h after disbudding. Fixed effects for statistical analysis of cortisol were baseline cortisol as a covariate, treatment, and the interaction of time and treatment, and time was a repeated measure using a spatial power covariance structure. For behavior, fixed effects were treatment and the interaction of treatment and time, with time as a repeated measure using a first-order autoregressive covariance structure, and pen was a random effect. At 30 and 90 min after disbudding, the D (28.2 ± 0.28 and 8.4 ± 0.09 ng/mL, respectively) calves had greater ($P < 0.01$) cortisol than S (9.3 ± 0.09 and 3.3 ± 0.04 ng/mL, respectively) calves. The D and L calves had similar ($P = 0.93$) cortisol across all time points after disbudding. Calves had similar ($P > 0.13$) behavior frequencies at disbudding. The L (11.1 ± 0.16) calves had greater ($P < 0.01$) head jerks 1 to 2 h after disbudding compared with D (2.8 ± 0.16) calves. The D and L calves had similar ($P = 0.57$) ear flicks at all time points. The D and L calves had greater ($P < 0.05$) cortisol and pain behaviors after disbudding compared with S calves. In summary, Dull It may be an effective alternative to lidocaine to mitigate pain and stress during and after hot iron disbudding in dairy calves.

Key Words: organic, disbudding, behavior

372 Outcome based welfare measures of high producing Holstein freestall housed dairy cattle across regional benchmarks in the USA. K. M. Luchterhand*, L. Collings, C. Cook, K. Ito, J. Kelsey, M. Mouw, and K. Wood, *Novus International Inc., St. Charles, MO.*

The objective of this observational study was to describe animal welfare in freestall housed cattle by creating regional benchmarks across the USA. The data were collected from 535 commercial freestall dairy farms from November 2012 to July 2017. Herd size averaged 1,434 (range 105–13,000) lactating cows. The high production Holstein pen was used and benchmarks were created by geographical region: West (WE; n = 87), Midwest (MW; n = 112), Northeast (NE; n = 277), Pacific Northwest (PNW; n = 42) and Southeast (SE; n = 17). Prevalence of clinical lameness was determined by locomotion scoring (LS; 1 = normal, 5 = severely lame; LS ≥ 3 considered lame) were (lsmean ± SE): 24.5 ± 1.5, 29.7 ± 1.3, 30.6 ± 0.8, 24.1 ± 2.0, and 27.38 ± 3.0 for WE, MW, NE, PNW and SE, respectively. Both WE and PNW regions had lower lameness prevalence than MW ($P = 0.01$, $P = 0.02$, respectively) and NE ($P < 0.01$, $P < 0.01$, respectively), but were not different from SE. No differences in lameness prevalence among MW, NE and SE. Hock injury prevalence (hair loss and/or swollen) were: 22.6 ± 2.6, 29.1 ± 2.4, 32.1 ± 2.3, 19.8 ± 3.0 and 23.1 ± 3.9 for WE, MW, NE, PNW and SE, respectively. Both WE and PNW had fewer hock injuries than MW ($P < 0.01$, $P < 0.01$, respectively), NE ($P < 0.01$, $P < 0.01$, respectively), but did not differ from SE. Northeast had more hock injuries than the SE ($P = 0.01$), but were not different from MW. Knee injury prevalence were 3.8 ± 0.5, 5.1 ± 0.5, 4.7 ± 0.4, 5.1 ± 0.6 and 3.9 ± 1.0 for WE, MW, NE, PNW, and SE, respectively. West had fewer knee injuries than MW ($P < 0.01$), NE ($P = 0.04$), PNW ($P = 0.02$) and were not different from SE. No differences in knee injury prevalence were observed among MW, NE, PNW and SE. Daily lying times (h/d) were 10.14 ± 0.12 for WE, 11.35 ± 0.10 for MW, 11.06 ± 0.06 for NE, 10.05 ± 0.16 for PNW, and

10.00 ± 0.26 for SE. Lying times in the MW were longer than WE ($P < 0.01$), NE ($P = 0.02$), PNW ($P < 0.01$), SE ($P < 0.01$). Northeast lying times were longer than WE ($P < 0.01$), PNW ($P < 0.01$) and SE ($P < 0.01$). There were no differences in lying time among WE, PNW and SE. Based on these results WE and PNW have better cow comfort than other regions in the USA.

Key Words: freestall, welfare, lameness

373 Remote assessment of herd-level welfare status based on indicators from routinely collected milking records. D. Warner[1,2], E. Vasseur*[2], S. Adam[1], M. Villettaz Robichaud[3], D. Pellerin[3], D. Lefebvre[1], and R. Lacroix[1], [1]*Valacta, Dairy Production Centre of Expertise Quebec-Atlantic, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*McGill University, Department of Animal Science, Sainte-Anne-de-Bellevue, QC, Canada,* [3]*Université Laval, Département des sciences animales, Québec, QC, Canada.*

A welfare assessment tool was developed with the aim to remotely assess welfare status of dairy herds. This tool will help producers benchmark their own performance relative to others, adopt tailored strategies, and monitor whether improvements are effective. The welfare assessment tool was based on 14 indicators, pre-recorded through routinely collected milking records and herd data. Indicators were selected based on advice of dairy experts covering longevity, nutrition, production, health, reproduction, and young stock. All values were normalized and percentile ranks were calculated. Ranks were averaged and agglomerated to a composite herd welfare index (HWI). The percentile rank of each indicator allows us to evaluate herd strengths and weaknesses, and the HWI situates the herd within the population of herds. Based on this approach, a HWI was calculated for 4463 dairy herds in Quebec, Canada. In addition, animal-based measures of herd welfare status (prevalence of lameness and lesions) were collected on 86 tie and free stall herds during summer 2017 to validate the selected indicators and the HWI. Pearson correlation coefficients between the HWI and animal-based measures were −0.41 (lameness), −0.41 (hock lesions), and −0.60 (knee lesions) for tie stall herds (n = 64; $P < 0.05$) but were not significant ($P > 0.05$) for freestall herds (n = 22). Based on the HWI, 5 tie stall herds among the 86 herds were situated within the worst 10% of the entire population of herds in Quebec, and showed deficiencies (among worst 25%) in knee lesions (4 herds), hock lesions (3 herds) and lameness (2 herds). In addition, 5 tie stall herds were within the best 10% in Quebec and excelled (best 25%) in lameness (4 herds), and hock and knee lesions (3 herds, respectively). The welfare assessment tool described in this study allows remote assessment and monitoring of the welfare status of dairy herds based on pre-recorded data and can be a powerful tool for producers to evaluate their herd relative to peers and to highlight opportunities for improvements.

Key Words: dairy herd improvement, national dairy database, cow welfare

375 Spatial heterogeneity and interactions of microbiota in raw milk and teat skin of dairy cows. H. Yan[1], S. Ji[1], C. Guo[1,2], J. Mao[1], Y. Du[1], F. Wang*[1], Y. Wang[1], Z. Cao[1], W. Du[1], and S. Li[1], [1]*State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, China.*

Microbiota in raw milk and teat skin played critical roles in human food quality and health of cows, yet knowledge of microbiota profiles and interactions in raw milk and teat skin was still limited. Here, we investigated the composition, diversity and co-occurrence network of bacterial community in raw milk and teat skin, respectively, as well as the bacterial interactions in these 2 adjacent spatial locations with 469 samples. We observed that raw milk and teat skin harbored distinguishable bacterial communities; however, bacterial interactions in these 2 adjacent spatial locations were also detected. With Source tracker pipeline (version 1.0.1) based on Bayesian algorithm, we were able to demonstrate that 92.1% of bacteria in raw milk were transferred from teat skin, while 63.6% of bacteria in teat skin were transferred from raw milk. Moreover, bacterial community in teat skin trended to be more similar with that in raw milk during milking, which suggests that milking period might be the window of bacteria transfer between raw milk and teat skin. These findings may expand our understanding of bacterial community in raw milk and teat skin, and highlighted that bacterial community in teat skin played comprehensive roles on that in raw milk.

Key Words: Source tracker, co-occurrence network, raw milk and teat skin microbiota

376 High-concentrate feeding and supplementation of a clay-mineral based mix modifies plasma metabolome in dairy cows. N. Reisinger*[1], E. Humer[2], I. Kröger[2], V. Neubauer[2], and Q. Zebeli[2], [1]*Biomin Research Center, Tulln, Austria,* [2]*Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria.*

Feeding high-concentrate diets to dairy cows increases the risk of rumen fermentation disorders, such as subacute ruminal acidosis (SARA). SARA might induce perturbations in the systemic metabolism. Clay minerals have shown the potential to counteract negative effects of high-concentrate feeding. This study investigated if supplementing concentrate-rich diets with a clay-mineral based mix (CM) modulates the plasma metabolome in dairy cows experiencing SARA. Eight ruminally cannulated nonlactating Holstein cows were arranged according to a change-over design, receiving either 50 g of CM per day or no additive (CON) (n = 8 per treatment). The CM was mixed and fed with the respective concentrates. Cows were first fed with a pure forage diet (Baseline), followed by 2-intermittent 65%-concentrate-diet phases (SARA 1, SARA 2; 1 and 2 weeks duration, respectively). SARA 1 and 2 were separated by a 1-week recovery, in which the pure forage diet was fed. During the Baseline and SARA 1 blood samples were collected and a targeted ESI-LC-MS/MS-based metabolomics approach was carried out. Statistical analysis was performed using the MIXED procedure of SAS, including the feeding phase and the diets (CON, CM), as well as a possible interaction between them, as fixed effects and run and cow as random effects. SARA caused a decrease in several amino acids, lysophosphatidylcholines, phosphatidylcholines, and sphingomyelins ($P < 0.05$). Cows receiving CM had higher concentrations of several amino acids (i.e., glycine, isoleucine, lysine, methionine, and threonine; $P \leq 0.01$) and phosphatidylcholines with diacyl-residues ($P \leq 0.10$) than CON-cows. In addition, lower concentrations of histamine and spermine were detected ($P < 0.01$). Data suggests that the induction of SARA caused strong changes in the plasma metabolome. Furthermore, CM may reduce negative effects of high-concentrate diets by counteracting SARA-induced perturbations in the systemic metabolism.

Key Words: SARA, metabolomics, clay minerals

377 Observational longitudinal study of feed additives as risk factors for herd diarrhea incidents on US dairy farms. G. Poppy*[1,2] and P. Morley[2], [1]*Colorado State University, Fort Collins, CO,* [2]*Fermented Nutrition, Luxemburg, WI.*

As measures are enacted to reduce antimicrobial use in agriculture, finding management, feeding practices, and feedstuffs that could decrease diarrhea on dairy farms would be of benefit to animal and human health. A longitudinal observational study was conducted to assess the incident rate of undifferentiated diarrhea events by month on the farm and study the impact of exposure to different direct fed microbial dietary additives, to assess the effect of different common farm management practices, and farm design to affect the incidence rate of diarrheal disease. The study design was stratified by region, to target 150 farms in 10 different regions that corresponded to somewhat distinct management and environmental examples. A cross sectional survey instrument was administered to collect data relating to disease incident risk factors. Only 76 farms completed both the surveys and supplied electronic backups that provided information on incidents of diarrhea recorded in the herd records system. Sixty-nine farms either did not provide electronic dairy records with the survey, or there was no evidence they electronically recorded diarrhea events. The remaining 76 herds were analyzed for risk factors for incident of diarrhea using Proc Glimmix in SAS (version 9.4, SAS Institute., Cary, NC), both as a Poisson distribution and as a negative binomial distribution. The final multivariable negative binomial included a yeast culture fermentation product of *Saccharomyces cerevisiae,* (YC) whether the farm vaccinated for BVD, and whether the herd was located in the Eastern or Western US. Feeding YC had an Incident Risk Ratio (*IR*) of 0.707 ($P = 0.043$, CI = 0.505, 0.989). Having a herd in the eastern US was associated with an increased *IR* 2.036 ($P = 0.066$, CI = 0.953, 4.39). Vaccinating the herd with a BVD vaccine was associated with a decreased *IR* of 0.213 ($P = 0.186$, CI = 0.022, 2.111). The study shows there is a small association with feeding a yeast culture based additive and lower incidence of diarrhea on farms. This association although small, (a decrease of 2 cases per 1000 cows per month), indicates that further research should be conducted in this area.

Key Words: undifferentiated diarrhea, *Saccharomyces cerevisiae,* longitudinal observational study

378 Metabolic changes in Simmental and Holstein cows after Pegbovigrastim injections during the periparturient period. V. Lopreiato*[1], E. Trevisi[2], D. Britti[1], V. M. Morittu[1], J. J. Loor[3], and A. Minuti[2], [1]*Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro, Italy,* [2]*Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [3]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL.*

Pegbovigrastim is a commercial long-acting analog of bovine granulocyte colony-stimulating factor (Imrestor, Elanco Animal Health, Greenfield, IN) that promotes increase count and functionality of neutrophils in dairy cows around parturition. However, the response may influence the inflammo-metabolic status of the cow during the periparturient period. A total of 54 cows from 2 commercial farms (Holstein and Simmental purebred, respectively) were randomly allocated in a 2 × 2 × 2 factorial design to assess the effect of treatment [pegbovigrastim (PEG) or saline (CTR) injection], breed, parity (primiparous and multiparous), and their interactions on blood inflammo-metabolic profile. The PEG or CTR treatments were administered approximately 7 d before (1st) and within 24 h from calving (2nd). Blood samples were collected at −21, −7 (before injection), 1, 3, and 28 d relative to calving (DRC). A MIXED model with repeated measures was fitted to the normalized data using Proc MIXED of SAS (ver. 9.3). A significant effect of treatment, interaction breed × treatment and breed × treatment × DRC were observed for total protein. Simmental cows that received PEG injections showed higher plasma protein concentration at d 1 and 3 after calving compared with CTR cows, while no differences were detected between PEG and CTR Holstein cows. Albumin was also greater in PEG Simmental cows at d 1 compared with control (breed × treatment × DRC effect). Blood concentrations of free fatty acids and bilirubin were lower in Simmental PEG cows at d 3 compared with CTR cows. However, both breeds had similar values between −21 d and 1 d. On the other hand, PEG cows had higher levels of gamma-glutamyl transferase (GGT) at d 1 and 3, and a tendency at d 28, and higher alkaline phosphatase (ALP) concentration at d 3, mainly due to PEG Holstein cows. Overall, pegbovigrastim injection treatment led to better liver functionality in Simmental cows after parturition and better biliary system (i.e., increased GGT and ALP) in both breeds. However, no changes in the post calving acute phase reaction were observed.

Key Words: immunometabolic profile, pegbovigrastim

379 Dynamics of rumination, activity, and milk yield around hoof trimming. S. Paudyal*[1], F. Maunsell[2], and P. Pinedo[1], [1]*Colorado State University, Fort Collins, CO,* [2]*University of Florida, Gainesville, FL.*

Preventive and therapeutic hoof trimming in dairy cattle is associated with stress and may result in temporary alterations in behavior and production. Our objective was to characterize the dynamics of rumination time (RT), activity (ACT), and milk yield (MY) in animals submitted for hoof trimming (HT) during early lactation. A total of 198 multiparous Holstein cows from the University of Florida Dairy Unit, were enrolled and monitored until 60 d in milk for daily RT (min), ACT (min), and MY (kg). Cows submitted for HT were differentiated as receiving corrective interventions (TRM) or as being diagnosed with a lameness disorder (LAM). Cows not submitted to HT were considered controls (CON). A rolling index was created for each parameter, based on the deviation from a specific day (d 0), relative to the average reading for the previous 3 to 5 d, standardized by this average (−3 to −5d). To determine the effect of HT, the index was calculated daily, considering d 0 at 5 different points (d of HT to d 4 post-treatment). Data were examined by repeated measures analysis, with the index value as outcome and the foot condition at HT as the independent variable, adjusting for DIM and parity. No significant differences were determined for RT index between groups. For TRM animals, the index on daily RT decreased at d 1 after HT to subsequently increase to reach the level of CON animals at d 4. In LAM animals, the RT index started to increase on the day of HT and surpassed the CON animals at d 3. The ACT index for TRM animals started to increase on the day of treatment and surpassed the CON animals on d 2 ($P = 0.002$). The ACT index for LAM animals followed a similar pattern; however, ACT indices were only different from the CON group on the day of treatment ($P = 0.02$). The ACT index surpassed the CON animals on d2 after treatment and remained similar to CON animals until d 4 post-treatment. Average MY index was greater in TRM animals than in CON animals on the day of HT ($P < 0.001$), whereas in the LAM animals, MY index was only significantly greater than in CON group at d 3 and d 4 after treatment. We conclude that the dynamics of RT, ACT, and MY around HT are specific and require different recovery times to reach normal levels.

Key Words: hoof trimming, rumination, activity

380 Efficacy of novel treatment options for digital dermatitis in organic dairy systems. S. Paudyal*[1], D. Manriquez[1], A. Velasquez[1], J. Shearer[3], P. Plummer[3], H. Bothe[2], J. Velez[2], and P. Pinedo[1], [1]*Colorado State University, Fort Collins, CO,* [2]*Aurora Organic Dairy, Boulder, CO,* [3]*Iowa State University, Ames, IA.*

Digital dermatitis (DD) is a major cause of lameness in dairy cows, resulting in pain and leading to reduced animal welfare and significant economic loss. Strict antibiotic regulations in organic dairy systems create the need for validation of non-antibiotic treatment options. Our objective was to evaluate the efficacy of the treatment of DD using different combinations of copper sulfate, honey, and iodine. Cows at early stages of DD (M1 = early stage, skin defect < 2 cm diameter; and M2 = acute active ulcerative lesion) were identified and enrolled. Study cows (n = 70) were randomly assigned to 1 of 3 topical treatment options: (1) Copper sulfate and iodine (CS-I); (2) honey and iodine (HO-I); and (3) nontreated controls (CON). A clean paper towel was impregnated with the corresponding formulation (10 mL) and applied topically to the lesion with a bandage and maintained for 3 d. Cows were subject to follow-up on d 3, d 12, and d 28 post-treatment to evaluate lesion stage and size, pain response, and lameness score. A subsample of 45 cows were monitored at d 120. All cows continued going through copper sulfate footbaths after milkings. Data were subject to repeated measures analysis for continuous and categorical data. Early erosive lesions (M1/M2) progressed into papillomatous mature forms (M4), irrespective of the treatment group. On d 3 and d 12, lesion size differed and was smaller for HO-I group ($P < 0.001$). Treatment effect changed in subsequent evaluations (d120), with smaller lesions for CS-I and CON groups vs. HO-I ($P < 0.001$). The interaction between time and treatment was significant for pain reaction ($P < 0.001$). The odds (95% CI) of pain for cows in the CON group at d 3 and at d 12 were 7.69 (1.70–3.33) and 4.00 (1.01–16.7) times the odds of pain for animals in CS-I group, respectively. However, at d 120 the odds of pain for cows in the HO-I group were 15.2 (1.60–147) times the odds of pain for animals in CON group. The odds of lameness score > 1 decreased until d 28 and again increased on d 120 ($P < 0.001$) for all the treatment groups, with the highest incidence in HO-I group. Non-antibiotic treatment options were effective in controlling DD early after treatment. The CS-I combination

was superior to both treatment with HO-I and no-treatment in CON group.

Key Words: digital dermatitis

381 Impact of inhibitors of choline product synthesis and signaling on the inflammatory response of innate and adaptive immune cells. M. Garcia*[1], M. Riley[1], L. K. Mamedova[1], B. Barton[2], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*Balchem Corporation, New Hampton, NY.*

Previous research suggested that one or more choline products altered the inflammatory response of bovine immune cells supplemented with free choline. The objective was to assess the impact of choline product inhibitors on the inflammatory response of monocytes and lymphocytes from cows in early lactation (3.8 ± 2.5 DIM, n = 5). Blood immune cells were isolated using density gradient media and were incubated at 37°C and 5% CO_2. Mononuclear cells were incubated for 2 h, then monocytes were separated from lymphocytes by adherence. Monocytes (15 min) and lymphocytes (30 min) were primed with chemicals reported to inhibit the activity of choline dehydrogenase (CDI), choline kinase (CKI), muscarinic acetylcholine receptors (MRI), nicotinic acetylcholine receptors (NRI), and control without inhibitor (CTL), and then supplemented with choline (0 or 10 μ*M*) for 1 h (monocytes) or 4 h (lymphocytes). Cells were treated with LPS (1 μg/mL, monocytes, 2 h) or concanavalin-A (5 μg/mL, lymphocytes, 48 h) to harvest spent media for cytokine measures. Another subset of monocytes (identical inhibitor and choline treatments) was treated or not with labeled *E. coli* for 40 min, followed by phagocytic and oxidative burst assays, and treated lymphocytes were challenged or not with concanavalin A for 48 h, followed by a 24-h proliferation assay. The data were transformed to attain normality and analyzed as a complete randomized design. Regardless of choline treatment, CDI markedly inhibited ($P < 0.01$) monocyte phagocytosis, oxidative burst, and TNF-α production and lymphocyte proliferation and IFN-γ production. Unlike with other inhibitors, inhibition of lymphocyte proliferation by CDI was reversed ($P < 0.01$) by activation with concanavalin A (~3 ×). Supplemental choline enhanced ($P < 0.01$) phagocytosis and oxidative burst of CTL- and CKI-treated monocytes but reduced that of MRI-treated monocytes. These findings confirm that choline affects the inflammatory response of innate and adaptive immune cells via its downstream products, contributing to the efforts to define or refine choline requirements in transition cows.

Key Words: choline, immune cells, transition cow

382 Long-term effects of clinical diseases postpartum on culling, production, and reproduction of dairy cows. M. R. Carvalho*, T. J. DeVries, B. McBride, and E. S. Ribeiro, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to evaluate the impact of clinical diseases in the early postpartum on culling, production and reproduction of dairy cows up to 305 d in milk (DIM). Detailed data regarding health, production, reproduction, and culling of 5,085 cows that calved in 2012 in a single herd were summarized. Of all cows, 1,534 cows had at least one clinical problem (i.e., metritis, mastitis, lameness, digestive, or respiratory problem) in the first 21 DIM (ClinDis21), which represented 65.7% of all cows presenting a clinical problem up to 305 DIM. Clinical cases diagnosed within 21 DIM represented 54.3% of all clinical cases occurring up to 305 DIM. The impact of ClinDis21 on dependent variables was then accessed. Statistical analyzes of binary and continuous dependent variables were performed using the GLIMMIX procedure of SAS fitting the correct data distribution. Survival analyses were performed using the PHREG procedure of SAS. The rate of culling was increased by ClinDis21 (adjusted hazard ratio [AHR]: 1.96, confidence interval [CI]: 1.75–2.18, $P < 0.01$) and resulted in a greater proportion of cows leaving the herd by 305 DIM (38.2 vs. 22.6%; $P < 0.01$). The effect of ClinDis21 on culling rate was observed ($P < 0.01$) even when cows that left the herd within 21 DIM were excluded. ClinDis21 did not affect ($P = 0.82$) time to first breeding, but reduced pregnancy rate (AHR: 0.82, CI: 0.77–0.89, $P < 0.01$) and the proportion of cows becoming pregnant by 305 DIM (85.1 vs 90.6%, $P < 0.01$). The effect of ClinDis21 on pregnancy rate was observed ($P < 0.01$) even when cows that became pregnant at the first breeding were excluded. Moreover, 305-d yields of milk, fat, and protein were all reduced ($P < 0.01$) by ClinDis21 (milk: 10,012 vs. 10,465 kg; fat: 359 vs. 378 kg; protein: 290 vs. 303 kg) and these differences could not be explained by the yields observed in the first 21 DIM. All effects of ClinDis21 described herein were augmented by the incidence of multiple diseases, characterizing additive effects. In conclusion, clinical diseases in the first 21 DIM have long-term effects on culling, reproduction and milk production of dairy cows.

Key Words: cow, health, performance

384 The effects of stocking density, heat stress, and combination on variations in cell-mediated and humoral immunity. A. R. Lee*[1], P. D. Krawczel[1], R. J. Grant[2], and G. M. Pighetti[1], [1]*University of Tennessee Knoxville, Knoxville, TN,* [2]*William H. Miner Agricultural Research Institute, Chazy, NY.*

Physiological adaptions to general stress may affect cell-mediated and humoral responses among dairy cows, with overstocking (OS) and heat stress (HS) serving as 2 common stressors. The objective was to evaluate the effects of OS, HS, and combined OS and HS (OSHS) on delayed type hypersensitivity (DTH) reactions and antibody generation to keyhole limpet hemocyanin (KLH). Intramuscular neck injections (1 mL) containing KLH (0.1 mg), Quil-A adjuvant (0.5 mg), heat killed *Candida albicans* (HKCA; strain CS5314, 2×10^6 cells) and sterile non-pyrogenic saline were administered on d 2 post-study initiation to 64 cows (parity and days in milk (DIM) ± SEM 1.7 ± 0.1 and 129 ± 8, respectively). To evaluate HS occurrence, cows' respiration rates (RR) were recorded at 16:00 ± 1 h 4×/weekly. Sera was collected at d 0 before administration and d 7 and 14 post-study initiation. Cell mediated immunity was assed via intradermal injections of 0.1 mL HKCA (d 7: 2×10^6 cells; d 14: 4×10^6 cells) or sterile non-pyrogenic saline in 6 neck spots. At 0, 24, 48, and 72 h post-HKCA administration, DTH was evaluated via calipers. A stimulation index (SI) was calculated as mean HKCA site over mean saline site. The MIXED procedure (SAS 9.4, Cary NC) evaluated the fixed effects of DIM, parity, temperature-humidity index, treatment, d, and time post-injection on SI, antibody generation, and all 2-way interactions. Manual backward elimination removed non-significant 2-way interactions, with all main effects remaining in the model, regardless of significance. Respiration rate was 15 to 16 breaths/min greater among HS and OSHS cows than non-HS cows ($P < 0.001$), indicating mild heat stress. The SI was greater at 24 h (1.15 ± 0.02) than at 0 h (1.02 ± 0.02, $P < 0.001$), and equal to 48 and 72 h post HKCA administration, but did not vary between treatments ($P = 0.26$). Antibody production was not affected by treatment, day, DIM, or parity ($P \geq 0.22$). Overstocking, HS, and OSHS did not limit immunity under low doses of antigen, suggesting greater environmental stress and antigen doses may be necessary to trigger response.

Key Words: overstocking, heat stress, immunity

\385 Molecular epidemiology of bovine anaplasmosis in Khyber Pakhtunkhwa Province of Pakistan. M. Ijaz*, S. H. Farooqi, A. I. Aqib, K. Hussain, and A. Khan, *University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.*

Anaplasmosis is endemic in Pakistan, and is economically one of the most important bovine diseases in Asia. It reduces livestock productivity and leads to high mortality especially in young animals. This study was aimed at identifying the potential risk factors responsible for the occurrence and spread of anaplasmosis in bovine. A total of 900 (cattle = 479, buffalo = 421) blood samples were collected irrespective of age and sex from 3 distinct temporal zones of Khyber Pakhtunkhhwa (KPK) province of Pakistan. Polymerase chain reaction (PCR) technique was used for the molecular detection of anaplasmosis in comparison with microscopy of stained blood smears to compare test sensitivity for its diagnosis. Data collected on a piloted questionnaire including 11 predicting variables which were analyzed using R-statistical software, and association between the dependent and independent variables was assessed using univaraible analysis. Automated and manual approaches were exercised, producing comparable models. Key risk factors identified in all the approaches included specie of the animal, breed of animal, sex of animal, tick infestation status, previous tick history, tick control status, and acaricides use (Odds ratio > 1). Also, 165 samples produced the 611bp DNA fragment specific for 16SrRNA gene of *Anaplasma* spp. The samples were confirmed for anaplasmosis through sequencing and BLAST queries. The findings of the current study are in line with the results of previous studies conducted in other countries identifying similar potential risk factors for anaplasmosis spread in bovine. Enhancing the protective measures for controlling the identified risk factors can reduce the spread of anaplasmosis in Pakistan.

Key Words: bovine PCR, spherical body protein-4, temporal zone

Breeding and Genetics III: Feed Efficiency, Crossbreeding, and Production

387 Progeny testing results in accurate genomic breeding values for feed efficiency in Holstein dairy sires. C. Heuer*, N. Deeb, C. Sun, D. Kendall, J. Moreno, and R. Vishwanath, *STgenetics, Navasota, TX.*

Feed efficiency is projected to be one of the most important traits in dairy cattle breeding for the upcoming decades. STgenetics has committed significant resources to establish a progeny testing scheme for sire evaluations in feed efficiency. More than 2,600 growing heifers from the genetic nucleus farm at STgenetics' Ohio Heifer Center went through feed efficiency trials so far. Heifers were not pregnant and between 200 and 400 d of age with an average of 304 d.The 70 d trials are taking place in pens of 50 heifers with 8 bunks per pen across 10 pens. Bodyweights were recorded on entry and exit and, on average, 3 additional times throughout the trial and ranged from 292 to 1087 pounds with an average (SD) of 650 (138) pounds. Average daily feed intake ranged from 11.48 to 64.40 pounds of fresh feed per day with an average (sd) of 41 (8.3). Average daily gain was estimated using a linear regression of weight on age and ranged from 0 to 7.6 pounds per day with an average (SD) of 2.2 (0.87). Residual feed intake was defined as the residuals from a linear model with daily feed intake as the response and fixed effects comprising average daily gain, metabolic body weight and age. A total of 2241 animals had genotypes and phenotypes. The heifers without genotypic data were incorporated using a single step marker regression model. The estimated heritability from the single step model was 0.16 with a posterior standard deviation of 0.03. There were 455 sires with number of daughters ranging from 1 to 73 with an average of 5. Eighteen sires had more than 20 daughters on test which resulted in reliabilities above 0.5 for that group. Generally, reliabilities of the genomic breeding values of the sires ranged from 0.2 to 0.72 with an average of 0.45. A large part of the heifers that go through test are part of the genetic heifer nucleus of STgenetics and are therefore offspring of young elite AI sires. The progeny testing scheme allows introducing high genetic merit sires with highly accurate breeding values for feed efficiency of growing heifers.

Key Words: feed efficiency, genomic prediction, single step

388 Dry matter intake, production, body condition score, body weight, and frame size of ProCROSS crossbred versus Holstein cows. B. N. Shonka-Martin*[1], B. J. Heins[2], and L. B. Hansen[1], [1]*University of Minnesota, St. Paul, MN,* [2]*West Central Research and Outreach Center, Morris, MN.*

ProCROSS (Montbeliarde, Viking Red, Holstein) rotational crossbred cows were compared with Holstein cows for DMI, production, BW, BCS, and height during the first 150 d of first, second, and third lactation. Primiparous and multiparous crossbred (n = 63 and n = 43, respectively) and Holstein (n = 60 and n = 37, respectively) cows calved from fall 2014 to spring 2017. Cows were fed the same total mixed ration twice daily, with refusals weighed once daily. The BW was recorded twice weekly, and BCS was evaluated weekly. Height at the hips was recorded monthly. Production estimates were obtained from Best Prediction from monthly test days. Daily DMI and fat plus protein production were summed up to 150 d in milk. Statistical analysis for primiparous cows included the fixed effects of year and breed group, and the multiparous model included breed group and the repeated effect of cow. Primiparous crossbred cows (2807 kg) had less ($P < 0.01$) mean DMI than Holsteins (2948 kg), but produced a similar ($P = 0.73$) amount of mean fat plus protein production (331 vs. 329 kg, respectively). The mean BW was not different ($P = 0.59$) between primiparous crossbred (562 kg) and Holstein (556 kg) cows, although crossbred cows (3.46) had higher ($P < 0.01$) mean BCS than Holsteins (3.20). For mean hip height, primiparous crossbred cows (142.3 cm) were shorter ($P < 0.01$) than Holsteins (144.3 cm). Multiparous crossbred cows (3360 kg) consumed less ($P = 0.02$) mean DMI than Holsteins (3592 kg). Mean fat plus protein production was similar for multiparous crossbred (445 kg) and Holstein (441 kg) cows. Also, multiparous crossbred (636 kg) and Holstein (644 kg) cows did not differ for mean BW, and multiparous crossbred cows (3.25) had higher ($P < 0.01$) mean BCS than Holsteins (3.06). Multiparous crossbred cows were similar ($P = 0.16$) for mean height at the hips (145.2 cm) compared with Holsteins (146.4 cm).

Key Words: crossbreeding, feed intake

389 Feed efficiency and residual feed intake of ProCROSS crossbred versus Holstein cows. B. N. Shonka-Martin*[1], B. J. Heins[2], and L. B. Hansen[1], [1]*University of Minnesota, St. Paul, MN,* [2]*West Central Research and Outreach Center, Morris, MN.*

Rotational 3-breed crossbred cows of Montbeliarde, Viking Red, and Holstein (ProCROSS) were compared with Holstein cows for feed efficiency and residual feed intake (RFI) during the first 150 d of first, second, and third lactation. Primiparous and multiparous crossbred (n = 63 and n = 43, respectively) and Holstein (n = 60 and n = 37, respectively) cows were fed the same TMR twice daily with refusals weighed once daily. Feed was analyzed for dry matter, net energy of lactation, and crude protein content. Fat and protein production were estimated from monthly test days with Best Prediction. Body weight was recorded twice weekly. Measures of 150-d efficiency were fat plus protein production (F+P) per unit of DMI, ECM per unit of DMI, DMI per unit of BW, ECM per unit of net energy of lactation intake (NEI), protein production per unit of crude protein intake (CPI), and income over feed cost (IOFC), which was revenue from F+P minus feed cost. The RFI for each lactation was the residual error remaining from regression of DMI on milk energy output (mcal), metabolic BW, and change in body energy (mcal). Statistical analysis of feed efficiency and RFI for primiparous cows included the fixed effects of year and breed and, for multiparous cows, breed was a fixed effect and cow was a repeated effect. Both primiparous and multiparous crossbred cows had higher mean F+P, ECM, and protein per unit of DMI, NEI, and CPI, respectively, compared with primiparous and multiparous Holstein cows (Table 1). Also, both primiparous and multiparous crossbred cows had higher mean IOFC compared with Holstein cows (Table 1). Mean RFI was significantly lower (more desirable) for both primiparous and multiparous crossbreds compared with Holsteins.

Table 1 (Abstr. 389). Feed efficiency between primiparous and multiparous crossbred and Holstein cows

	Primiparous		Multiparous	
Item	Holstein	Crossbred	Holstein	Crossbred
F+P/DMI	0.11	0.12**	0.12	0.13**
ECM/DMI	1.70	1.77**	1.89	2.01*
ECM/NEI	1.05	1.09*	1.16	1.23*
Protein/CPI	0.30	0.32**	0.32	0.35**
DMI/BW	5.31	5.03**	5.57	5.31*
IOFC	$825	$875**	$1208	$1296*
RFI	68.8	−65.5**	75.0	−64.5*

**$P < 0.01$, *$P < 0.05$ for differences between breed groups.

Key Words: crossbreeding, feed efficiency

390 Activity and rumination of Holstein versus crossbred cows in an organic grazing and low-input conventional herd. G. M. Pereira* and B. J. Heins, *University of Minnesota West Central Research and Outreach Center, Morris, MN.*

Holstein (HO) and crossbred cows from an organic grazing and low-input conventional herd were evaluated for activity and rumination from January 2014 to December 2017 at the University of Minnesota West Central Research and Outreach Center, Morris, MN. Within each herd (organic grazing or low-input), breed groups comprised HO (n = 114), HO maintained at 1964 breed average level (H64; n = 83); crossbreds comprised of Montbéliarde, Viking Red, and HO (MVH; n = 248), and Normande, Jersey, and Viking Red (NJV; n = 167). From May to October, organic grazing cows were on pasture, supplemented daily with 2.72 kg of corn per cow, and low-input cows were fed a TMR in an outdoor confinement lot. From November to April organic and low-input cows were fed a TMR in a compost barn or outwintering lot. Activity (reported in activity units per day) and rumination (reported in min per day) were monitored electronically using HR-LD Tags (SCR Engineers Ltd., Netanya, Israel). The PROC HPMIXED of SAS was used for statistical analysis and independent variables were herd, month, breed group, parity group and 2 and 3 way interactions of herd, month, breed group and parity group. Cow nested within breed group and herd was a random effect. All breed groups had similar daily activity in both herds. However, the H64 cows had lower rumination (495; $P < 0.05$) compared with HO (529), MVH (519) and NJV (513) cows in the organic herd. In the low-input herd the H64 cows had lower rumination (483; $P < 0.05$) compared with HO (512), MVH (507) and NJV (512) cows. In the primiparous group of the organic herd, H64 cows had lower rumination (495; $P < 0.05$) than HO (520) cows, and multiparous H64 cows had lower rumination (496; $P < 0.05$) than all other multiparous breed groups. In the primiparous group of the low-input herd, H64 cows had lower rumination (478; $P < 0.05$) than HO (498) and MVH (497) cows, and multiparous H64 cows had lower rumination (489; $P < 0.05$) than all other multiparous breed groups. In both herds, breed groups were active during the day and ruminated during the evening and night hours.

Key Words: crossbreeding, grazing, rumination

391 Incorporation of feed efficiency into a selection index for Holstein cattle. K. Houlahan*[1], F. Miglior[1,2], M. Kargo[3], Z. Wang[4], C. Maltecca[5], B. Gredler[6], A. Fleming[1], and C. F. Baes[1], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Bioscience, University of Guelph, Guelph, ON, Canada,* [2]*Canadian Dairy Network, Guelph, ON, Canada,* [3]*Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics, Aarhus University, Tjele, Denmark,* [4]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [5]*Department of Animal Science and Genetics, North Carolina State University, Raleigh, NC,* [6]*Qualitas AG, Zug, Switzerland.*

Feed efficiency (FE) is a trait currently under intense research worldwide. With feed costs accounting for over 50% of total operational costs on most dairy farms, there is a clear need for improvement in the efficiency of dairy cows' ability to convert feed to milk. Studies have shown there is genetic variation between FE of animals with similar production. Determining an optimal breeding strategy for improving FE in dairy cattle may be helpful in reducing feed costs, while maintaining or increasing production. The objective of this study was to estimate the expected genetic gains and monetary implications of including a measure of FE in a breeding program using different definitions of FE. Various breeding goals were modeled using ZPLAN+. A base scenario consisting of fat and protein yield, age at first service, first service to conception, days open and clinical mastitis was established. Five alternative scenarios of the base with the inclsion of various traits such as, RFI, DMI with a positive or negative economic value, production: feed intake ratio, and feed performance, were compared with results of the base scenario. Using genetic and phenotypic correlations, heritabilities and trait economic values, the effects of incorporating various measures of feed intake and FE were analyzed and compared. Annual genetic gain and monetary genetic gain were estimated for all scenarios. Long-term effects expected on both genetic gain and monetary genetic gain were assessed over a 20-year period. Preliminary results indicate an unfavorable trend for genetic gain and monetary genetic gain in relation to health and fertility traits when FE is included. The inclusion of various measures of FE appears to have minimal negative impact on the genetic gain and monetary genetic gain for production traits. Based on these results, further work is required to determine the optimal method to simultaneously improve health, fertility, production and FE.

Key Words: feed efficiency, breeding strategies, genetics

392 Methods to compute reliabilities for genomic predictions of feed intake. P. M. VanRaden and J. L. Hutchison*, *USDA Animal Genomics and Improvement Lab, Beltsville, MD.*

For new traits without historical reference data, cross-validation is often the preferred method to validate reliability (REL). Time truncation is less useful because few animals gain substantial REL after the truncation point. Accurate cross-validation requires separating genomic gain from pedigree contributions and assuming that other animals with pedigrees less connected to the reference data will have less REL than validation cows. Fiveway cross-validation of residual feed intake (RFI) used data from 80% of the 3,965 US research cows to predict the other 20% and repeated the process 5 times to test predictions for all cows. However, RFI records were excluded from validation data if the cow had progeny in the reference data to ensure correct prediction direction. Pedigree REL for the validation cows was 13%, and their genomic REL was only 18% compared with 21% expected. Pedigree REL for elite young calves was 3%. After adjusting the discount factor to match expected with observed REL, their genomic REL was only 9%, which was less than the 12% previously estimated. Research cows often have paternal sibs, maternal sibs, or dams with RFI records, whereas most calves in other herds are > 2 generations removed from any relatives with RFI records and thus have lower REL. Cross-validation of SCS records for these same 3,965 research cows gave REL estimates similar to those for RFI. Reliability of SCS was also estimated as genomic REL of national SCS predictions (72%) multiplied by the correlation of research herd

predictions with national predictions (0.39) squared, which resulted in 11% REL. Some evaluations report feed saved, which includes RFI plus the economic value for body weight composite (BWC). Inclusion of BWC added only about 7% to the REL of feed saved because RFI contributed much more genetic variance than BWC. Correlated yield traits contribute much variance to feed intake but not to RFI, which is independent of yield. Increases in genomic REL with further RFI data can be forecast. For young calves, such REL could be 12% with 5,000, 19% with 10,000, 31% with 20,000, and 52% with 50,000 cows in the reference population.

Key Words: feed efficiency, cross-validation, reliability

393 Indicator traits to predict dry matter intake in Holstein cattle. S. C. Beard*[1], F. Miglior[1,2], F. Schenkel[1], B. Gredler[3], Z. Wang[4], A. Fleming[1], and C. F. Baes[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Canadian Dairy Network, Guelph, ON, Canada,* [3]*Qualitas AG, Zug, Switzerland,* [4]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The cost of feed is the largest expense of a dairy farm, and this cost is rising. Feed intake traits such as DMI are highly desirable traits for dairy breeding programs. Measuring individual feed intake, however, is difficult and expensive. The use of predictor traits may be better suited to measure feed intake, as they are less expensive and easier to measure. Information such as mid-infrared spectroscopy (MIR), BW, and BCS have the potential to be predictor traits for DMI. MIR is used to analyze molecular vibration and rotation when a material is exposed to electromagnetic radiation. It is currently used worldwide to quantify milk components during routine milk analysis. MIR technology may provide a cost-effective opportunity to obtain predicted phenotypes for feed intake on many animals by taking advantage of technology that is currently used in regular milk recording. The objective of this study was to evaluate the efficacy of MIR, BW, and BCS to predict DMI and to determine the optimal predictor trait for DMI. Weekly milk samples were collected for 143 Canadian Holsteins (n = 2,775) during routine milk recording and were sent to CanWest DHI for MIR spectral analyses. Milk, fat and protein yields (n = 2,775), BW (n = 1,656), and BCS (n = 1,656) were also collected. Daily DMI (kg/d) for all cows (n = 2,775) was recorded and averaged per lactation week with a mean (SD) of 85.33 (43.99). A prediction equation for DMI was produced using partial least squares regression from the MIR spectra of milk samples. Equations to predict DMI using BW and BCS were calculated to determine the optimal predictor trait for DMI. Further steps are needed to determine the ability of the prediction equations to estimate DMI.

Key Words: feed efficiency, mid-infrared (MIR), genetics

394 Dairy Wellness Traits from genomic testing with a control Holstein cow population compared to contemporary Holstein cows in a pasture production system. B. J. Heins*[1], G. M. Pereira[1], L. C. Hardie[2], and C. D. Dechow[2], [1]*University of Minnesota, Morris, MN,* [2]*Penn State University, State College, PA.*

The University of Minnesota initiated a designed study of yield selection in 1964 that included an unselected control group of cows. The study continues at the West Central Research and Outreach Center (WCROC), Morris, Minnesota, and is currently part of the low-input sustainable and organic grazing dairy project. Control Holstein cows (n = 45) were compared with contemporary Holstein cows (n = 64) for wellness traits from the Clarifide Plus genomic test. Herd average production during first lactation were 4,114 kg milk, 143 kg fat, 129 kg protein, and 3.2 SCS for the control cows and 6,563 kg milk, 241 kg fat, 209 kg protein, 2.5 SCS for the contemporary Holsteins. The control line of cows has not been evaluated in a pasture-based dairy production system. Cows were housed at the WCROC, a grazing herd, and calved for a first time from March 2011 to June 2017. All Holstein and control cows were tested during the summer of 2017 with Clarifide Plus and Dairy Wellness Traits (mastitis, lameness, metritis, retained placenta, displaced abomasum, and ketosis) were received during fall 2017 for all 109 cows. Independent variable for statistical analysis with PROC GLM was the fixed effect of genetic line. The control cows had significantly ($P < 0.01$) lower Dairy Wellness Profit (−$745 vs. +$402) compared with the contemporary Holsteins cows, respectively. The control cows were significantly higher ($P < 0.01$) for mastitis (108 vs. 102), lameness (106 vs. 101), and displaced abomasum (105 vs. 102) than contemporary Holstein cows, respectively. The control cows and contemporary Holsteins cows were not different for metritis (102 for both genetic lines) and retained placenta (101 for both genetic lines). The contemporary Holstein cows were higher ($P < 0.05$) for ketosis (102 vs. 101) compared with the control cows. The control cows had a higher Wellness Trait Index ($235 vs. $103) compared with the contemporary Holstein cows. Future studies will compare the 2 genetic lines with genome-wide association analysis for production and health traits.

Key Words: genetic selection, organic, health

395 Heritability and genetic correlations of shape and size of lactation curves in Israeli Holsteins using geometric morphometrics. A. A. Duron-Benitez[1], J. I. Weller*[1], and E. Ezra[2], [1]*ARO, The Volcani Center, Rishon LeZion, Israel,* [2]*Israel Cattle Breeders Association, Caesaria Industrial Park, Israel.*

Routinely, genetic evaluations are based on 305-d lactations, which represent the size or magnitude of lactation curve but ignore the shape, an inherently multidimensional feature. We combined the methods of quantitative genetics and the geometric morphometrics (GM) to evaluate the heritability and genetic correlations of size and shape of lactation curves. The data were daily records of first parity milk production from January 2014 through January 2017 from 43 communal herds distributed throughout Israel. We proposed 2 geometrical shapes of lactation curves, one depicted by line graph and the other by orbital graph; using monthly records as the basis for estimating landmark coordinates, superimposition, and genetic evaluation. Lactation curve shapes were represented by a set of 2-dimensional coordinates. These landmarks were then superimposed using the Procrustes technique to extract shape information. The size of the line- and orbital-depicted lactation curve was recorded as the centroid size (CS), computed as the square root of the summed squared distances of each landmark from the centroid. Heritabilities and genetic and environmental correlations computed by the MTC REML individual animal model program for CS of the proposed shapes and total lactation are in Table 1. Similar heritability values and complete genetic and environmental correlations of the CS of the orbital lactation curve with the traditional method validate it as proxy for the size measure of lactation curves. The heritability of shape was 0.179 for the orbital-depicted lactation curve and 0.082 for the line-depicted lactation curve. These results demonstrate GM as a promising new approach to study the patterns of phenotypic and genetic variation of shape and size of lactation curve in dairy cattle.

Table 1 (Abstr 395). Heritabilities (on the diagonal) and genetic (above diagonal) and environmental (below diagonal) correlations of centroid size (CS) and total lactation among 3,492 first-parity cows[1]

Variable	CS-line	CS-orbital	Total lactation
CS-line	**0.09**	0.49	0.48
CS-orbital	0.02	**0.39**	1.00
Total lactation	0.02	1.00	**0.39**

[1]Standard errors of heritabilities were <0.05.

Key Words: geometric morphometrics, lactation shape, heritability

396 Montbeliarde- and Viking Red-sired crossbred cows compared with Holstein cows for health treatment cost in high-performance dairies in Minnesota. A. R. Hazel*[1], B. J. Heins[2], and L. B. Hansen[1], [1]*University of Minnesota, St. Paul, MN,* [2]*West Central Research and Outreach Center, Morris, MN.*

A 10-yr designed study on crossbreeding compared a 3-breed rotational crossbreeding program (ProCROSS) with Holstein (HO) cows for health treatment cost during parities 1 to 3. Crossbreds and HO cows were housed in 7 high-performance dairies. Two-breed crossbreds were Montbéliarde × HO (MH, n = 576) and Viking Red × HO (VH, n = 604) versus HO (n = 1,239) cows that calved for a first time from 2010 to 2015. Three-breed crossbreds were Montbéliarde × VH (n = 386) and Viking Red × MH (n = 401) versus their HO contemporaries (n = 871) that calved a first time from 2012 to 2016. A fixed treatment cost including veterinary fees, pharmaceutical expense, and labor cost was assigned to 14 types of health treatments. Total health cost (THC) was summed across each lactation and subsequent dry period for each cow. The 2-breed and 3-breed crossbreds were compared with HO separately with a phenotypic mixed model including the fixed effects of parity, herd-year-season of calving nested within parity, breed, crossbred group nested within breed, interaction of parity and breed, interaction of parity and crossbred group nested within breed, random sire effect nested within sire breed, and random cow nested within breed group. All fixed effects were statistically significant except for some interactions that were required to estimate solutions. The 2-breed cows had mean THC of $34, $49, and $73 during their first 3 parities, respectively; however, the HO cows had significantly more ($P < 0.01$) mean THC of $45, $66, and $93, respectively by parity, compared with the 2-breed cows. For the 3-breed cows, the crossbred ($33) and HO ($40) cows did not differ ($P = 0.31$) for mean THC during first lactation. During second ($60 vs. $81) and third ($80 vs. $136) lactations, mean THC was significantly less ($P < 0.01$) for 3-breed crossbreds vs. HO, respectively. The reduction of THC for 2-breed (−22% to −24%) and 3-breed (−18% to −41%) crossbred cows vs. HO cows should increase the profitability of crossbreeding.

Key Words: crossbreeding, total health cost

397 Elucidating the contribution of *Listeria monocytogenes* plasmids to survival in dairy foods and production facilities. A. L. Naditz*, D. Dhar, and S. Schmitz-Esser, *Iowa State University, Ames, IA*.

Listeria monocytogenes is a food-borne pathogen responsible for the disease listeriosis and is commonly isolated from dairy products and facilities. Most strains of the same sequence type (ST) contain highly conserved plasmids, though little is known of the contributions of plasmids on survival in dairy foods and environments. To determine the contribution of plasmids to survival under such conditions, we studied 3 *L. monocytogenes* ST5, ST8, and ST121 strains, which were isolated from dairy foods and production facilities, and contain plasmids ranging from 62.2 to 90.5 kb harboring putative stress response genes. Plasmid curing was conducted by exposure to subinhibitory novobiocin concentrations (0.3 µg/ml) in tryptic soy broth + yeast extract at 44°C. Wildtype (wt) and plasmid-cured (Δ) strains were exposed to sublethal concentrations of oxidative stress (H_2O_2), salt (NaCl), acid (lactic acid), heat stress, or disinfectant (benzalkonium chloride, BC) for 2 h at 20°C. After stress exposure, cells were plated on tryptic soy agar for colony forming unit (cfu) counts. When exposed to 0.01% H_2O_2, 15% wt/vol NaCl with pH 5, or 1% vol/vol lactic acid, cfu counts for all wt strains were significantly higher ($P < 0.001$, $P < 0.05$, and $P < 0.001$ respectively) than those of the Δ strains. cfu counts for the ST5 wt strain exposed to 10 µg/mL BC were significantly higher ($P < 0.001$) than the Δ strain. The ST8 and ST5 wt strains exposed to an elevated temperature (50° and 55°C respectively) had a significantly higher ($P < 0.01$ and $P < 0.001$) cfu count than the Δ strains. Our data revealed that *L. monocytogenes* ST5, ST8, and ST121 plasmids confer increased tolerance against elevated temperature, salinity, acidic environments, oxidative stress and disinfectants. Further examination of the phenotypic and functional characteristics of plasmids is required to better understand the mechanisms used by *L. monocytogenes* for survival in dairy food and food production facilities.

Key Words: *Listeria monocytogenes*, plasmid, stress survival

398 Transcriptomic analysis of high exopolysaccharide-producing dairy starter bacterium *Streptococcus thermophilus* ASCC 1275 in milk. Q. Wu and N. P. Shah*, *The University of Hong Kong, Pokfula Road, Hong Kong.*

Streptococcus thermophilus ASCC 1275, a typical dairy starter bacterium that has been completely sequenced, could produce the highest known amount (~1000 mg/L) of exopolysaccharide (EPS) in milk under the optimal condition among the species of *Str. thermophilus*. However, little is known about the mechanistic insights into the effects of environmental factors such as pH, temperature and whey peptides on its EPS production. In this study, RNA-seq was applied to this organism cultivated in skim milk under different conditions: 1) Cd1 – pH 6.5 and 37°C; 2) Cd2 – pH 5.5 and 37°C; 3) Cd3 – pH 5.5 and 40°C; 3) Cd4 – pH 5.5 and 37°C with whey peptide supplementation. In condition 1, fermentation was carried out at pH 6.5 at 37°C. Similarly, for condition 2, the fermentation was carried out at pH 5.5 at 37?. For condition 3, the fermentation was carried out at pH 5.5 at 40°C. For condition 4, the fermentation was carried out at pH 5.5 at 37°C with whey peptide supplementation. Samples for RNA seq analysis were collected at 6 h after the start of fermentation. These conditions are based on previous studies carried out in our lab. Total RNA extraction was extracted using Ambion RiboPure-Yeast kit. The high quality RNA from each condition was taken further for transcriptomics library construction. Results showed that 526 genes were upregulated in Cd2 compared with that in Cd1; specifically, ribosomal protein synthesis and EPS assembly genes were upregulated in Cd2 which may explain the enhanced EPS production in Cd2. As for temperature effect, only 49 genes were upregulated in Cd3 compared with that in Cd2; purine and lactose metabolism were enhanced in Cd3 suggesting that more amino sugar may have been synthesized for EPS assembly in Cd3. Sixteen genes were upregulated with whey peptide supplementation in Cd4 compared with that in Cd2. It was found that cysteine and methionine metabolism and some unidentified amino acid/peptide transporters were improved for bacterial growth. This study indicates that regulation of amino acid sugar synthesis, EPS assembly and bacterial growth are responsible for an enhanced EPS production from *Str. thermophilus* ASCC 1275.

Key Words: *Streptococcus thermophilus* ASCC 1275, transcriptomic analysis, gene expression

399 Impact of farm management on microflora of raw bovine milk: A Swedish farm-based study. L. Sun*[1], G. Bernes[4], J. Dicksved[2], A. Höjer[3], K. H. Saedén[3], M. Johansson[1], M. Hetta[4], and Å. Lundh[1], [1]*Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [3]*Norrmejerier Ek. Förening, Mejerivägen* 2*, Umeå, Sweden,* [4]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden.*

Manufacturers of long-ripened hard cheese worldwide share the problem with a costly variation in ripening time required to achieve the characteristic flavor of a specific cheese. The variation is partly considered to originate from the composition and properties of the raw milk, which in turn is influenced by on-farm management. Non-starter lactic acid bacteria stemming from the raw milk, especially *Lactobacillus*, have been identified as crucial for flavor development in cheese. Thus, the aim of this study was to explore the impact of farm management on raw milk microflora. Bulk milk samples from 45 farms located in northern Sweden were collected monthly during one year and samples from 6 of these months were used in the current study. Farm visits were conducted on 2 occasions to collect data on management factors, e.g., feeding and forage production, milking system, hygiene and udder health. The microbial community of the raw milk was investigated with DNA based amplicon sequencing approach targeting 16S rRNA with Illumina Miseq platform. Canonical correspondence analysis (CCA) was used to elucidate correlations between microbial data and farm management factors. Amplicon sequencing indicated that the overall microbial diversity richness was at similar level, no variation was observed between farms or between months of sampling. However, taxa evenness as measured by Simpson index was lower in December. The 2 psychrotrophic genera *Pseudomonas* and *Acinetobacter* were the most commonly dominant bacteria observed. Lactic acid bacteria – order *Lactobacillales* – had an average relative abundance of 11.8%, varying from 0.1–77%. CCA analysis showed a strong correlation between milking system and microflora present in raw milk. For instance, all OTUs from *Streptococcus* had higher relative abundance in robot milking systems; the only OTU from *Lactobacillus* was higher in conventional milking systems. Results are currently being validated with additional sequencing data from extra months. In conclusion, several on-farm management factors correlating

with milk microflora were identified. Results from our research will be used in the development of best practice protocols for production of raw milk that will benefit manufacture of long-ripened cheese.

Key Words: farm management, microflora, bovine milk

400 Time for change—Indicators of public health concern for raw milk and processed dairy products. S. Murphy*, *EAS Consulting Group LLC, Alexandria, VA.*

Regulatory standards used as indicators of conditions considered public health risks for grade "A" milk and milk products fall under FDA's grade "A" Pasteurized Milk Ordinance (PMO). These standards have not changed in decades. This overview presents a perspective based on current science on where changes are warranted. Requirements for grade "A" raw milk include temperature (≤7°C); bacterial limits (≤100,000/mL producer); drug residues (Not Found); and SCC limits (≤750,000/mL). While lower temperatures are ideal, limited storage time reduces public health risks. Although exceeding limits for bacteria and SCC indicate deficiencies in production practices and potential quality effects, from a public health perspective, no need for more stringent standards was found assuming a 5 log reduction of pertinent pathogens is sufficient. Somatic cells are not associated with public health, but may present a negative image. The public health impact and testing requirements for drug residues continues to be debated. Requirements for pasteurized milk products include temperature (≤7°C); bacterial limits (≤20,000/mL non-cultured milk products; ≤ 10,000/g dried milk products); coliform bacteria (≤10/mL or g); alkaline phosphatase (ALP; 350 mU/L). Refrigeration at ≤7°C is not in line with the FDA Food Code, which considers risk for *Listeria* growth within 7 d with foods held > 5.0°C; spore-forming pathogens should be considered especially with extended product storage times. Bacteria count limits of pasteurized milk products provide no information on potential pathogen contamination and are often performed without consideration for microbial growth over shelf-life. Coliform bacteria historically used as indicators of post-processing contamination (PPC) are unacceptable at any level; their absence however does not confirm PPC is prevented. Alternative microbial methods are needed. ALP test results used to indicate proper pasteurization typically are < 100mU/L; milk with a result of 300mU/L while acceptable would likely contain raw milk or be underpasteurized. This overview suggests it is time for change for some indicators and that further research is needed, especially with pasteurized milk products.

Key Words: standards, Grade "A", testing

401 Physical removal of bacteria from raw milk by centrifugation: effect of force and temperature. E. R. Griep* and C. I. Moraru, *Cornell University, Ithaca, NY.*

Bacterial spores cause major quality and shelf life issues in fluid milk and other dairy products. Spores can only be destroyed at sterilization temperatures, which have negative effects on product quality and taste. Alternatively, bactofugation can be used as a nonthermal method to physically remove spores from milk. The objective of this work was to evaluate the impact of centrifugal force and temperature on spore removal from milk, using *Bacillus licheniformis* and *Geobacillus* spp. as challenge microorganisms. Whole raw milk was inoculated with 10^6 to 10^7 cfu/mL spores and centrifuged for 15 min in a lab centrifuge at g-forces representative of cream separation (2,000 × *g*) and bactofugation (10,000 × *g*). Experiments were conducted at 4°C and 50°C. Uninoculated whole milk was used as a control. Cream, skim, and pellet were collected aseptically. All fractions and the whole milk were analyzed for total aerobic bacteria (TPC), mesophilic spores (MSC), thermophilic spores (TSC) and somatic cells (SCC). A plant study was completed at a dairy plant in NY State, which has a one-phase bactofuge installed before warm (60–65°C) separation. Samples of whole raw milk, bactofuged raw milk, skim, cream, and pasteurized milk were analyzed for TPC, MSC, SCC, and psychrotrophic spores (PSC). Experiments were completed at least in triplicate, and statistical differences among means were evaluated using ANOVA at 0.05 significance level. TPC, MSC, TSC, and SCC were significantly reduced in skim by centrifugation at 4°C, regardless of centrifugal force, while bacteria and occasionally SCC significantly increased in cream. At 10,000 × *g* and 50°C, only TPC and TSC were significantly reduced in skim. No differences between whole, skim, or cream were observed at 2,000 × *g* and 50°C. In the plant trials, bactofuged whole, skim, and pasteurized milk had significantly lower TPC and SCC. MSC and PSC were reduced below detection level by centrifugation. Overall, the effectiveness of bacterial removal was primarily affected by centrifugation temperature, rather than centrifugal force. This data provides useful information about the effectiveness of centrifugation for spore removal from milk, which can positively impact dairy product quality and shelf life.

Key Words: bactofugation, spore removal, bacteria removal

402 Interaction of lactic acid bacteria with MFGM phospholipids – Surface adherence. J. Ortega-Anaya*, I. García-Cano, D. Rocha-Mendoza, and R. Jiménez-Flores, *The Ohio State University, Colulmbus, OH.*

It is widely known that probiotic lactic acid bacteria attaches to membranes of intestinal cells triggering signals that result in beneficial effects to human health. Strong evidence also indicates that milk phospholipids (PLs) derived from the MFGM have an impact in lowering cholesterol, improving anti-inflammatory, chemotherapeutic and anti-neurodegenerative responses. However, little is known about the role of milk PLs once they come in contact with lactic acid bacteria arising many questions: Do they bind intact PL molecules? Do they utilize them, producing metabolites that could be further absorbed by human intestine? In this work, we studied the primary surface interaction and adherence of 5 strains of lactic acid bacteria with PL from the MFGM. We tested *L. casei, L. pentosus, L. plantarum, P. acidilactici*, and *P. lolli* which have been proved by our research group to grow in a media enriched with milk PLs (0.5% wt/vol). We prepared supported lipid bilayers (SBL) in a SiO_2 surface using a specific mixture of milk PLs kindly donated by Fonterra Co-Op, NZ (1.5 mg/mL in PBS buffer pH 7.4). Interaction studies were investigated using a quartz crystal microbalance with dissipation (QCM-D) which measures in real time, changes in the resonance frequency (ΔF) and dissipation energy (ΔD) of acoustic waves when a layer of molecules (milk PLs and cells) is adsorbed on the active surface (SiO_2) of the sensor. Our results indicate that *L. pentosus* and *plantarum* bind irreversibly to milk PLs at different stages of cell growth. Additionally, we observed and characterized the mass loss of PL molecules from the supported bilayer indicating that the bacterial cells utilize and metabolize them to further produce metabolites with different bioactivities. In conclusion, our experimental work shows that some strains of lactic acid bacteria such as *Lactobacillus pentosus* and *L. plantarum* unequivocally bind to phospholipids isolated from bovine MFGM. This information is helpful in understanding and modeling the phospholipid–bacterial response and behavior in either the intestinal lumen or in fermented dairy products.

Key Words: lactic acid bacteria adherence, milk phospholipids, quartz crystal microbalance

403 The effect of bovine milk consumption on in vivo porcine adipose stem cells. K. Swanson*, D. Hendrix, D. Jiang, and M. Bionaz, *Oregon State University, Corvallis, OR.*

Milk is known to contain many bioactive compounds as well as bovine exosomal miRNA that can have effects on various cells, including stem cells. Among them, adipose stem cells (ASC) are of particular interest due to their role in adipose tissue growth, thus, obesity. In this study we aimed to determine if the consumption of milk had an effect on ASC in growing piglets. For this study, twelve 8- to 9-wk-old Duroc-Berkshire cross piglets were used and were all fed a normal growing diet supplemented with 750 mL of whole milk (n = 6; TRT) or an isocaloric maltodextrin solution (n = 6; CTR). At the end of 11 wk, the pigs were euthanized to isolate ASC from their subcutaneous back fat. Amount of isolated cells/g of tissue and colony forming units (cfu) were assessed. A proliferation assay and RNA extraction and sequencing were performed using passage 1 ASC. All cell data were analyzed using R with a linear mixed effects model with treatment as main effect and pig as random effect for proliferation and cfu and a Welch 2 sample *t*-test for amount of cells/g tissue. mRNA sequencing data were analyzed using Kallisto for alignment and annotation and the expression analysis was conducted by fitting a negative binomial model using the edgeR package in R. There was a tendency for more ASC/g tissue isolated from TRT ($P = 0.09$) as well as significantly more ASC proliferation ($P < 0.05$) when compared with CTR. There were no differences between TRT and CTR for the cfu ($P = 0.15$). RNA sequencing analysis identified 509 genes affected by milk supplementation with a FDR < 0.1. Analysis with Dynamic Impact Approach and DAVID revealed an induction by milk supplementation of lipid metabolism-related pathways and cGMP-PKG that are associated with a decrease in obesity-related inflammation. Analyses also indicated that milk supplementation inhibited immune-related pathways such as T cell differentiation and leukocyte migration along with MAPK signaling. In addition, pathways associated with insulin resistance and type II diabetes were both inhibited in TRT vs. CTR. Our data indicate that supplementing milk can improve the status of ASC via transcriptomic effects with a possible beneficial effect on preventing obesity and related problems.

Key Words: milk, adipose stem cells, obesity

404 Effect of milk supplementation on bone growth in pre-pubertal pigs. B. Batty*[1], M. Kutzler[1], S. Campbell[1], A. Torres[1], N. Enos[1], K. Swanson[1], S. Busato[1], N. Aguilera[1,2], E. Plancarte[1], and M. Bionaz[1], [1]*Oregon State University, Corvallis, OR,* [2]*Universidad Zamorano, Francisco Morazan, Honduras.*

Achieving peak bone mass during childhood and adolescence is associated with a decrease in the risk of osteoporosis and osteoporotic related fractures later in life. Milk has always been associated with increased bone health, strength, and development, but assessing the direct effect of milk on multiple properties of bone development is difficult with a human model. Therefore, this study aimed to use a pig model to evaluate the effect of milk consumption on the growing skeleton. For this, we used 24 pre-pubertal pigs fed a normal growing diet supplemented with 750 mL of whole milk or an isocaloric maltodextrin solution for 12 wk. The experiment was run in 2 trials: trial 1 utilized 4 male and 2 female 5-wk-old Yorkshire pigs per group while trial 2 utilized 7-wk-old Duroc × Yorkshire pigs of 6 males per group. Ultrasonography was used throughout the trial to record *in vivo* bone growth. After 12 wk, the pigs were euthanized and bones of the appendicular skeleton and the mandible were collected. All bones were analyzed with dual energy x-ray absorptiometry (DEXA). A 3-point bending test was used for biomechanical testing. After fracture, the cortical bone thickness was taken at 3 regions of the femur. Data were analyzed using GLM procedure of SAS with TRT and Experiment (and Sex for Trial 1) as fixed effect and pig as random. To evaluate differences between groups, a Student's *t*-test was used. Significance was declared with $P < 0.05$. Of the DEXA measurements taken, only the bone mineral density of the mandible was significantly lower in pigs receiving milk ($P = 0.04$). Upon further evaluation, the difference was in the mandibular body ($P = 0.03$). There was no difference in maximum force, stiffness, or extension of the femur from the biomechanical tests. The medial cortical thickness of the femur was larger in the control group (4.4 ± 0.1 mm vs. 4.0 ± 0.4 mm, $P = 0.03$), while the lateral thickness was larger in the group receiving milk (7.4 ± 0.8 mm vs 6.2 ± 0.9 mm, $P = 0.05$). Short-term milk supplementation did not increase bone mineral density, growth, and strength. The biological significance and the link between milk and the larger lateral cortical thickness in the femur and the lower bone density in the mandible remain unclear.

Key Words: milk, bone, dual energy x-ray absorptiometry (DEXA)

405 Milk consumption and childhood obesity: Does milk really deserve the bad rap? K. Swanson*[1], S. Akers[1], C. Penix[1], N. Aguilera[2,1], S. Busato[1], B. Batty[1], M. Kutzler[1], and M. Bionaz[1], [1]*Oregon State University, Corvallis, OR,* [2]*Universidad Zamorano, Tegucigalpa, Honduras.*

It is difficult to study direct causes of childhood obesity due to varied diets and genetics of human children. In the present study, we used piglets as a model to determine the effect of milk on adipose tissue. We hypothesized that milk consumption would not affect production of adipose tissue. Two studies were conducted using 5-wk-old male Yorkshire piglets (n = 8) for a pilot study and 8- to 9-wk-old male Berkshire-Duroc crosses (n = 12) in the main study. The piglets were all fed a normal growing diet and randomly assigned to get daily 750 mL of whole cow milk or an isocaloric maltodextrin solution. For 11 wk, body weight, feed intake, and subcutaneous back fat thickness determined ultrasonographically were recorded. At euthanasia, back and neck fat thicknesses were measured and samples of back fat were collected for adipose histology. All data were analyzed using R with a linear mixed effect model with treatment as main effect for histology data and all back fat data and with additional interaction of time and treatment for the weight gain, feed conversion ratio (FCR), average daily intake (ADI), average daily gain (ADG), and ultrasonography data. Pig was used as random variable. In the pilot study there were significant ($P < 0.002$) time and treatment interactions for FCR, ADI, weekly weight gain, and overall food intake. In all instances but FCR, the piglets fed with milk had a larger value at the end of the study. In the main study, there was a trend toward control piglets eating more ($P = 0.07$) but having less ADG ($P = 0.08$) as the study progressed. There were no differences in ultrasound back fat thickness for either study, but in the pilot study both back fat and neck fat thickness were larger in the milk-fed piglets ($P < 0.05$). In the pilot study the milk-fed piglets had higher frequency of small adipocytes ($P = 0.09$) and lower frequency of intermediate and large adipocytes compared with control ($P < 0.05$). In the main study, the control pigs had significant greater ($P < 0.05$) frequency of intermediate adipocytes but the milk-fed piglets tended to have higher frequency of the largest adipocytes ($P = 0.06$). Based on the data from both studies, we conclude that milk has no apparent causal effect on amount of adipose tissue in growing individuals.

Key Words: milk, obesity, piglet

Dairy Foods Processing Symposium: Emerging Processing Technologies to Improve Quality and Functionality of Dairy Ingredients

287 Opportunities for novel dairy ingredients—End-user perspective. P. Upreti*, *Nestle R&D Center Inc., Solon, OH.*

Dairy ingredients are frequently used for enhancing flavor, texture, appearance, and nutrient-density of finished products. These ingredients often excite marketers because of the ability to make product-claims and create competitive gaps. At present, with the changing consumer preferences and awareness, these ingredients can play even more significant role because of their nutritious and wholesome connotation and consumer-friendly labeling.
The presentation will provoke fellow academic researchers and dairy industry associates to ensure the new dairy ingredients and processes maintain the cleaner-label aspects and transparency expected by consumers from the novel dairy ingredients. This presentation will also highlight the opportunities to consider wholesomeness of dairy ingredients, in conjunction with "simple-to-explain" in-process transformations, in order to create novelty. Such discoveries, their development, and (ultimately) deployment takes time; so patience and persistence are key to successfully launch in the marketplace the products that are nutritious and flavorful.

Key Words: dairy, processing, ingredient

406 High-pressure-jet spray-drying to create novel dairy products. F. Harte*, *Department of Food Science, Pennsylvania State University, University College, PA.*

High-pressure technologies are gaining relevance throuhgout the Food and Dairy Industries. This presentation will review current state of the art in high hydrostatic pressure (HHP), high-pressure homogenization (HPH), and high-pressure-jet processing (HPJ). It will focus on the effect of HPJ on milk proteins and the potential to create milk powders with targetted functionality. Research in HPJ is showing that the physicochemical properties of dairy powders—from flow properites to interfacial properties—can be modified by nonthermal processes that are amigable with current 'clean label' trends among consumers. This presentation will also highlight the need for better understanding the basic structure-funtion properties of the casein protein fraction as a unique means toward the development of the next generation of dairy-based ingredients.

Key Words: nonthermal, casein, structure-function

407 Use of forward osmosis as a non-thermal method of concentration for the manufacture of high quality milk concentrates and powders. C. I. Moraru*, *Cornell University, Ithaca, NY.*

Concentration is commonly used in the manufacture of condensed milk or milk powders, and it can be achieved by thermal evaporation or, more recently, reverse osmosis (RO). Thermal evaporation is energy intensive, leads to undesirable product quality changes, and the outgrowth of spores in the concentration units. RO is affected by membrane fouling, and limited achievable final product concentration. Forward osmosis (FO) is emerging as an attractive nonthermal method of concentration, which is less prone to fouling and can achieve higher concentration levels compared with RO. This presentation will discuss practical aspects related to FO concentration of whole and skim milk, in terms of concentration level and final product quality. Pasteurized skim and whole milk (Cornell Dairy, Ithaca, NY) were concentrated at 4°C and 15°C using a micro pilot-scale FO unit (Ederna, France), equipped with a modified spiral-wound cellulose triacetate membrane. Potassium lactate 60 °Brix was used as draw solution, and a vacuum evaporator was used for its regeneration (Evaled, Italy). The cumulative water flux for RO and FO was determined gravimetrically. Batches of 8L of milk were concentrated in triplicate, and their physico-chemical properties evaluated. The water flux during FO decreased exponentially with time, while sample concentration, evaluated using a refractometer, increased exponentially. Flux values were comparable for both types of milk, although the decrease in flux for whole milk was slightly more pronounced than for skim milk. For whole milk, flux values of 0.656 L/(m^2h) after 8h at 15°C and 0.784 L/(m^2h) at 4°C, while for skim milk flux values of 0.96 L/(m^2h) after 7h at 4°C and 0.87 L/(m^2h) after 7 h at 15°C) were obtained. FO processing of both skim and whole milk had virtually no impact on the color and overall quality of the final product. Overall, the experimental data suggests that FO can be a very attractive alternative to thermal concentration or RO concentration of milk.

Key Words: forward osmosis, nonthermal concentration, concentrated milk

408 Innovations in micro- and nano-bubble technology to improve dairy powder functionality. J. Amamcharla*, *Food Science Institute/Animal Sciences and Industry, Kansas State University, Manhattan, KS.*

High-protein dairy powders such as milk protein concentrates (MPC) and milk protein isolates (MPI) are added to a variety of dairy and food products to improve the nutritional, sensory, and functional properties. Various factors such as processing conditions, the composition of the powder, storage conditions, and dissolution conditions affect the overall solubility of MPC and MPI. MPCs have the best possible solubility instantly after production and the solubility decreases as the storage time and temperature increases. Moreover, it was reported that increasing the protein content from 85% to 90% led to an overall reduction in solubility. The MPC and MPI must be soluble to give the products the desired characteristics. In this work, it is proposed that the microstructure of MPC powders can be altered using nano- and micro-bubbles (MNB) to improve the reconstitution properties of the MPC powder. In recent years, MNB are gaining interest due to their wide applications in a variety of fields. The physical properties of MNBs are different from those of milli-scale bubbles. The milli-bubbles rise very rapidly, burst on the liquid-air interface, and disappear. On the other hand, the MNBs are stable for considerably long periods. The objective of the present work was to modify the microstructure of the MPC powder particles to create channels for subsequent movement of water during rehydration. The presentation will compare the dissolution properties of MPC powders obtained by the conventional spray drying process as well as the proposed MLB process.

Key Words: micro- and nano-bubbles, microstructure, high-protein dairy powders

409 Single droplet drying—A new technology for optimization of drying conditions for dairy ingredients. L. E. Metzger* and H. N. Vora, *South Dakota State University, Brookings, SD.*

As a result of their extended shelf-life, dried dairy ingredients are a major product category produced for global markets. During the development of dairy ingredients, several drying trials are typically conducted to determine optimum drying conditions. The results of these trials can be critical in determining optimum dryer design. However, these trials can be expensive and time consuming. An alternative that has recently been developed is the use of a new technique called single droplet drying (SDD). The SDD technique involves a single droplet suspended on the tip of a glass filament, where changes in droplet diameter, mass, and temperature are measured during drying. This makes it possible to create a pictorial view of the drying process. Once the drying process is complete, particle morphology can be determined using microscopy or the rehydration behavior can be visually studied. A predictive model generated using SDD can then be used to optimize the drying conditions and dryer design. The modeling will help reduce costly plant trials and accelerate the development of new ingredients with novel functionalities.

Key Words: single droplet drying, drying kinetics

Growth and Development/Ruminant Nutrition Symposium: Post-Weaning and Beyond

410 Integration of post-weaning nutrient requirements and supply with growth and mammary development in modern dairy heifers. M. E. Van Amburgh*, *Cornell University, Ithaca, NY.*

To optimize first lactation and lifetime milk yield, growth benchmarks were established to help nutritionists meet the appropriate growth objectives of breeding weight and age at an economically viable time and to achieve the optimum body size and composition at first calving (NRC, 2001). However, there are still concerns that mammary development is impaired when BW gain exceeds a certain threshold and this impairment negatively affects milk yield. The objective of this review will be to integrate concepts of body growth and composition, mammary development and milk yield to provide a systems based perspective on first lactation milk differences that have been associated with mammary development. The seminal work by Sejrsen et al. (1982; 1983) describing the effect of high energy intake on mammary development and the relationship with circulating growth hormone linked the relationship between pre-pubertal growth, mammary development and future milk yield. The primary outcome of Sejrsen et al. was to provide an intuitive mechanism to explain why rapid growth during the pre-pubertal phase resulted in reduced milk yield. The observation of reduced mammary development could be repeated in almost every experiment (Pritchard et al., 1972; Petitclerc et al., 1984; Mäntysaari et al., 1995; Capuco et al., 1995; Meyer et al., 2006). These repeatable observations lead to the conclusion that high energy intake and increased ADG reduced mammary development through altered hormone status or some signaling processes. However, Meyer et al. (2006) were the first to recognize that mammary development was not reduced by high energy intake, and instead a function of the time to reach puberty and the associated signals to change from allometric mammary growth. The mammary gland, like all reproductive organs, grows in proportion to the size of the body and not in proportion to nutrient intake during the post-weaning, pre-pubertal phase. First lactation milk yield, mammary development and body composition will be further discussed in the context of mechanisms and opportunities.

Key Words: heifer, mammary development, milk yield

411 Macronutrient metabolism in the growing calf. W. J. J. Gerrits*, *Animal Nutrition Group, Wageningen University & Research, Wageningen, the Netherlands.*

Under conventional milk feeding systems, calves are provided restricted amounts of milk or milk replacer and weaned after a few weeks of age. With increasing awareness that early nutrient intake may increase milk yield in first lactation, high milk replacer schemes in early life are currently intensively researched. In the veal industry, high milk replacer schemes for calves are common practice, but in Europe, the intake of concentrates in this sector is strongly increasing, at the expense of milk replacer. Combining knowledge on nutrient metabolism of these areas provides valuable insights for future development of targeted feeding schemes for young calves. Protein efficiency in calves drops dramatically in early life, particularly related to metabolic inefficiencies. Typically for calves and unlike in monogastric species, protein and energy simultaneously limit protein retention. With the possible exception of the first weeks of life, amino acid imbalance is rarely a cause of protein inefficiency. Fats and lactose are well digested, but digestion of starch appears limited. After absorption, fats are oxidized or stored as body fat, but lactose is predominantly oxidized. Recently, it was revealed that calves do possess the enzyme systems for de novo lipogenesis from glucose, but use it only at very high lactose intakes. Consequently, the increase in fat deposition following an increase in feed intake is almost exclusively from dietary fat. Insulin sensitivity rapidly drops in the first 6 weeks of life to levels that are invariably low. This decrease seems independent of diet, as it occurs in milk fed as well as in weaned calves. Meal responses of glucose and insulin largely reflect portal glucose appearance rather than insulin sensitivity. Nonetheless, available glucose is virtually completely oxidized. Apparently, despite the low insulin sensitivity, glucose transport into cells is almost complete. In veal calves of 4-6 months of age, the efficiency of utilization of lactose, fat and protein for growth was found to be independent of the level of intake of solid feeds, suggesting that feeding scheme's for milk (replacers) and solid feeds for calves may be developed independently.

Key Words: macronutrient, metabolism, calf

412 Re-evaluation of NRC energy estimates in calf feeds. J. D. Quigley*, T. M. Hill, J. R. Knapp, X. Suarez-Mena, T. S. Dennis, and W. Hu, *Provimi, Brookville, OH.*

Estimates of energy content of dry feeds used by the National Research Council (NRC, 2001) are based on equations to estimate truly digestible non-fiber carbohydrate (NFC), CP, fatty acids and NDF from composition of feed. Total digestible nutrients, digestible and metabolizable energy (ME) are then calculated from these fractions with appropriate corrections and adjustments. Although this approach is efficient for adult animals, errors may occur when calculating ME in calf starters (CS). Digestion of nutrients, but particularly starch and NDF, is low in young calves consuming little CS and with negligible rumen development. Apparent total-tract digestion (TTD) of ADF and NDF in calves fed > 0.8 kg/d MR are < 50% of estimates in calves fed < 0.8 kg/d until 12–13 wk (6–7 wk post-weaning). Although CS intake increases rapidly post-weaning, inability to digest CS nutrients may partially explain post-weaning depressions in BW gain in the first few weeks post-weaning when calves are fed > 0.8 kg/d MR. Further, nutrient content of CS may influence TTD of nutrients. Calves fed a 41% starch, texturized CS had lower ADF and NDF digestion at 8 wk (1 wk post-weaning) compared with calves fed a 10% starch, pelleted CS, which may be due to low ruminal pH and depression of fiber digestion. We calculated ME in CS fed to Holstein bull calves from 0 to 16 wk using TTD data from 3 published studies. Calves were fed varying amounts of MR, and water and CS were available ad libitum. The TTD of NDF, NFC and total DM in CS increased with increasing NFC intake from CS in a logarithmic fashion. Calculated ME in CS in calves fed > 0.8 kg/d of MR (lower CS intake) were lower than calves fed less MR (greater CS intake) at similar ages. Natural logarithm of NFC intake from CS accounted for 70% of variation in calculated ME in CS. Calculated ME in CS was < 75% of NRC estimated ME when calves consumed 0.2 kg/d of NFC and 98% of NRC estimated ME when calves consumed 1 kg/d of NFC. Ability of the calf to extract energy from CS changes with age and rumen development and depends on intake of NFC from CS. Feeding management that delays NFC intake may reduce ME in CS. Equations using NFC intake may provide greater accuracy in estimating ME in CS.

Key Words: calves, energy, digestion

413 Growing and developing dairy heifers from birth to weaning. A. J. Heinrichs*, *The Pennsylvania State University, University Park, PA.*

We have made dramatic changes in the nutrition and management of dairy heifers over the past 20 years. Significant basic nutrition research and applied management studies have moved our knowledge of the dairy heifer forward. Increasingly, the industry has become more progressive in adopting management practices based on the physiology and nutrient needs of the heifer. The US has experienced consistent progress toward optimizing heifer growth rates and reducing age at first calving. Part of this change has to be a result of economic analyses showing that age at first calving drives the overall cost of the heifer enterprise. Studies using data from the early 1990s demonstrated the economic advantage of calving at 23 to 24 mo, yet the industry lagged far behind at that time. Recent industry trends show a marked reduction in age at first calving, from 28 mo in 1980 to 25.5 mo in 2004 and a sustained decline since then. Research into growth rates and standards for body size and stature have been instrumental in developing rearing programs that provide heifers with adequate nutrients to support growth and improve milk production in first lactation. Free stall and bedded pack housing along with use of higher quality forage in total mixed rations and limit feeding systems have improved the feed efficiency of heifers to allow improved growth rates and breeding at younger ages. Transitioning the weaned heifer to this feed efficient system remains a problem on many farms. There remains opportunity for research to more fully understand management and feed efficiency of heifers as they continue to mature at younger ages. Currently, calving at 22 to 23 mo appears to best balance the cost of growing heifers with their lifetime production and income potential.

Key Words: dairy heifer, growth rates, age at first calving

414 Period2 gene silencing impacts on the proliferation, apoptosis and cell cycle progression of bovine mammary epithelial cells. L. Hu*[1], M. Wang[1], L. Wei[1], Y. Jing[1], Q. Xu[1], and J. J. Loor[2], [1]*College of Animal Science and Technology, , Yangzhou University, Yangzhou, China,* [2]*Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL.*

Studies showed that the aberrant of Period2 (*PER2*) gene, a core component of circadian oscillator, is responsible for rhythm alteration of physiological activities including growth, metabolic and endocrine regulation. This study employed bovine mammary epithelial cells (BMEC) as a model to investigate the effect of the suppression of *PER2* on cell proliferation, apoptosis and cell cycle progression. BMEC cells were established by enzymatic digestion of mammary tissue from mid-lactation cows and cultured in growth medium (DFEM/F12 as basis). The fourth passage BMEC were transiently-transfected with siRNA specific for *PER2* to downregulate transcription. After incubating for 24 h and 30 h, the alterations in proliferative activity of BMEC by cell counting Kit-8, apoptosis and cell cycle distribution by flow cytometry, DNA fragmentation by agarose gel electrophoresis and mRNA expression alterations in the important genes related to cell cycle network and circadian rhythm by qPCR were all detected. Statistical analysis of data were performed using an independent sample *t*-test module (SPSS16.0). The results showed that cell proliferation was increased ($P < 0.05$) while cell apoptosis rate declined ($P > 0.05$), the DNA fragmentation did not significantly respond to *PER2* silence and the number of cells in the G1/G0 phase was decreased both for 24h and 30h transfection ($P < 0.01$). The gene expression levels of *cyclin D1*, *CDK2*, *p21*, *PER1* and *BMAL1* were dramatically decreased ($P < 0.05$), whereas the *CDK4* mRNA level was significantly increased ($P < 0.05$) compared with control cells after 24h incubation. In conclusion, the present study supports that *PER2* regulates proliferation, apoptosis and cell cycle progress of BMEC and effects expression of core circadian clock gene *BMAL1* at the transcriptional level. The multiple implications of our findings for further studies lies in understanding how *PER2* regulates bovine mammary gland development and circadian mammary organic functions.

Key Words: Period2 gene, bovine mammary epithelial cell, cell cycle

415 Temporal effect of dry period heat stress on mammary gland gene expression and structure. T. F. Fabris*, J. Laporta, A. L. Skibiel, Dado-Senn B., D. R. Silva, S. Wohlgemuth, and G. E. Dahl, *University of Florida, Gainesville, FL.*

Dry period (DP) heat stress (HT) impairs subsequent milk yield. Our objective was to evaluate the effect of HT during early and late DP on mammary gland (MG) gene expression and structure. Cows were dried-off 45d before parturition and randomly assigned to one of 4 treatments: HT (n = 20) or cooling (CL, n = 20) during the entire DP, HT first 21d dry then CL to calving (CLHT, n = 19) or CL first 21d dry then HT to calving (HTCL, n = 20). In the DP, data were divided into 2 periods: first 21 d of the DP and from 22d until calving (after switch). Mammary biopsies (5–8 cows per treatment) were performed at −3, 3, 7, 14, and 25d relative to dry-off to evaluate MG gene expression and histology of alveolar structure. Autophagy related genes, ATG3 and 5, and beclin-1 (BECN1); apoptosis related genes, caspase 3 and 8, heat shock proteins (HSP70 and 90), and heat shock factor 1 (HSF1); insulin growth factor 1 receptor (IGF1R); and prolactin signaling related genes, prolactin receptor long form (PRLR-L), STAT5a and β-casein were analyzed by qRT-PCR. Mammary alveoli number and luminal area were visualized by hematoxylin and eosin staining and analyzed using Image J software. Cooling tended to or increased caspase 3 ($P = 0.08$), IGF1R ($P = 0.07$), HSP90, HSF1, BECN1, ATG3, ATG5, PRLR-L (all $P \leq 0.05$) expression in the early dry period. But, late in the DP after the switch, CLHT cows increased caspase 3, STAT5a and PRLR-L across all treatments ($P \leq 0.05$); CLHT cows increased caspase 8, HSP70 and 90 and β-casein relative to CL and HTCL ($P < 0.05$); CLHT cows increased ATG3 relative to CL ($P = 0.08$) and HTCL ($P < 0.01$); and CLHT cows increased ATG5, BECN1 and IGF1R relative to HTCL ($P \leq 0.05$). In the early DP there was an interaction between treatment and days relative to dry-off ($P < 0.01$). In the early DP, cows exposed to HT had higher alveoli number relative to CL ($P < 0.01$), and HT decreased expression of genes related to autophagy and apoptosis in the early DP, consistent with a delay in involution with HT. Also, exposing cows to CLHT triggers early PRLR-L activation that may be detrimental to mammary development and compromise milk yield in the next lactation.

Key Words: autophagy, apoptosis, prolactin receptor

416 The impact of pre-weaning plane of nutrition on the myoepithelial cell population within the immature bovine mammary parenchyma. A. J. Geiger*[1], C. L. M. Parsons[2], and M. R. Akers[2], [1]*Zinpro Corporation, Eden Prairie, MN,* [2]*Virginia Tech, Blacksburg, VA.*

An enhanced pre-weaning diet improves development of the immature, bovine mammary gland. Alterations in the population of myoepithelial cells (MYEC) can also affect mammary development. Our objective was to determine the impact of a higher plane of nutrition (HPN) on the MYEC population within the mammary gland of dairy calves. Thirty-six Holstein heifer calves were reared on (1) a control milk replacer (MR) fed at 454 g powder/d (R; 20% CP and 20% fat) or (2) an enhanced MR fed at 1,135 g powder/d (EH; 28% CP and 25% fat). At weaning (wk 8), a subset of calves (n = 6/diet) were harvested. Post-weaning, half of the remaining calves from each diet were supplemented with exogenous estradiol (E_2) to determine if a 2 week mammogenic stimulation further affected the MYEX population in response to diet consumed. At harvest, the mammary parenchyma (PAR) was removed and fixed. MYEC nuclei were identified by immune-staining for p63 in sections from lower, middle and distal portions of PAR. Images were analyzed using CellProfiler to measure nuclear characteristics of the MYEC population within each image. Comparisons between treatments were determined using PROC GLIMMIX in SAS. Nuclear area of MYEC was greater for calves fed EH compared with R (675 vs. 620 pixels; $P < 0.05$). Additionally, calves fed EH had a greater number of neighboring MYEC surrounding a given cell compared with calves fed R (5.2 vs. 4.9 neighbors; $P < 0.01$) and had a greater nuclear perimeter (105 vs. 99 pixels; $P < 0.05$). Nuclear compactness and eccentricity (degree of elongation) were not affected by dietary treatment ($P = 0.99$ and 0.84, respectively). Additionally, diet did not affect the distance between a given MYEC and its closest neighboring cell ($P = 0.28$). Finally, E_2 supplementation did further impact the MYEX population. These results

suggest that diet-induced changes in prepubertal mammary growth are linked to alterations in MYEC population.

Key Words: calf, milk replacer, myoepithelial cell

417 Heat stress negatively affects the transcriptome related to overall metabolism and milk synthesis in mammary tissue of mid-lactating dairy cows. D. Bu*[1,3], L. Ma[1,2], S. Gao[1], Z. Zhou[1], L. H. Baumgard[4], J. Duo[5], and M. Bionaz[5], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China,* [3]*Hunan Co-Innovation Center of Safety Animal Production, Changsha, Hunan, China,* [4]*Department of Animal Science, Iowa State University, Ames, IA,* [5]*Animal and Rangeland Sciences, Oregon State University, Corvallis, OR.*

Study objectives were to examine transcriptome related to casein synthesis in lactating cows under heat stress (HS) by using RNA sequencing. Four multiparous Holstein dairy cows (101 ± 10 DIM; 38 ± 2 kg milk/d) were randomly assigned to 1 of 4 environmental chambers with a crossover design. Following a 9-d adaptation period, cows were either subjected to HS [36°C during the day and 32°C during the night; temperature-humidity index (THI) = 87.2 and 81.8] for 9d or kept in thermal neutral conditions [TN: 20°C; THI = 65.5] for 9 d, but pair-fed (PF) with heat-stressed cows. There was a 30-d washout between periods. Mammary biopsies were obtained at the end of each period. HS decreased milk yield (17%) and protein content (4.1%). HiSeq2000 platform was used to measure the mRNA profile. Alignment to the cattle reference genome (Bos_taurus.UMD3.1) was performed using spliced read mapper Tophat2 and a quasi-negative binomial model was used to analyze the normalized data, which included as covariates the cow effects and the order in which the treatment and control conditions were implemented. The effects of HS on gene expressions were tested using quasi-likelihood F tests. The analysis was performed with the edgeR package in R. There were 1,718 differentially expressed genes (DEG; FDR < 0.01): 870 upregulated and 848 downregulated genes in HS vs. PF. Major casein genes (*CSN1S2*, *CSN1S1*, *CSN3*, and *LALBA)* and amino acid transporters *(SLC38A10*, *SLC38A3*, *SLC38A1*, *SLC30A2*, *SLC34A2*, *SLC1A2*, *SLC38A3*, *SLC1A4*, *SLC1A5*, *SLC38A3*, *SLC7A5*, *SLC7A8*, *SLC1A1*, and *SLC1A2)* were downregulated in HS vs PF. Inhibited casein genes and amino acid transporters likely explains the observed decrease in protein synthesis. Besides decrease in protein synthesis, data analyzed using the Dynamic Impact Approach and Database for Annotation, Visualization and Integrated Discovery revealed a general inhibition of metabolic pathways in HS vs. PF, in particular, most impacted pathways were related to lipid, lactose, and amino acid synthesis. In summary, transcriptome analysis revealed that HS directly (independent of reduced feed intake) markedly altered mammary metabolism.

Key Words: transcriptome, milk synthesis, heat stress

418 Comprehensive analysis of fatty acid biohydrogenation intermediates involved in milk fat depression over time in dairy cows. H. M. Leskinen*, L. Ventto, P. Kairenius, T. Stefanski, K. Shingfield, and J. Vilkki, *Natural Resources Institute Finland (Luke), Jokioinen, Finland.*

The factors driving the progress of diet-induced milk fat depression (MFD) were studied by combining data from the flow of specific ruminal biohydrogenation (BH) intermediates of fatty acids (FA) at the omasum and the proportions of FA in milk fat over time. Four Nordic Red cows in mid-lactation were used in a 4 × 4 Latin square with a 2 × 2 factorial arrangement of diets and 35-d periods. Diets were based on grass silage: low (65:35, forage:concentrate, FC) or high (35:65) concentrate diets supplemented with 0 g (diets L and H, respectively) or 50 g/kg dry matter of sunflower oil (SO; LSO and HSO, respectively). Data were analyzed using the MIXED procedure of SAS with fixed effects of period, FC, SO and FC × SO interaction. HSO lowered (FC × SO $P < 0.01$) milk fat synthesis up to 31.9% relative to other diets. MFD was associated with shift in ruminal BH toward the *trans*-10 pathway (*trans*, *t*; *cis*, *c*) and increases in *t*10 containing intermediates in milk. However, the flow of *t*10,*c*12 conjugated linoleic acid (CLA) at the omasum was greater ($P < 0.01$) on both H and HSO compared with L and LSO indicating that *t*10,*c*12 CLA was not the only factor causing MFD. Contrary to this, MFD on the HSO was accompanied by an increase in *t*10,*c*12 CLA in milk compared with other diets (FC × SO $P < 0.05$). HSO resulted in the formation of *t*10 18:1 and *t*9,*c*11 CLA in the rumen relative to other diets, causing increased proportions of these intermediates also in milk (FC × SO $P < 0.05$), which support the previous evidence that *t*10 18:1 and *t*9,*c*11 CLA have a role in the regulation of milk fat synthesis. BH intermediate *t*10,*c*15 18:2 was found in omasal digesta and milk on HSO providing more support to the existence of alternative *t*10 pathway of 18:3n-3 metabolism in the rumen that is pronounced in cows fed diets causing MFD. In conclusion, ruminal *t*10,*c*12 CLA formation was not accompanied by MFD suggesting that other BH intermediates (*t*10 18:1, *t*9,*c*11 CLA, *t*10,*c*15 18:2) or additional mechanisms have a role in the regulation of fat synthesis in the bovine mammary gland.

Key Words: bovine, lipid metabolism, conjugated fatty acid

419 Photoperiod affects mammary clock gene expression during late pregnancy and lactation in dairy goats. S. J. Mabjeesh*[1], M. Kalyesubula[1], C. Sabastian[1], N. Reicher[1], A. Shamay[2], Y. Wein[1], E. Bar-Shira[1], K. Plaut[3], and T. M. Casey[3], [1]*The Hebrew University of Jerusalem, Rehovot, Israel,* [2]*The Volcani Center, Rehovot, Israel,* [3]*Purdue University, West Lafayette, IN.*

Short-day photoperiod (SDPP; 8 h light:16 h dark) in the dry period increases milk yield in the subsequent lactation compared with long-day photoperiod (LDPP; 16 h light:8 h dark) in goats. Photoperiod, is the most potent environmental cue that entrains circadian clocks. Our previous studies showed that disruption of circadian clocks impact mammary proliferation, differentiation and milk yield. We therefore, hypothesized that photoperiod effects on lactation are mediated in part through the mammary circadian clock system. The current study aimed to investigate the effect of photoperiod on the mammary expression of the core clock genes: *ARNTL*, *CLOCK*, *CRY1*, *CRY2*, and *PER1*. To study these effects, 12 Israeli Saanen goats were blocked at dry off (~45 d prepartum) into 2 treatments of SDPP and LDPP. Two mammary biopsies were taken – one in the middle of the light phase and another in the middle of the dark phase – at approximately 3 wk prepartum and 5 wk postpartum. mRNA abundance of the core clock genes was measured using qPCR. Milk yield was greater ($P < 0.0001$) in SDPP than in LDPP (3.15 ± 0.04 vs. 2.7 ± 0.05 kg/d). PRL concentration was greater ($P < 0.0001$) in LDPP than in SDPP at 3 wk prepartum (823.4 ± 56 vs. 40.2 ± 10.2 ng/mL), 3 wk postpartum (471.3 ± 40.3 vs. 41.1 ± 5.75 ng/mL) and 5 wk postpartum (365.4 ± 26.6 vs. 20.9 ± 4.21 ng/mL). The light/dark schedule affected the relative expression of *PER1,* being

greater in the light period ($P = 0.003$). Generally, there was a greater relative expression of *CLOCK* ($P = 0.03$), *CRY1* ($P = 0.04$), *CRY2* ($P = 0.03$), and *PER1* ($P = 0.02$) in mammary of SDPP versus LDPP exposed goats. There was an increase in the expression of *ARNTL* ($P = 0.014$), *CLOCK* ($P < 0.0001$), *CRY1* ($P = 0.009$), and *CRY2* ($P = 0.0001$) from late pregnancy to lactation. Thus, homeorhetic changes associated with differences in photoperiod and physiological state are accompanied by changes in mammary circadian clocks. Since circadian clocks regulate cell proliferation and metabolism, there is the potential that changes in mammary core clock dynamics are regulating the photoperiod effects on milk yield in dairy goats.

Key Words: circadian clock, photoperiod, mammary gland

420 Effects of dietary zinc source on inflammatory biomarkers and PMN function following lipopolysaccharide challenge in lactating cows. E. A. Horst*[1], E. J. Mayorga[1], S. L. Portner[1], M. Al-Qaisi[1], C. S. McCarthy[1], M. A. Abeyta[1], B. M. Goetz[1], H. A. Ramirez-Ramirez[1], D. H. Kleinschmit[2], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*Zinpro Corporation, Eden Prairie, MN.*

Objectives were to evaluate the effects replacing 40 ppm of zinc from zinc sulfate (CON) with zinc amino acid complex (Zn; Availa-Zn, Zinpro Corp., Eden Prairie, MN) on PMN function and acute phase protein production following an i.v. lipopolysaccharide (LPS) challenge in lactating cows. Cows were enrolled in a 2 × 2 factorial design and assigned to 1 of 4 treatments: (1) pair-fed (PF) control (PF-CON; 5 mL saline; n = 5), (2) PF Zn (PF-Zn; 5 mL saline; n = 5), (3) LPS-euglycemic clamp control (LPS-CON; 0.375 μg/kg BW LPS; n = 5), (4) LPS-euglycemic clamp Zn (LPS-Zn; 0.375 μg/kg BW LPS; n = 5). Prior to study initiation, cows were fed their respective diets for 42 d. During P1 (3 d), cows received their respective dietary treatments and baseline data was obtained. At the initiation of P2 (2 d), a 12-h LPS-euglycemic clamp was conducted or cows were pair-fed to their respective dietary counterparts. Mild hyperthermia (1°C, relative to PF cows) was observed in LPS cows at 3 h postbolus ($P < 0.01$). Throughout P2, rectal temperature of LPS-Zn cows was decreased (0.3°C), relative to LPS-CON cows ($P = 0.01$). Administrating LPS increased circulating LBP and SAA (3- and 9-fold, respectively), relative to PF cows ($P < 0.01$). Compared with LPS-CON, LPS-Zn cows had increased circulating SAA (38%) 24 h postbolus ($P = 0.03$). Ionized calcium was decreased (33% at 12h) post-LPS administration and dietary Zn tended to alleviate this response ($P = 0.10$). Compared with PF cows, circulating WBC and neutrophil counts initially decreased, then gradually increased ($P < 0.01$). Relative to baseline, oxidative burst of LPS-Zn cows was increased (44%; $P = 0.05$) throughout P2 and tended to be increased (31%; $P = 0.07$) in LPS-CON cows. Myeloperoxidase released after in vitro stimulation increased in all treatments at 12 h postbolus. Throughout P2, myeloperoxidase was increased in LPS-CON and tended to be increased in LPS-Zn cows, relative to baseline (37 and 22%, respectively; $P < 0.01$ and $P = 0.09$). In summary, supplemental Zn increased circulating SAA, reduced fever, and tended to alleviate hypocalcemia following immunoactivation, but appeared to have little or no effect on PMN function.

Key Words: LPS, zinc

421 Effects of maintaining eucalcemia following immunoactivation in lactating cows. E. A. Horst*, E. J. Mayorga, M. Al-Qaisi, M. A. Abeyta, S. L. Portner, C. S. McCarthy, B. M. Goetz, H. A. Ramirez-Ramirez, and L. H. Baumgard, *Iowa State University, Ames, IA.*

Objectives were to evaluate effects of maintaining eucalcemia on production and immunity following an i.v. lipopolysaccharide (LPS) challenge in lactating cows. Cows were enrolled in a study containing 2 experimental periods (P); during P1 (3 d), cows consumed feed ad libitum and baseline values were obtained. At the initiation of P2 (4 d), cows were assigned to 1 of 2 treatments: (1) LPS administered (LPS-CON; 0.5 μg/kg of BW LPS; n = 6) and (2) LPS administered + eucalcemic clamp (LPS-Ca; 0.5 μg/kg of BW LPS; Ca infusion; n = 6). Effects of treatment, time, and treatment by time interaction were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Cows were fasted for 12 h during P2. After LPS administration, ionized Ca (iCa) was determined every 15 min for 6 h and every 30 min for an additional 6 h and Ca infusion was adjusted in LPS-Ca cows to maintain eucalcemia. Blood iCa was decreased 32% for the first 12 h post-LPS in LPS-CON cows, and by design Ca infusion prevented hypocalcemia. Collectively, 12 ± 0.9 g of Ca was infused to maintain eucalcemia. Mild hyperthermia (0.8°C) was observed for ~6 h post-LPS administration, relative to P1. From 6 to 7 h post-LPS rectal temperature of LPS-Ca cows was increased (0.6°C), relative to LPS-CON cows ($P < 0.01$). Overall, DMI did not differ due to treatment, but hypophagia was observed for 2d post-LPS, relative to baseline (34 and 45% for LPS-CON and LPS-Ca, respectively; $P < 0.01$). On d 1 of P2, milk yield decreased (61%) in both treatments relative to P1, however, the decrease tended to be more pronounced with Ca infusion (15%, relative to LPS-CON cows; $P = 0.07$). Overall circulating LPS-binding protein (LBP) continuously increased post-LPS, at 24 h circulating LBP in LPS-Ca cows increased (68%), relative to LPS-CON cows. While not significant ($P = 0.28$), circulating SAA was numerically increased (47%) in LPS-Ca cows throughout P2, relative to LPS-CON cows. Neutrophil counts of LPS-Ca cows were reduced from 9 to 12 h post-LPS, relative to LPS-CON cows ($P = 0.03$). In summary, maintaining eucalcemia during an immune challenge decreased milk yield and neutrophil counts whereas rectal temperature and circulating LBP increased.

Key Words: LPS, calcium

422 Nutrient-sensing kinase signaling and energy storage in bovine immune cells during the transition period. S. Mann*[1], A. Sipka[1], F. Leal-Yepes[2], D. Nydam[1], T. Overton[2], and J. Wakshlag[3], [1]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [2]*Department of Animal Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY,* [3]*Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Transition cows experience a nutrient deficit, particularly immediately postpartum. The inflammatory balance is altered at this time, and cows exhibit an immune response primed for inflammation. Studies in other mammals demonstrate a role of nutrient-sensing kinases in determining immune phenotypes and inflammatory balance. Our primary objective was to investigate changes in energy storage (glycogen) and signaling through the protein kinase B (AKT)/mammalian target of rapamycin (mTOR) and adenosine monophosphate-activated kinase (AMPK) pathway in bovine immune cells in the transition period. Glycogen concentration was measured by an enzymatic-fluorometric method in peripheral blood mononuclear cells (PBMC) of multiparous Holstein cows (n = 72) at 3 time points (21 d before, 7 and 28 d after calving). Phosphorylation of AKT, mTOR substrates 4EBP1 and S6RP, and AMPK were assessed by immunoblotting in PBMC from 60 animals at the same time. Repeated measures ANOVA was performed in Proc MIXED (SAS v. 9.4) and pairwise comparisons controlled with Tukey's procedure. The average (95% CI) concentration of glycogen decreased from 1.5 (1.35 to 1.66) μg/10^6 PBMC on d 21 prepartum to 63% of the prepartum levels on d 7 postpartum [0.95 (0.86 to 1.04) μg/10^6 PBMC, $P < 0.0001$)]. On d 28 postpartum, average concentrations increased to 84% of the prepartum values [1.25 (1.12 to 1.41) μg/10^6 PBMC], $P < 0.01$ to both other time points. Compared with prepartum values, activation of AKT in bovine PBMC on d 7 was reduced by 9% ($P = 0.001$), non-phosphorylated 4EBP1 increased by 5% ($P = 0.07$) whereas no change was observed in the phosphorylation of S6RP ($P > 0.27$).

The activation of AMPK increased by 42% postpartum ($P < 0.001$). We conclude that decreasing energy storage at the time of the nadir in nutrient balance goes along with changes in nutrient-signaling pathways in bovine PBMC postpartum. Temporal associations with changes in immune response at this time and the documented role of the studied pathways in immune-phenotype determination of other species are intriguing and warrant further investigation as a possible link between nutrient deficit, metabolism, and bovine immune response postpartum.

Key Words: transition, immune cell, nutrient-sensing kinase

423 Hormone-sensitive lipase activity modulates the biosynthesis of oxidized linoleic acid metabolites in adipose tissue of periparturient dairy cows. G. A. Contreras*, C. S. Barboza, J. De Souza, J. Gandy, R. Nelli, J. De Koster, A. L. Lock, and L. M. Sordillo, *Michigan State University, East Lansing, MI.*

Oxidized linoleic acid metabolites (OXLAM) are products of adipocyte lipolysis with the potential to modulate adipose tissue (AT) lipid metabolism and inflammation. In periparturient cows, linoleic acid is preferentially mobilized from AT by hormone-sensitive lipase (HSL) during lipolysis compared with other polyunsaturated fatty acids. Enzymatic and non-enzymatic reactions generate OXLAM. Among OXLAM, 9, 10, and 12- hydroxy-octadecadienoic acids (HODE) have pro- inflammatory functions whereas 9- and 13-oxo- octadecadienoic acids (oxoODE) and 13-HODE can facilitate anti-inflammatory responses and promote lipogenesis. High 9- HODE:9-oxoODE (R1) and 13-HODE:13-oxoODE (R2) ratios are associated with dysregulated inflammation. This study evaluated the effect of HSL activity on OXLAM biosynthesis by subcutaneous AT explants collected from multiparous dairy cows (n = 3) at 10 d before and 10 and 24 d after calving. Explants were treated for 3 h with the β adrenergic agonist isoproterenol (CON 0M; ISO, 10^{-6}M) to induce HSL activity. HSL contribution to OXLAM biosynthesis was determined by inhibiting its activity with CAY10499 (10^{-4}M) in the absence (CAY) or presence (CAYISO) of adrenergic stimulation. After treatments, media and explants were collected for lipidomic analysis using HPLC- MS/MS. The statistical model included animal as random effect and the fixed effects of treatment, time relative to calving, and their interaction. ISO increased the biosynthesis of 9-, 12-, and 13-HODE and 9- and 13-oxoODE, and reduced R1 and R2 compared with CON and CAY ($P < 0.05$). CAYISO partially reversed ISO effects on HODE and 13-oxoODE ($P < 0.05$); however, 9-oxoODE was not reduced by CAYISO ($P = 0.26$). The biosynthesis of 12-, 13-HODE and 13-oxoODE was higher and R1 and R2 were lower in ISO at −10d compared with 10d and 24d ($P < 0.05$). Our ex-vivo model demonstrates for the first time a direct effect of HSL activity on the biosynthesis of OXLAM in AT especially 10 d before parturition. However, biosynthesis of anti-inflammatory OXLAM is limited postpartum and may promote AT inflammation and lipolytic responses to negative energy balance.

Key Words: lipolysis, oxylipins, hormone-sensitive lipase (HSL)

424 Short-chain fatty acids regulate the inflammatory response and peripheral blood mononuclear cells recruitment via G protein-coupled receptor 41 in bovine rumen epithelial cells. M. Jiang*, K. Zhan, X. Gong, G. Zhao, and M. Lin, *Institute of Animal Culture Collection and Application, College of Animal Science and Technology, Yangzhou University,, Yangzhou, JiangSu, China.*

Short-chain fatty acids (SCFA) produced by colonic microbiota fermentation of dietary fiber regulate cell responses via G protein-coupled receptor 41 (GPR41) for non-ruminant animals. However, the regulation of cell responses by SCFA and GPR41 remain unknown in ruminant animals. Therefore, the objective of study is to investigate the functions of SCFA and GPR41 in inflammatory responses in bovine rumen epithelial cells (BREC). Bovine ruminal epithelium tissues from 3 young Holstein calves were obtained from the Experimental Farm of Yang Zhou University. The wild type (WT) BREC were cultured in DMEM medium for 24 h. Inflammatory responses were induced in WT BRECs and GPR41 knockdown (GPR41KD) BRECs with 20 m*M* SCFA (12 m*M* sodium acetate, 5 m*M* sodium propionate, and 3 m*M* sodium butyrate) for 24 h of incubations. Statistical analysis was performed by one-way ANOVA followed by the least significant difference (LSD) test for post-hoc correction for multiple comparisons of treatment means using the SPSS 16.0 software. $P < 0.05$ was considered significant. These results showed that the concentrations of 20 m*M* SCFA significantly enhanced IL1B, TNF, and CCL20, CXCL2, CXCL3, CXCL5, CXCL8, CXCL14 chemokines in WT BREC compared with WT BREC in the absence of 20 m*M* SCFA ($P < 0.01$). In comparison with WT BREC with 20 m*M* SCFA, the GPR41KD BREC with 20 m*M* SCFA significantly enhanced the proinflammatory cytokine of IL1B and TNF expression ($P < 0.05$), and reduced the expression of CCL20, CXCL2, CXCL3, CXCL5, CXCL8, CXCL14 chemokines, Occludin, and ZO1 ($P < 0.05$). Remarkably, GPR41KD BREC markedly decreased the peripheral blood mononuclear cells (PBMC) recruitment compared with WT BREC. The regulation of PBMC recruitment by GPR41 correlated well with immunological barrier protection. These findings revealed that SCFA regulate GPR41-mediated immune cell migration and thereby mediate protective immunity in BREC.

Key Words: bovine rumen epithelial cells, GPR41, inflammatory response

425 Beta-hydroxybutyrate enhances kisspeptin-stimulated expression of gonadotropin releasing hormone in GT1-7 cells. L. L. Amelse*[1], J. T. Mulliniks[2], J. A. Daniel[3], and B. K. Whitlock[1], [1]*College of Veterinary Medicine, University of Tennessee, Knoxville, TN,* [2]*West Central Research and Extension Center, University of Nebraska, North Platte, NE,* [3]*Department of Animal Science, Berry College, Mount Berry, GA.*

Metabolic stress can inhibit reproduction at least partially through altered serum gonadotropin concentrations. Kisspeptin (KP) and its receptor, Kiss1R, are critical for the function of the hypothalamic-pituitary-gonadal axis. Disruption of either KP or Kiss1R results in delayed puberty or infertility. β-Hydroxybutyrate (BHB) is a ketone body in the plasma of cattle in the negative energy state of early lactation. To determine the effects of metabolic stress on reproduction at the hypothalamus, GT1-7 cells [from mouse hypothalamic tumors which express Kiss1R and release gonadotropin releasing hormone (GnRH) in response to KP] were incubated with or without 6 m*M* BHB (Sigma Aldrich) for 6 h before administration of 10 n*M* KP (KP-10; human Metastin 45–54, Peptides International Inc.). Cells were collected at 0, 5, 15, 30, 60, 90 and 120 min following KP, and total RNA was extracted. Relative expression of GnRH (normalized to expression of GAPDH) was determined by QPCR at each time, and data were tested for effects of treatment, time, and their interaction with JMP software (version 10.0.0; SAS Inst. Inc., Cary, NC). There was an overall effect of treatment ($P < 0.0001$), as KP-stimulated expression of GnRH mRNA was increased by BHB [1.47 ± 0.03 (SD)] compared with those of control (1.06 ± 0.03). There was also an effect of time ($P < 0.0001$) and treatment x time interaction ($P < 0.0001$) such that KP-stimulated expression of GnRH mRNA was increased ($P < 0.05$) by BHB at all but one time (15 min) compared with those of control. Enhanced KP-stimulated GnRH

mRNA by BHB supports published in vivo derived data indicating that feed-restricted rodents and early lactation dairy cows have greater KP-stimulated plasma gonadotropin concentrations. Utilization of GT1-7 cells, or other similar cell lines, will aid in the determination of the mechanism(s) by which BHB affects KP/Kiss1R system, providing greater understanding of the effects of metabolic stress on hypothalamic control of reproduction.

Key Words: hypothalamus, reproduction, stress

426 Assessing tissue-specific metabolic rate to predict production. Y. Xiao* and B. J. Renquist, *University of Arizona, School of Animal and Comparative Biomedical Sciences, Tucson, AZ.*

We developed a method for monitoring energy expenditure of tissue biopsies using the cell culture viability reagent AlamarBlue to assess reduction potential (NADH, NADPH, F ADH_2, and $FMNH_2$) within the organism or tissue sample. We applied this assay to compare the metabolic rate of biopsies taken from a variety of mouse tissues and predict milk production. Tissue biopsies (<10 mg) of murine heart, kidney, liver, brown adipose, red skeletal muscle and white skeletal muscle were collected into wells of a 96-well plate containing DMEM without Phenol Red (4.5 g/L glucose) immediately following decapitation under bell jar isoflurane anesthesia. Fluorescence (ex: 530 nm, em: 590 nm) was measured on a SpectraMax M2 plate reader at tissue collection and 1, 2, and 4 h later. Plates were incubated in 5% CO_2 at 37?C between measurements. The change in fluorescence/mg tissue increased linearly with time to 4h, suggesting that the tissue remained viable throughout the experiment. Fluorescence change/mg tissue which varied up to 8.9 times was highest in kidney, and in descending order the remaining tissues included brown adipose tissue, liver, heart, red skeletal muscle, and white skeletal muscle ($P < 0.0001$). We subsequently, tested the application of this assay to assess mammary gland metabolic function, while concurrently measuring ex vivo lactose production. On the day of parturition, mammary glands were collected after sacrifice, and assessed for ex vivo lactose production and metabolic rate. To induce variability in ex vivo lactose production, mice were exposed to differing environmental conditions during the final 5 d of gestation (thermoneutral environment (22°C, 50% relative humidity), heat stress (HS; 35°C, 50% relative humidity) and thermoneutral pair-feeding (PF). Heat stress decreased lactose production (46%; $P < 0.05$) and fluorescence change (36%; $P < 0.05$) relative to PF control mice. Moreover, ex vivo metabolic rate and lactose production correlated linearly ($R^2 = 0.68$, $P = 0.0035$). Combined these studies show that this assay can be applied to assess metabolic rate across several tissues, assess the effect of physiological perturbations on metabolic rate in a given tissue, and predict production.

Key Words: metabolic rate, reducing equivalents, lactation

427 Identification of immune cells migrated into the jejunum of heat-stressed dairy cows using RNAseq. F. Koch*[1], U. Thom[2], E. Albrecht[3], R. Weikard[2], B. Kuhla[1], and C. Kühn[2], [1]*Institute of Nutritional Physiology "Oskar Kellner," Leibniz Institute for Farm Animal Biology, Dummerstorf, MV, Germany,* [2]*Institute of Muscle Biology and Growth, Leibniz Institute for Farm Animal Biology, Dummerstorf, MV, Germany,* [3]*Institute for Genome Biology, Genome Physiology Unit, Leibniz Institute for Farm Animal Biology, Dummerstorf, MV, Germany.*

High temperatures jeopardize animal welfare of dairy cows. During periods of hot weather, leaky gut syndrome, characterized by impaired gut integrity, may occur. Diminished intestinal barrier facilitates penetration of toxic and bacterial compounds, leading to increased inflammation in jejunum and reflected by increased appearance of immune cells. This study aimed at identification of immune cell populations infiltrated in the lamina propria and underneath the muscularis mucosae of jejunum of heat-stressed dairy cows. Three German Holstein dairy cows (240 ± 25 d in milk) were exposed to 28°C (52 ± 2% RH; THI = 76) for 4 d, with ad libitum feeding tempered to 28°C, to induce heat stress (HS). Cows were then slaughtered to obtain jejunum samples. Infiltrated cells were collected from dehydrated cryo-sections by laser microdissection and RNA was extracted. RNaseq libraries were prepared with NuGEN's Ovation Solo RNA-Seq Kit and sequenced on the Illumina HiSeq2500 platform using 2x100bp paired-end sequencing cycles. The resulting reads were checked for quality, mapped to the bovine reference genome with HISAT2 and analyzed using IG viewer. Mucosa from a cow kept under thermoneutrality and ad libitum feeding served as control. Immunohistochemistry was used to detect CD3, CR2, CD163 and SIRPA in serial sections of jejunum. The dendritic cell marker *SIRPA* was detected in all samples. Macrophage-related genes including *ITGAM*, *CD14*, *FCGR3A*, *CD68*, and *MRC1* were also expressed in all samples, whereas *CD163* was only present in cells from HS cows but not in control. Furthermore, the B cell marker *CR2,* and T cell markers *CD4* and *CD3E* were found in all samples. Immunofluorescence confirmed that infiltrated cells were $CD163^+$ and $SIRPA^+$; but $CD3^+$ and $CR2^+$ cells were only detected in different regions. Heat-stress induced infiltration of immune cells in the lamina propria and underneath the muscularis mucosae. Immune cells were identified as macrophages and dendritic cells in concordance between RNaseq and immunohistochemistry results, whereas expression of markers for B and T cells could not be confirmed on protein level.

Key Words: heat-stress, gut, RNAseq

428 "Feeding" the transcriptome: Nutrigenomic effect of NEFA on peroxisome proliferator-activated receptor activity. S. Busato* and M. Bionaz, *Oregon State University, Corvallis, OR.*

The combination of a sudden increase in metabolic demands and the reduced dry matter intake in early postpartum cows is regarded as the culprit of a sudden increase in nonesterified fatty acids (NEFA), indicative of the rapid mobilization of lipid reserves. We hypothesized that the increase in concentration of circulating NEFA would alter gene expression profiles via modulation of peroxisome proliferator-activated receptors (PPAR), transcription factors known to respond to fatty acids and with a well-defined role in metabolism and inflammation-associated gene regulation. To mimic the natural cellular environment, we ran a series of experiments using whole cow serum with different NEFA concentrations (0.41 mEq/L, SLN; 0.71 mEq/L, SHN) to treat a bovine mammary epithelial cell line (MAC-T) and human liver hepatocellular carcinoma cells (HepG2) using either a bioluminescent or a fluorescent reporter assay to assess PPAR activation and assess cell viability. Data were analyzed using general linear model procedure of SAS. Both SLN and SHN activated PPAR in MAC-T cells when compared with the control ($P < 0.001$) but these results were not recapitulated in HepG2. To assess directly the effect of NEFA, cells were treated with NEFA isolated from each serum and resuspended at equimolar concentration in BSA-free medium. NEFA in medium had high ($P < 0.0001$) cytotoxicity in both cell types. The cytotoxicity decreased as NEFA was spiked into the serum at decreasing concentration. Despite their clear detrimental effect on cellular viability, extracted NEFA had a dramatic effect on PPAR activation, especially in MAC-T (>3.5-fold; $P < 0.001$). To assess if NEFA released from VLDL activate PPAR, 2 ng/μL of bovine lipoprotein lipase (LPL) was added to the serum. The addition of LPL

resulted in significant activation of PPAR in HepG2 cells (>2-fold; $P < 0.001$), although viability was negatively affected ($P < 0.001$), but not MAC-T cells. Our preliminary results clearly indicated that extracted NEFA, but also NEFA released by LPL, especially for HepG2, are cytotoxic. Despite this, our data indicated that NEFA stimulate PPAR activity, suggesting the possible involvement of the PPAR pathway in the metabolic response to the increasing circulating NEFA during the transition period.

Key Words: NEFA, peroxisome proliferator-activated receptors (PPAR), bovine

429 What's the norm in normalization? A note on the use of RTqPCR in livestock-related studies. S. Busato*[1], N. Aguilera[2,1], M. Mezzetti[3,1], and M. Bionaz[1], [1]*Oregon State University, Corvallis, OR,* [2]*Universidad Zamorano, Tegucigalpa, Honduras,* [3]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Reverse transcription quantitative PCR (RTqPCR) is regarded as the most sensitive method to quantify RNA, but its extreme sensitivity makes it prone to methodological errors affecting the reliability of results. One of the most critical aspects to obtain reliable RTqPCR data is the normalization of raw data using reference genes (internal control genes or ICG). Pivotal to obtain reliable normalization is the use of multiple ICG that have been tested for their reliability. Several algorithms, such as GeNorm or NormFinder allow the user to select a suitable set of ICG. Despite the clear advantages of this method, far too often RTqPCR studies are normalized using a single, unverified ICG, often leading to unreliable results. With the purpose of determining what is common practice of RTqPCR normalization in our field, we performed a meta-analysis of all RT-qPCR studies published from 2013 to 2017 in the 5 most prominent journals related to livestock science (n = 227). To evaluate the proper use of normalization we developed a score accounting for number of ICG used (<2 = 0, 2 = 23, >2 = 35), the validation of ICG (No = 0, Yes = 50), and the report of indexes of the validation (No = 0, Yes = 15). The mean ± SD score was 31.4 ± 38.9. Less than 20% of the papers received a score of 100, while > 45% of the papers scored 0. The use of a single ICG accounted for 57.6% in 2013 and increased to 83.3% in 2017. The absence of any validation of ICG accounted for 68.7% of the publications. The studies where the ICG were validated using an algorithm (YAL) encompassed a wider variety of ICG (54 vs. 36) than the ones that did not use an algorithm (NAL). The 5 most recurrent ICG in NAL were *GAPDH*, *ACTB*, *RNA18S*, *RPS9*, and *B2M,* that were used to normalize 72.5% of the publications, as opposed to YAL, where they accounted for 43.3% of the publications. As multiple publications have demonstrated the danger of improper normalization, our data clearly reveal serious problems in the normalization of RTqPCR jeopardizing the reliability of results from studies carried out in livestock.

Key Words: reverse transcription quantitative PCR (RTqPCR), method, normalization

Reproduction Symposium: Recent Innovations in Reproductive Management

430 Focusing on bull management and puberty attainment in the genomic era. B. Harstine*, *Select Sires Inc., Plain City, OH.*

The advent and implementation of genomic selection in dairy cattle has prompted significant changes within the industry from an animal management and breeding perspective. An increased emphasis on decreasing generation intervals has greatly influenced the management of bulls at artificial insemination (AI) organizations. Prior to genomic selection, the genetic value of an AI sire was determined by examining the phenotype and production traits of his daughters (i.e., proofs), and the average age at which sires were debuted to the industry (proven) was between 4 to 5 years. Today, only one decade after the introduction of genomics to the industry, unproven genomically tested sires between 1.5 and 4 years of age account for greater than 50% of AI unit sales for most major AI organizations. Accordingly, bulls destined for AI use receive optimum care and nutrition from birth to diminish developmental delays, and there is a renewed interest in examining the effects of nutrition and endocrine modulation on the attainment of puberty and testicular development in bulls. A recent report from our laboratory demonstrated that a follicle-stimulating hormone (FSH) treatment regimen administered prepubertally hastened puberty attainment (ability to ejaculate 5×10^7 sperm, 10% motile) to 9 mo of age in Holstein bulls. This treatment regimen also positively affected testicular development (increased number of Sertoli cells per seminiferous tubule cross section at 5 mo of age in beef bulls) and altered the endocrine profiles of several key reproductive hormones related to puberty attainment. Importantly, the treatments had no negative effects on bull health or semen production later in life. In practice, hastening puberty in bulls can be used concurrently with modern techniques of oocyte collection in prepubertal heifers to minimize generation intervals in cattle, thus increasing the rate of genetic advancement. The usage of young genomic bulls will likely increase or remain stable in the future, and AI organizations will continue to focus on optimizing bull management to ensure top tier genetics are available to the industry.

Key Words: bull, semen, artificial insemination

431 Impact of early life nutrition on the molecular and physiological regulation of puberty onset in the bull. D. A. Kenny*, *Animal Bioscience Research Department, Teagasc Grange, Dunsany, Co. Meath, Ireland.*

The advent of genomic selection has accentuated interest in procuring saleable semen from young genetically elite bulls, as early in life as possible. However, the timing of availability of semen, for commercial use, will be determined by the age at which these young animals reach puberty and subsequent sexual maturity. Enhancing early life nutrition stimulates the functionality of the hypothalamic-pituitary-testicular (HPT) axis, mediated through a complex biochemical interplay between metabolic and neuro endocrine signals and culminating in enhanced testicular growth, steroidogenesis, spermatogenesis and ultimately earlier onset of sexual maturation. Indeed, recent evidence suggests that the timing of sexual precocity in the bull is determined by prevailing metabolic status during calfhood and is not compensable following nutritional augmentation, once calves reach 6 mo of age. However, despite this, the precise neuronal mechanisms regulating these developmental processes remain to be elucidated for the bull. While early life nutrition clearly affects the sexual maturation process, there is little evidence for latent effects on post pubertal semen characteristics. Equally, post pubertal fertility measured as in vitro fertilisation and early embryogenesis is not influenced by nutritional status during early life. Current efforts employing high throughput nucleic acid and proteomic sequencing and targeted immunofluoresence and couple with systems biology based gene network analyses, are providing further insight on how nutrition may mediate the biochemical interaction between neuroendocrine and testicular processes. Such information can be harnessed to identify potential genomic targets as part of genomically assisted breeding programmes as well as facilitating development of nutritional regimens to optimize sexual maturation and subsequent semen availability from genetically elite young bulls.

Key Words: neuroendocrine, hypothalamus, testes

432 Effects of the adoption of automated monitoring systems for mornitoring transition cows and reproduction on performance. R. Chebel*[1,2], [1]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Dairy producers have an evident need to monitor individual cows and groups of cows to adjust management (e.g., feeding, comfort, heat abatement), to identify ill cows and cows at risk for disease, and for reproductive management. The quest for automated monitoring systems is not new. Several monitoring systems are now readily available to producers that seek what is believed to be more precise and consistent management of individual cows and groups of cows. Such systems, however, will only positively impact dairies when the data produced by the systems are translated into a clear language that allows prompt proactive measures to solve problems or to make decisions. In recent experiments, we and others have demonstrated that automated health monitoring systems have the potential to expedite the diagnosis of metabolic diseases compared with clinical evaluation alone or cowside tests at predetermined intervals from calving. Questions remain, however, about the advantages of such earlier diagnosis to the health and performance of the cow and whether different therapy strategies may be adopted according to a cow's patterns of rumination, feeding, and activity. Several experiments have demonstrated that the benefits of automated estrous detection monitoring systems is highly dependent on the herd's current reproductive strategy and performance. Generally, herds with poor estrus detection efficiency and accuracy are the herds that could potentially gain the most with the incorporation of automated estrus detection systems into their reproductive management. Finally, in recent experiments we have demonstrated that genetic selection for cattle with greater reproductive aptitude may changed physiological responses during the estrous cycle to the point of altering phenotypes associated with estrus (e.g., acceptance of mount, walking/activity, rumination), which may ultimately facilitate detection of estrus. Automated monitoring systems are becoming more accessible to dairies of all sizes, however, considering the initial cost of such technologies, one should be cautious not to seek a problem to make use of a new tool, but instead seek a new tool to solve an existing problem.

Key Words: automated monitoring system, dairy cow, health

433 Detection and management of pregnancy loss in a cow herd. K. G. Pohler*[1], M. F. Smith[2], J. A. Green[2], and J. L. M. Vasconcelos[3], [1]*Department of Animal Science, University of Tennessee, Knoxville, TN,* [2]*Division of Animal Sciences, University of Missouri,*

Columbia, MO, [3]*Departamento de Produção Animal, Faculdade de MedicinaVeterinária e Zootecnia, UNESP, Botucatu, São Paulo, Brazil.*

Reproductive wastage is a major contributor to the overall efficiency of a cattle operation. Management issues, cow or bull infertility, heat stress, and embryonic mortality are all contributing factors to reproductive inefficiency. Minimizing reproductive inefficiency, specifically embryonic mortality (EM) is vital. Embryonic mortality is generally considered to be the primary factor limiting pregnancy rates in cattle and occurs early (<d 28) or late (≥d 28) during gestation (d 0 = estrus). In cattle, the incidence of early EM is approximately 25% and the incidence of late EM is varied, approximately 3.2 to 42.7%. Significant effort has been directed toward understanding the mechanisms resulting in early EM; however, relatively little is known about the causes or mechanisms associated with late EM, most of which occurs around the time of placentation. To understand the mechanisms associated with reproductive loss around these time periods, specific markers must be characterized to help identify pregnancies that lead to success or failure. Extracellular microRNAs have been a recent area of biomarker discovery and based on recent evidence seem to be a promising target for an embryonic viability biomarker. Additionally, binucleate trophoblast cells constitute 15–20% of the ruminant placenta trophoblast population, appear around d 19–20 of gestation in cattle and secrete pregnancy associated glycoproteins (PAGs) along with other products in to the circulation. Bovine PAGs are commonly used to diagnose pregnancy success in cattle and have recently been reported to be a potential marker of late embryonic mortality in dairy and beef cattle. Using predicative value analysis, we have identified circulating concentrations of PAG that are 95% accurate in predicting EM at d 28 of gestation. This talk will highlight some of the work our group is focusing on to detect pregnancy loss during these pivotal periods of pregnancy loss and potential management aspects to mitigate reproductive inefficiency. *This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017–67015–26457 from the USDA National Institute of Food and Agriculture.*

Key Words: cattle, pregnancy, placenta

434 Embryo transfer as a reproductive management tool. P. S. Baruselli*[1], R. Ferreira[1], C. A. Rodrigues[2], and G. A. Bo[3], [1]*Departamento de Reprodução Animal, FMVZ-USP, São Paulo, Brazil,* [2]*SAMVET, São Carlos-SP, Brazil,* [3]*Instituto de Reproducción Animal Córdoba (IRAC), Córdoba, Argentina.*

Embryo transfer is a reproductive technology commonly used around the world to reproduce animals with high genetic merit. However, the application of embryo transfer (ET) technology in combination to artificial insemination (AI) can improve the reproductive performance of high producing dairy herds. The main reason for that potential improvement is the higher fertility reported after embryo transfer in cows experiencing heat stress and those diagnosed as repeat-breeders. Heat stress has a deleterious effect on fertility in dairy herds around the world, especially in tropical areas in which summers are hot and humid. Studies have shown that embryo transfer is an effective tool to increase fertility during heat stress because it bypasses the damage to the oocyte and early embryo caused by hyperthermia. Therefore, a useful management tool to maintain high pregnancy rates throughout the year would be to produce embryos during the cooler months and use them for ET during the periods of heat stress. Repeat breeder cows are usually defined as cows that do not become pregnant over a period of time (usually after 3 or 4 unsuccessful breedings). The reasons for poor fertility in these cows are still controversial. However, the transfer of embryos to repeat breeder recipients resulted in increased repeat breeder pregnancy rates compared with AI, supporting the notion that the fertility problem in some repeat-breeders may be associated with oocyte quality and/or failure of early embryo development. In summary, although the embryo transfer technology has been used primarily to reproduce animals with high genetic merit, this technology can be used to resolve reproductive problems such as the reduced fertility found during heat stress and in repeat breeder cows.

Key Words: heat stress, repeat breeder, reproductive efficiency

435 Relationships between birth season and protein and energy consumed from milk replacer and starter on calf growth and first lactation production performance of Holstein dairy cows. J. J. Rauba*[1], B. J. Heins[2], H. Chester-Jones[3], H. L. Diaz[1], D. Ziegler[3], J. Linn[1], and N. Broadwater[4], [1]*Milk Specialties Global, Eden Prairie, MN,* [2]*University of Minnesota West Central Research and Outreach Center, Morris, MN,* [3]*University of Minnesota Southern Research and Outreach Center, Waseca, MN,* [4]*University of Minnesota Extension, Rochester, MN.*

The objective was to determine relationships between protein and energy consumed from milk replacer and starter for heifer growth and first lactation performance of Holstein cattle. Data were collected from 4,534 Holstein animals from birth year of 2004 through 2014 to analyze growth of calves, and 3,627 cows were analyzed for production during first lactation. Calves were received from 3 commercial dairy farms and assigned to 45 trials at the University of Minnesota Southern Research and Outreach Center. Milk replacer (MR) metabolizable energy (ME), starter ME, MR protein intake, and starter protein intake consumed from 0 to 8 wk were (mean ± SD): 102.7 ± 13.3 Mcal/kg, 151.9 ± 41.4 Mcal/kg, 4.8 ± 1.0 kg, and 9.5 ± 2.6 kg, respectively. Birth season, year, 6- and 8-wk ADG class, and herd were included in the model with calf trial as a random effect. Calves born in the fall and winter consumed more ME ($P < 0.001$) and protein ($P < 0.001$) from MR and starter than calves born in the spring and summer. Increased protein and ME consumption led to greater growth ($P < 0.05$) and first lactation production ($P < 0.02$). Variation was high in all estimates suggesting numerous factors contribute to growth and milk production.

Table 1 (Abstr. 435). Effect of birth season on 8-wk MR and starter protein (kg) and ME (Mcal/kg; n = 3,627)

Variable	Spring	Summer	Fall	Winter	Season *P*-value
MR protein intake	4.7[ab]	4.7[a]	4.6[b]	4.7[a]	0.057
Starter protein intake	9.0[b]	9.1[b]	10.2[a]	10.2[a]	<0.001
MR ME intake	101.8[a]	101.5[a]	100.1[b]	102.9[a]	0.025
Starter ME intake	143.6[b]	145.0[b]	162.9[a]	162.8[a]	<0.001
Combined protein intake	13.7[b]	13.9[b]	14.8[a]	14.9[a]	<0.001
Combined ME intake	245.9[b]	246.8[b]	262.9[a]	265.8[a]	<0.001

Key Words: milk replacer, starter, first lactation

436 Growth performance of dairy heifers fed carinata meal compared with canola meal and a control diet. K. Rodriguez-Hernandez*[1,2] and J. L. Anderson[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Matamoros, Coahuila, México.*

Carinata meal is a developing oilseed meal and has similarities to canola meal. Our objective was to compare the growth performance of dairy heifers fed diets containing carinata meal, canola meal, or a control diet. A 16-wk randomized block design experiment with 32 Holstein heifers [6.3 ± 0.7 mo of age, and 207 ± 20 kg of body weight (BW)] was conducted. Heifers were blocked by age. Treatment diets were (1) carinata meal (CRM), (2) canola meal (CAN), both at 10% of diet dry matter (DM); and (3) control diet (CON) with most of the protein provided from soybean meal. All test meals were solvent extracted. The remainder of the diets were grass hay, distillers dried grains with solubles, ground corn, soybean hulls and mineral mix. Diets were isocaloric and isonitrogenous. Heifers were individually limit-fed rations at 2.4% of BW on DM basis using a Calan gate system. Frame sizes, BW, and BCS were measured at 4 h post feeding on 2 consecutive days during wk 0 and then every 2 wk throughout the feeding period. From all heifers rumen fluid was collected via esophageal tubing at the same time in wk 12 and 16 and during wk 16 fecal grab samples were collected for analysis of total-tract digestibility of nutrients (TTD) using acid detergent insoluble ash as an internal marker. Data were analyzed using MIXED procedures with repeated measures in SAS 9.4. Significance was declared at $P < 0.05$. The DMI (6.31, 6.16, and 6.05 kg/d, SEM = 0.114 for CRM, CAN, and CON, respectively), average daily gain (1.01, 1.02, and 1.03 kg/d, SEM = 0.033) and BCS (3.1, 3.1, and 3.1, SEM = 0.02) were similar ($P > 0.05$). There were no effects of treatment on withers heights (122.2, 122.6, and 122.4, SEM = 0.34) or other frame measurements. Rumen fermentation was not influenced as indicated by similar acetate to propionate ratios (2.6, 2.6, and 2.6, SEM = 0.04). The TTD of DM (71.6, 71.6, and 73.7, SEM = 2.30) was similar, as were individual nutrients. Feeding CRM at 10% of the diet, maintained intakes, growth performance, rumen fermentation, and TTD compared with CAN or CON. Therefore, CRM shows potential as a new feedstuff for growing dairy heifers.

Key Words: dairy heifer, carinata meal, growth

437 Evaluation of carinata meal included in a total mixed ration fed ad libitum to dairy heifers. R. D. Lawrence* and J. L. Anderson, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Our objective was to determine the effects on growth performance of dairy heifers when fed carinata meal in a TMR containing corn silage. Previous research from our group has found that limit-feeding carinata meal at 10% of the total diet in the concentrate mix with grass hay as the major forage maintained heifer growth. A 12-wk randomized complete block design study was conducted using 24 Holstein heifers [242.4 ± 34 d of age; BW 272.8 ± 45 kg]. Treatments were (1) control (CON) a TMR with grass hay, corn silage, and soybean meal and dried distillers grains with solubles as major concentrate ingredients; and (2) a TMR with 10% (DM basis) carinata meal (CRM) replacing a portion of the soybean meal in the grain mix. Diets were fed for ad libitum intakes and formulated to be isonitrogenous and isocaloric. Rations were fed to target 10% refusal rate and intakes were measured using Calan gates. Frame sizes, BW, and body condition scores (BCS) were measured on 2 d during wk 0, 2, 4, 6, 8, 10, and 12. Data were analyzed in MIXED procedures of SAS 9.4 with repeated measures. Heifer DMI was greater ($P < 0.01$) for CON (9.2, and 8.4 kg/d for CON and CRM, respectively; SEM = 0.25). Body weights (327.0 and 327.1 kg; SEM = 2.53), ADG (1.09 and 1.05 kg/d; SEM = 0.05), and gain: feed (0.12 and 0.13; SEM = 0.01) were similar ($P > 0.05$) between treatments. Withers height (128.0 and 128.1 cm; SEM = 0.28), body length (119.5 and 120.0 cm; SEM = 0.44) and BCS (2.99 and 2.97; SEM = 0.02) were also similar ($P > 0.05$). Greater heart girth ($P < 0.01$) was found for the CON heifers (153.3 cm and 152.0 cm; SEM = 0.39). Differences were not found ($P = 0.18$) for paunch girth (187.0 cm and 187.8 cm; SEM = 1.90) between

treatments. The CRM fed heifers had greater ($P < 0.01$) hip width (40.0 cm and 40.4 cm; SEM = 0.24). Results indicated that replacing soybean meal with carinata meal at 10% of the ration and feeding as a TMR for ad libitum consumption maintained heifer body frame growth while decreasing DMI. Carinata meal shows potential as a feedstuff to be included in TMR fed to dairy heifers.

Key Words: carinata meal, dairy heifer, growth performance

438 Comparative analysis of host tissue transcriptomics and rumen wall microbial metatranscriptomics in neonatal calves treated with artificial dosing of rumen content from adult donor cow. W. Li*[1], A. Edwards[1], M. Cox[4], S. Raabis[5], J. Skarlupka[4], A. Steinberger[4], and G. Suen[4], [1]*USDA Dairy Forage Research Center, Madison, WI,* [2]*US Dairy Forage Research Center, Madison, WI,* [3]*Department of Genetics, University of Wisconsin-Madison, Madison, WI,* [4]*University of Wisconsin, Department of Microbiology, Madison, WI,* [5]*University of Wisconsin, School of Veterinary Medicine, Madison, WI.*

Rumen microbial community is important to host health and development in neonatal ruminants. However, molecular and host physiological changes associated with early bacterial colonization are largely unknown in cattle. The aim of this study was to investigate changes in host tissue transcriptomics (in liver and rumen), and in the rumen wall microbial metatranscriptomics in neonatal calves, treated with artificial dosing of exogenous rumen content. Ruminal content from adult cow was used as inoculum. Four newborn Holstein calves were treated with fresh inoculum, and 4 of them were treated with sterilized rumen inoculum at 2, 4, and 6 weeks. Bacterial community diversity of administered rumen inoculum was analyzed by 16S rRNA gene sequencing. All administered inoculum samples had Good's coverage ≥97%, with *Bacteroidetes* as the most abundant phylum (53.31 ± 1.85% SE) followed by *Firmicutes* (34.32 ± 1.82% SE). Liver and rumen tissues were collected at 12 weeks at necropsy. Whole transcriptome RNA sequencing was used to characterize transcriptome changes. A total of 158 differentially expressed genes (DEGs) (fold-change, FC ≥ 1.5, $P \leq 0.05$) was identified in rumen, and 338 DEGs (FC ≥ 1.5, $P \leq 0.05$) was identified in liver. Functional annotation of these DEGs indicated enrichment of extra cellular matrix ($P \leq 0.001$) and inflammatory response ($P \leq 0.001$) in rumen, and enrichment in immune response ($P \leq 0.001$) and sphingolipids metabolism ($P \leq 0.001$) in liver. Specifically, upregulation of SGPLl, a polar sphingolipid metabolite, suggested an adaptive response in host to the influx of *Bacteroidetes* introduced by artificial dosing. Metatranscriptome analysis of rumen wall microbes indicated significant increase ($P \leq 0.001$) of mRNA transcripts from *Bacteroidetes* and *Firmicutes* in treated calves, suggesting functional establishment of artificially dosed microbial communities. Our study provided insights into transcriptome changes in host and rumen microbial community resultant from early colonization of microbial species in young calves. Follow-up studies will focus on microbes mediated rumen development, health and nutrition in ruminants.

Key Words: calves, artificial dosing, transcriptome changes

439 Growth performance, nutrient utilization, and health of calves supplemented with condensed whey solubles. N. D. Senevirathne*[1], J. L. Anderson[1], J. S. Osorio[1], L. Metzger[1], and B. St Pierre[2], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Department of Animal Science, South Dakota State University, Brookings, SD.*

Our objective was to evaluate growth performance, nutrient utilization, and health of calves supplemented with condensed whey solubles (CWS). Twenty-four Holstein calves (2 d old) in hutches were used in a 12-wk randomized complete block design study. Calves were blocked by birthdate and sex. Treatments were (1) control (CON) with no supplement and (2) 40 mL/d CWS. Pre-weaning CWS was fed with milk and post-weaning CWS was top-dressed on starter pellets. Calves were fed 2.83 L of pasteurized milk 2×/d during wk 1 to 5, 1 × /d in wk 6, and weaned at d 42. Starter pellets and water were fed ad libitum. Individual intakes of milk and starter pellets were measured daily. Fecal scores (0 = firm, 3 = watery) and respiratory scores (healthy ≤3, sick ≥5) calculated from the sum of scores for rectal temperature, cough, ocular, and nasal discharge were recorded daily. Body weights (BW), frame growth and jugular blood samples were taken 1 d every wk at 3 h post morning feeding. Fecal grab samples were collected in wk 12 for total-tract digestibility (TTD). Data were analyzed using MIXED procedures of SAS 9.4 with repeated measures. Significant differences were declared at $P < 0.05$ and tendencies were $0.05 \leq P < 0.10$. Total DMI was greater ($P < 0.01$) in CWS (1500 and 1580 g/d; SEM = 45.8 for CON and CWS, respectively). Calf BW (89.6 and 93.5 kg; SEM = 1.47) tended to be greater ($P = 0.06$) during post-weaning in CWS. Gain:feed (0.55 and 0.53 kg/kg; SEM = 0.02), ADG (0.81 and 0.86 kg/d; SEM = 0.03), withers height (92.3 and 93.7 cm; SEM = 0.55), plasma urea nitrogen (12.5 and 12.5 mg/dL; SEM = 0.38) and glucose (94.7 and 93.2 mg/dL; SEM = 0.96) were similar. β-hydroxybutyrate (BHB; 34.4 and 36.1 mg/dL; SEM = 1.03) was greater ($P = 0.03$) in CWS. The TTD of DM, CP, and ADF were similar and NDF (57.6 and 48.9%; SEM = 5.05) tended to be greater ($P = 0.10$) in CON. Fecal scores were similar overall with a treatment × wk interaction ($P < 0.01$). Respiratory scores were similar. Supplementing CWS improved starter intake, post-weaning BW, BHB, fecal scores, and maintained frame growth.

Key Words: condensed whey solubles, dairy calf, growth performance

440 Dietary transition from hay to silage-based TMR in weaned dairy calves: Effect on sorting behavior, intake, growth performance, and blood metabolites. M. A. Rashid*[1], Z. A. Qamar[1], H. U. Rehman[2], M. S. Yousaf[2], and M. Raza[2], [1]*Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan,* [2]*Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.*

Experiment aimed to determine dietary adaptability to transition from hay to silage based TMR during the post weaning period. Other objectives were to determine effects of dietary hay inclusion in TMR on sorting behavior, intake, growth performance, and feed efficiency (FE). Eighteen (Friesian × Jersey) weaned calves were randomly assigned to 3 TMR treatments (n = 6 calves/treatment): RG13, RG26 and RG39 containing 13%, 26%, and 39% chopped rhodes grass (RG) hay on DM basis. Total duration of experiment was 8 wk including 2 phases (4 wk each). During 1st phase (1–4 wk), weaned calves were fed on TMR diets (RG13, RG26 and RG39); thereafter, during 2nd phase (5–8 wk), all calves were shifted to a corn silage based TMR. Calves were housed in individual pens and were given free choice access to water and feed. Samples of diets fed and orts were collected twice weekly to calculate sorting % using Penn state particle separator. Data for ADG, daily DMI, feed sorting and blood metabolites were analyzed using repeated measures ANOVA; whereas, BW and FE were analyzed using one way ANOVA. To determine effect of increasing hay level in TMR diet polynomial orthogonal contrasts were used. Results were declared significant at $P < 0.05$. During 1st phase, calves sorted for short and fine

particles ($P < 0.05$) with increasing level of RG hay. However, during 2nd phase, sorting behavior was not affected ($P > 0.05$) by hay level during 1st phase. Overall, daily DMI and ADG was not affected ($P > 0.05$) by dietary treatments. However, FE was higher ($P > 0.05$) in the RG26 compared with the RG13 and RG39 calves. Glucose concentration decreased linearly ($P < 0.05$) by increasing hay level in the diets. Although calves sorted for fine particles when chopped hay inclusion was increased in post weaning TMR; however, higher ADG, FE and lower blood glucose level in RG26 suggest positive impact of hay inclusion on transition to silage based TMR.

Key Words: total mixed ration, feed preference, weaned calf

442 Growth performance and health of dairy calves supplemented with flax and soy oil. C. R. Schossow*, J. L. Anderson, and J. S. Osorio, *Dairy and Food Science Dept., South Dakota State University, Brookings, SD.*

Our objective was to investigate supplementing flax and soy oil in milk and then on starter pellets on growth performance and health of dairy calves. Thirty-six female Holstein calves in individual hutches were used in a 12-wk randomized complete block design study. Treatments were (1) control (CON) with no oil, (2) 80 g/d of flax oil (FLAX), and (3) and 80 g/d of soy oil (SOY). Pre-weaning oils were fed with the milk and post-weaning were top-dressed on starter pellets. Calves were fed 2.83 L of pasteurized milk 2×/d during wk 1 to 5 and 1 × /d during wk 6. Pellets and water were fed ad libitum. Fecal scores (0 = firm, 3 = watery) and respiratory scores (healthy ≤3, sick ≥5) calculated from the sum of scores for rectal temperature, cough, ocular, and nasal discharge were recorded daily. Body weight (BW) and frame growth were measured weekly at 3 h post-morning feeding. Results were analyzed using the MIXED procedure of SAS 9.4 with repeated measures. Significant differences were declared at $P < 0.05$. Dry matter intake (1792, 1487 and 1650 g/d; SEM = 79.5 for CON, FLAX, and SOY, respectively) was greater ($P < 0.01$) in CON than FLAX with SOY similar to both. There was a treatment × wk interaction ($P < 0.01$) with calves on FLAX eating less in the last 2 weeks of the study. Calf BW (69.8, 67.2, and 68.0 kg; SEM = 2.39) and gain: feed (0.58, 0.58, and 0.54 kg/kg; SEM = 0.03) were similar ($P = 0.38$) but had treatment × wk interactions ($P < 0.05$). The ADG (0.78, 0.71 and 0.76 kg/d; SEM = 0.05), body condition score (2.43, 2.47, 2.41; SEM = 0.027), withers height (82.1, 82.2, and 83.0 cm; SEM = 0.68) and other frame measures were similar ($P > 0.05$) among treatments. Fecal score (0.57, 0.64, and 0.70; SEM = 0.070) was similar overall but had an interaction of treatment × week ($P < 0.01$) with SOY having greater fecal scores during weaning and the last 2 wk. Body temperature and respiratory score were similar ($P > 0.05$). Supplementing flax and soy oil maintained growth performance compared with CON in the pre-weaning period when fed with milk, but decreased intake and BW during the last 2 weeks of the post-weaning period when fed with starter pellets.

Key Words: flax oil, dairy calf, growth performance

443 Amino acid supplementation in calf milk replacer. M. Terré*[1], M. Font-i-Furnols[2], A. Bassols[3], M. Vidal[1], A. Brun[2], and A. Bach[4,1], [1]*Institut de Recerca i Tecnologia Agroalimentàries, Caldes de Montbui, Spain,* [2]*Institut de Recerca i Tecnologia Agroalimentàries, Monells, Spain,* [3]*Universitat Autònoma de Barcelona, Bellaterra, Spain,* [4]*Institución Catalana de Recerca i Estudis Avançats, Barcelona, Spain.*

Supplementation of Lys, Met, and Thr in milk replacers (MR) has been widely studied, but scarce information exists about potential roles of other amino acids (either essential or not). The effects on growth performance of supplementation of 4 different amino acid (AA) combinations in a MR (25.4% CP and 20.3% fat) based on skim milk powder and whey protein concentrate were evaluated in 76 dairy Holstein male calves (2.8 ± 0.14 d old): Control without additional AA supplementation; PG providing 0.3% Pro and 0.1% Gly, respectively; FY supplying 0.2% Phe and 0.2% Tyr, respectively; KMT containing 0.62% Lys, 0.22% Met, and 0.61% Thr, respectively. All calves received the same MR feeding program and were weaned at 56 d of study. Concentrate intake was limited to minimize interference of potential differences in solid feed intake among treatments. Animals were weighed weekly, intakes recorded daily, and blood samples obtained at 2, 5, and 7 wk of study to determine plasma AA concentrations. At 7 wk of study, carcass composition was evaluated using CT in 8 calves per treatment. Data were analyzed with a mixed-effects model with repeated measures. There were no differences in growth rate among treatments (0.84 ± 0.022 kg/d), but fat carcass content was greater in Control than in FY calves, and similar among the other MR supplementation (5.95 vs 5.23 vs 5.29 vs 5.36 ± 0.181% in Control, FY, PG, and KMT, respectively). Plasma concentration (both in absolute values or relative to total AA concentration) of all AA that were supplemented increased ($P < 0.01$) with the exception of Gly that maintain similar values in all 4 treatments. However, only KMT resulted in a reduction ($P < 0.05$) of Asn/Ser, His, Pro, Val, Leu, and Ile relative proportions in plasma, suggesting an improved use of these AA when KMT was supplemented. Although no differences in growth performance were observed when supplementing the AA combinations tested herein, some metabolic changes could be envisaged considering carcass fat deposition when supplementing FY and changes in plasma AA profile changes when supplementing KMT.

Key Words: amino acid, calves, milk replacer

444 Offering drinking water at birth could improve growth performance and fiber digestibilty in Holstein heifer calves. H. K. J. P. Wickramasinghe*, A. J. Kramer, and J. A. D. R. N. Appuhamy, *Iowa State University, Ames, IA.*

Although offering drinking water from birth is recommended, the average age of dairy calves when first offered drinking water is 17 d in the US The objective of this study was to examine the impact of the 17 d delay on water and starter intake, nutrient digestibility, health, and growth performance in dairy calves during the first 70 d of age. Thirty newborn Holstein heifer calves, balanced for parity of the dam (PD), birth weight, and month of birth (MB), were randomly assigned (n = 15) to receive water at birth (W0) or at 17 d of age (W17). Calves were bottle-fed with pasteurized whole milk 3 × daily (2 to 3 L per feeding) and weaned on d 49. Body weight (BW), hip height (HH), and body length (BL) were measured weekly. Blood (jugular) was drawn on d 14 and 21 and analyzed for hematocrit and haptoglobin concentration. On d 70, total feces weight (kg/d) were measured and analyzed for nutrient composition to calculate the total-tract digestibility of nutrients. Treatment effects were analyzed using linear mixed-effect models including fixed effects of treatment, PD, MB and random effect of the calf. W0 calves drank a significant amount of free water (0.704 ± 0.123 kg/d) during the first 17 d. W17 calves consumed more grain during the first 17 d ($P = 0.015$) and drank more water ($P = 0.009$) from d 18 to 42 than W0 calves. The starter intake was similar between W0 and W17 calves from d 18 to 70. The scours prevalence was significant in both groups between d 7 and 21 but the scours severity, hematocrit, and plasma hap

toglobin concentrations were similar between the groups. The weekly BW and average daily gains were similar between W0 and W17 calves throughout the 70 d. BL and HH were similar between groups before weaning but W0 calves had greater BL ($P = 0.087$), and HH ($P = 0.068$) than W17 calves after weaning (d 50 to 70). Despite the similar grain intake, the NDF digestibility of W0 calves was greater than W17 calves on d 70 ($P = 0.075$). Offering drinking water at birth could have a positive impact on rumen development and growth performance after weaning.

Key Words: body length, hip height, NDF digestibility

Animal Health Symposium: Bovine Tuberculosis—An Ongoing Animal Health Challenge

445 Ferret transmission model for tuberculosis. T. Gupta, S. Helms, K. Sakamoto, S. Harvey, T. Ross, C. Whalen, R. Karls, and F. Quinn*, *University of Georgia, Athens, GA.*

Tuberculosis (TB) is a persistent infectious disease that threatens the health of people and numerous mammalian animal species worldwide. Estimates are that 9 million new human TB cases occur annually while animal illness rates are higher but the actual numbers are unknown. Epidemiology studies indicate that transmission among close contacts is the driving force behind TB epidemics. Until successfully treated or until infected animals are removed, cases will continue to transmit bacilli to contacts. Since antibiotic treatment for most infected animals is not practical, and a vaccine that generates sterilizing immunity has not been developed, a vaccine that controls disease transmission may be the current best path forward. Unlike rodents, infected larger animals including cattle and non-human primates (NHPs) can effectively transmit the bacilli to naïve hosts via the aerosol route; however, routine work with statistically significant numbers of cattle and NHPs is cost-prohibitive. Thus, an appropriate small animal TB transmission model for assessing vaccines is needed. Ferrets are widely employed to study the transmissibility of influenza viruses and other respiratory agents, but thus far, no studies examining these parameters for *Mycobacterium bovis* (*Mb*) and *M. tuberculosis* (*Mtb*) have been reported. We know that mice and guinea pigs intratracheally or intravenously infected with virulent *Mtb* and *Mb* bacilli develop acute disease and ultimately succumb. This is not the disease process observed in humans and animals in natural settings. In high-exposure locations, TB contacts have been found to develop acute disease but remain TST-negative and free of the pathogen, remain TST-negative but carry viable bacilli in their airways, or become TST-positive but remain culture negative. In other words, natural transmission results in variable disease responses. Our team has generated exciting preliminary data demonstrating that ferrets can become infected when given intratracheal *Mtb* bacilli, but most importantly, transmission occurs when infected transmitter animals interact with naïve sentinels co-housed over the course of at least 2 mo. Additionally, the TB disease states described above also are observed in naturally-infected ferrets.

Key Words: tuberculosis, tranmission model

446 The current status of bovine tuberculosis in the world. A. Perera*, *USDA APHIS VS/IS Mexico, Mexico City, Mexico.*

Tuberculosis is an important disease in animals and humans causing substantial morbidity, mortality, and economic loss. Significant variation exists in ways organisms of the *M. tuberculosis* complex affect animals and humans; however, there are also important intersections between animals and humans. The best example is the occurrence of *M. bovis* disease in humans and animals. The genus *Mycobacterium* includes several species that cause TB in humans and other animals. The *M. tuberculosis* complex includes *M. tuberculosis*, *M. cannettii*, *M. africanum*, *M. bovis*, *M. pinnipedii*, *M. mungi*, *M. caprae*, and *M. microti*. The tubercle bacillus infects an estimated 2 billion people with 95% of the cases in developing countries. TB is a leading cause of infectious disease-related death worldwide, estimated to cause 1.5 to 2 million yearly. Zoonotic tuberculosis (TB) in people is caused by *Mycobacterium bovis*, which belongs to the *M. tuberculosis* complex. Cattle are the most important animal reservoir for exposure of humans, but the disease affects other species and can become established in wildlife. It often affects sites other than the lungs, such as lymph nodes. In many cases, it is clinically indistinguishable from TB caused by *M. tuberculosis*. In 2015, there were an estimated 149,000 new human cases of zoonotic TB globally, and 13,400 deaths due to zoonotic TB. Africa carries the heaviest burden of disease and death, followed by Southeast Asia. The true burden is likely underestimated due to a lack of routine surveillance data Risk factors: The most common route of transmission to humans is through food Airborne infections and direct contact with infected animals pose a risk to people with frequent direct contact with infected animals or contaminated animal products. Common laboratory diagnostic procedures do not differentiate *M. tuberculosis* from *M. bovis* leading to under-diagnosis of zoonotic TB. Zoonotic TB poses special challenges for patient treatment and recovery. *M. bovis* is naturally resistant to pyrazinamide, one of 4 medications used in standard first-line anti- TB treatment regimen. Most TB patients begin treatment without drug susceptibility testing, so patients with zoonotic TB may receive inadequate treatment. Zoonotic TB in humans is often extra-pulmonary and may be misdiagnosed, and therefore initiation of treatment delayed.

Key Words: tuberculosis, diagnostic procedures

447 Development of a subunit vaccine for bovine tuberculosis. N. Guy[1], N. Rawlyk[1], M. Bains[2], O. Ihedioha[1], Z. Lim[1], K. Bock[1], S. Walker[1], C. Wheler[1], V. Gerdts*[1], J. Chen[1], R. E. Hancock[2], and A. A. Potter[1], [1]*Vaccine and Infectious Disease Organization-InterVac, Saskatoon, SK, Canada,* [2]*Centre for Microbial Disease and Immunity Research, Vancouver, BC, Canada.*

Bovine tuberculosis remains a major threat to the dairy industry in North America. Although the disease has nearly been eliminated in the United States and Canada, sporadic outbreaks occur with significant economic consequences to the industry. For example, >45,000 cattle were quarantined and >12,000 animals were destroyed in a recent outbreak in Western Canada that could be traced back to 6 cows that initially tested positive for bovine tuberculosis. Wildlife reservoirs, including bison and elk, that are known to transmit the disease to cattle, significantly hamper effective disease control and our ability to internationally trade live animals and meat products. Vaccines are urgently needed that not only prevent the disease in animals but also distinguish infected from vaccinated animals (DIVA vaccine). We are currently using reverse vaccinology methodology to develop a novel subunit vaccine and companion diagnostics that will meet these requirements. Reverse vaccinology is a relatively new approach to vaccine development. It is based on an unbiased screening of large numbers of potential vaccine candidates (proteins) in animal models. We have produced >80 different proteins that are currently being evaluated for their ability to protect calves from bovine tuberculosis. Promising vaccine candidates will be further characterized and optimized by formulation with adjuvants. The talk will provide an update on the status of the project.

Key Words: bovine tuberculosis, vaccine

448 Bovine tuberculosis—An ongoing animal health challenge.
V. Kapur*, *Pennsylvania State University, University Park, PA.*

Bovine tuberculosis (bTB) is a chronic disease that limits livestock productivity and represents a significant threat to human health with annual economic losses estimated at over $3 billion. While the disease is well controlled in the developed world, it remains endemic in many developing countries. Given the importance of the dairy and livestock industry to inclusive agricultural transformation, improved nutrition, and overall economic development, there is an increased emphasis on improving animal productivity and intensive farming techniques. However, in the absence of an effective bTB control program, intensification of dairy production is expected to drive an increase in bTB prevalence and associated animal and human disease and reduction in animal productivity (~10% reduction each in milk productivity and reproductive efficiency, and 20% loss in weight of bTB infected animals). While the implementation of bTB control programs has been documented to result in reduction in human death and suffering together with an almost 10-fold return on investment in animal productivity and economic benefit to farmers, the upfront costs associated with implementing the test and cull program in developing countries where the majority of livestock are owned by small and marginal farmers is not feasible for social and economic reasons. Hence, there is an increasing recognition of the urgent need to accelerate the development and implementation of rational evidence-based approaches to control bTB. This presentation will describe a recently initiated program in India and Ethiopia to assess vaccines to block onward transmission (cattle-to- cattle or cattle-to- other livestock, human or wildlife), as well as develop and validate fit-for- purpose diagnostic assays to differentiate vaccinated from infected animals, together providing a strong foundation for the development of effective bTB control programs in developing countries.

Key Words: bovine tuberculosis, vaccine

Breeding and Genetics: Joint ADSA and Interbull Session: Phenotyping and Genetics in the New Era of Sensor Data from Automation

449 The value of precision technologies in the genetic evaluation of dairy cows. M. van der Voort*[1], C. Kamphuis[2], and H. Hogeveen[1], [1]*Wageningen University, Business Economics Group, Wageningen, the Netherlands,* [2]*Wageningen Livestock & Research, Animal Breeding and Genetics Centre, Wageningen, the Netherlands.*

Previously, milk production recording (MPR) programs were introduced by breeding organizations to provide farmers with production information on one hand, and to evaluate genetic potential of bulls at the same time. For decades, MPR data have been the major source of production data on farms. Today, more and more data can be collected automatically on dairy farms through many innovative precision dairy technologies. These data are of potential value in genetic evaluations. However, because farmers have to invest first, a fair question is what the value of these technologies is for the farmer. Precision dairy technologies concern all the measurements on physiological, behavioral, and production variables on individual animals. This includes all hardware and software needed to capture the data, transform this into information, and support decision making. Technologies are advancing at a rapid rate and today it is possible to automatically collect data on milk yield, milk components, temperature, milk conductivity, SCC, rumen pH, progesterone, daily body weight, behavioral patterns of feeding, rumination, and activity, positioning, body condition scoring and (increasingly) more. As a result, farm management is better positioned to change from herd or group level to management by exception (i.e., cow-specific management for only those cows that require attention). Despite widespread availability, the adoption of on-farm technologies remains limited. An important reason is the uncertain economic benefit of investment. Some cost-benefit analyses have been made, e.g., for estrus detection systems economic benefits have been shown (in Europe). It is, therefore, no surprise that estrus detection systems, such as 3- dimensional accelerometers, are increasingly implemented on dairy farms. But for other promising technologies (e.g., rumen wireless telemetry for monitoring metabolic status), a lack of knowledge on what has to be measured and how to relate these measurements to actual management hinders proper economic evaluations. When cost-benefits to farmers for management purposes are unclear, implementation into genetic evaluations will remain limited; however, there is a promising future for precision phenotyping when the value proposition is clear and farmers readily adopt the technology.

Key Words: precision technologies, milk production recording programs, farm management

450 Early prediction of lactational milk, fat and protein yields using daily milk data. O. Nir (Markusfeld), G. Katz*, and L. Reuveni, *Afimilk, Kibbutz Afikim, Israel.*

Prediction of complete lactations is essential for selection decisions and production planning (quota, summer and winter production). A large-scale retrospective study (Weller and Ezra) concluded that daily milk analysis may represent more accurately cow lactation production than periodic analysis. The study objective was to adapt Weller and Ezra's methods for prospective endpoints. Performance to d 305 in lactation was predicted for individual herds and the individual cows comprising them. Such predictions should allow farmers to make decisions regarding selection and production before breeding.Annual calibration models, were constructed by using calving events with complete 305 d production data. The research employed data collected over 2 years from 15 Israeli Holstein herds with 175 to 721 annual calving events and 11,840 to 13,635 kg annual milk production. Cow and production variables (milk, fat, protein and economy corrected milk) were taken from Afilab in-line milk spectrometer and AfiMilk MPC milk meter. Stepwise multiple regression was used to construct models from training data. For validation, the models were applied to predict the consecutive year's performance employing the test data.The correlations between prediction and actual ECM/305 d was ($r = 0.688$ to $r = 0.934$) in 54 DIM when applying the model on the consecutive year's validation data set. However, success criteria for decision-making should be the deviation from actual production (−1.8% to 5.4% of ECM) and cows culled by mistake for selection, 1 − specificity (0.0% to 9.1%). Respective values at 34 DIM were −2.7% to 6.6% for deviation from 305 d production of ECM, herd level and 0.0% to 18.9% for erroneous culling. Predictions as early as 34 DIM were superior to those using routine monthly milk tests. Adding genomic predicted transmitting abilities to models may improve prediction based on early lactation data. Rates of erroneous culling for low fat (kg) in Herd 9 dropped from 21.6 to 16.2% and 11.1 to 8.3% at 54 and 84 DIM respectively, when gPTA was employed.The results allow for low risk selection, culling and production planning at 54 DIM or earlier in lactation.

Key Words: lactation prediction, genomics

451 Comparison of milk composition and somatic cell count estimates from automatic milking systems sensors and milk recording laboratory analyses. L. Fadul-Pacheco[1,2], R. Lacroix[1], M. Séguin[1], M. Grisé[1], E. Vasseur[2], and D. Lefebvre*[1], [1]*Valacta, Ste-Anne-de-Bellevue, QC, Canada,* [2]*McGill University, Ste-Anne-de-Bellevue, QC, Canada.*

Some automatic milking systems (AMS) are equipped with sensors to estimate milk components and SCC. However, the accuracy of estimates produced by these sensors is unknown. For these data to be used for herd management, benchmarking and genetic evaluations, AMS estimates should be compared against standardized milk recording laboratory analyses. The aim of this study was to characterize estimates from AMS sensors. Milk samples were collected from all milkings (2.7 ± 1.0 milkings per cow) during a period of 24-h on 10 farms using Lely Astronaut A4 AMS. Samples were analyzed for milk components and SCC. Data from the same period on milk production per individual milking and number of milkings were extracted from the AMS software. Milk composition from laboratory analyses, calculated as 24-h average weighted by milk yield, was compared with the 24-h estimate provided by the AMS. Only records based on 3 or more samples were considered in the comparison ($n = 501$). Cows were divided according to their DIM as DIM1 (5 to 100), DIM2 (101 to 200) and DIM3 (>201). Statistical analysis was done in R Studio using agricolae package. Concordance Correlation Coefficients (CCC) between AMS sensor estimates and laboratory analysis results were 0.61, 0.59, and 0.69 for fat, protein and lactose percentages, respectively. The CCC for SCC and linear score (LS) were 0.52 and 0.32, respectively. Overall mean differences between AMS and laboratory for fat and protein were small (−0.05 ± 0.5% and −0.001 ± 0.23%, respectively) but mean absolute differences for fat, protein and lactose percentages and LS were (0.38 ± 0.31%, 0.17 ± 0.13%, 0.09 ± 0.06% and 1.32 ± 0.84, respectively). Mean difference

for fat percent was larger for DIM1 compared with DIM2 and DIM3 (−0.21 vs. 0.10; $P < 0.001$), whereas for protein the DIM3 had greater difference (−0.15 vs. 0.05; $P < 0.001$), than DIM1 and DIM2. No differences were found for SCC; however, LS differences were greater for DIM3 compared with the other groups (−0.42 vs. 0.11; $P > 0.05$). Results suggest that accuracy of the estimates may differ according to the stage of lactation.

Key Words: automatic milking systems, milk recording, sensors

452 Challenges and opportunities for evaluating and using the genetic potential of dairy cattle in the new era of sensor data from automation. N. Gengler*, *ULiege-GxABT, Gembloux, Belgium.*

Sensor data from automation are becoming available on an increasingly large scale. This leads to many challenges, but also new opportunities for assessing and using the genetic potential of dairy cattle. The first challenge is data quality as all uses of sensor data require careful data quality validation, potentially using external references. The second issue is data accessibility. Indeed sensor data generated from automatization often are designed to be available on farm in a given system. However to make these data useful for genetic improvement, the data also must be made available off-farm. By nature, sensor data often are very complex and diverse; therefore, a data consolidation and integration layer is required. Moreover, the traits we want to select have to be defined precisely when generated from these raw data. This approach obviously also is beneficial to limit the challenge of extreme high data volumes generated by sensors. An additional challenge is that sensors always will be deployed in a context of herd management; therefore, any efforts to make them useful should focus both on breeding and management. However, this challenge also leads to opportunities to use genomic predictions based on these novel data for breeding and management. Access to relevant phenotypes is crucial for every genomic evaluation system. The automatic generation of reference data is a major opportunity to get access to novel, precise, continuously updated and relevant data. If challenges of bi-directional data transfer between farms and external databases can be solved, new opportunities for continuous genomic evaluations integrating genotypes and the most current local phenotypes can be expected to appear. Several examples will be given to illustrate opportunities and challenges. In particular, illustrations based on fine milk composition involving on-farm sensors using on-line colorimetry or immune-assays, and also in-line infrared spectrometric solutions will be given.

Key Words: genomics, phenotyping, management

453 High-throughput computing in support of dairy science. M. Livny*, *University of Wisconsin, Madison, WI.*

For over 3 decades, we have developed technologies to offer capabilities that address the needs of researchers with high-throughput applications. Our technologies have been adopted worldwide and the capabilities we support on the UW-Madison campus as part of the Center for High Throughput Computing (CHTC) have delivered to researchers and collaborators almost 400M core hours in the past 12 mo. These cycles have been used across the entire spectrum of science domains ranging from single PI efforts to the largest science endeavors yet undertaken in areas such as high-energy physics and astrophysics. Many of these high throughput applications involve the extraction, processing, and analysis of information from very large ensembles of observational data. A cornerstone of our technologies are tools that automate the management of very large (hundreds of thousands) interdependent computational tasks. Annually, the CHTC serves more than 200 projects across UW-Madison. Some of these high throughput projects harness more than 100K core hours per day from more than a dozen campus based computer resources. Autonomous resources are managed by the HTCondor resource and job management system and are federated by the CHTC to facilitate sharing. The CHTC also is an active member of the Open Science Grid national consortium that offers High Throughput services across more than 60 sites in the US. The CHTC has been closely involved in several UW-based phenotyping studies ranging from human health to corn yield. These studies involved machine learning, statistical inference, and image processing. In a recent partnership with UW dairy and optimization researchers, we initiated an effort to build a repository of dairy farm data and decision engine. The effort leverages the distributed computing capabilities of our technologies to automate the collection, cleaning, processing, and analyses of the collected data. Our talk will provide an overview of the open source technologies we offer, the principals that guided us in developing and deploying these technologies and how they have been leveraged by researchers in diverse fields.

Key Words: high-throughput applications

454 Image-based phenotyping: Examples from plant breeding. N. Miller*, *University of Wisconsin, Madison, WI.*

Driven by inexpensive data acquisition, storage, and compute cycles, commercial plant breeding is in the midst of a revolution in phenotyping. We will present 3 cases of video and images for plant phenotyping. (1) Maize yield is the product of component traits, such as weight per kernel, kernels per row, rows per cob, and ear and kernel shape. Tedious manual processes can be replaced by processing images of maize ears/cobs/kernels at a resolution of 1200 dots per inch. Core methods used to extract complex maize traits from images include windowed Fourier transform, counting, and Bayesian-eigen. Concordance of manual and automated measurements was high (R^2 of 0.76–0.99), and these high-throughput phenotypes have been used successfully for GWAS studies. Publicly available software for automated phenotyping has been used to analyze >250K images of ears/cobs and 5 million kernels collected over 4 years at multiple research institutions across North America. (2) Cellular-level phenotypes may prove to be a novel and rich source of information for early screening in plant breeding programs. Machine vision approaches are being studied to extract cellular level traits, including expansion and signaling, from digital microscope imagery. As storage costs continue to drop and microscope automation advances, machine vision phenotypes extracted at this level likely will become part of the breeding/screening process. (3) In contrast to high capital outlay systems, low-cost high-throughput maize imaging platforms, which acquire and stream images to a cloud provider are being developed. For one low-cost system, images contain 3 plants, a barcode to encode metadata, and a resolution standard. Software extracts traits such as leaf color, number of leaves, plant width and height, stem diameter, digital biomass, and leaf curvature. These complex traits are stored in JSON/XML format for downstream processing. In comparison to fully automated systems high cost systems, this phenotyping station costs less than $5K, is mobile, scalable, and can image ~100 plants per hour. Cost effective phenotyping to capture new and more complex traits is driving developments in automation, new analysis techniques, and high-throughput computing.

Key Words: phenotyping, high-throughput computing

CSAS Symposium: Genomic Alterations and Implications on Health—Gut and Beyond

455 Application of omics to understand host-microbial interactions in dairy cows. N. Malmuthuge[1] and L. L. Guan*[2], [1]*Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK, Canada,* [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The recent advances in next-generation sequencing (NGS) has accelerated the research studying host-microbial interactions using omics-based approaches such as transcriptomics, metagenomics and metatranscriptomics. Transcriptomics analysis reveals the functional changes of host (tissue and/or cells) at molecular level under different metabolic and physiological conditions, while metagenomics and metatranscriptomics analyses capture the whole genome (DNA) and transcriptome (RNA) repertoire of the microbiome, providing both taxonomic and functional information of microbiota. In the last decade, the rumen microbiome has been widely explored to understand phylogenetic diversities and functional characteristics, especially with the advancement of molecular biological techniques. To date, hundreds to thousands of microbial phylotypes have been identified from various rumen samples via sequencing of marker gene PCR amplicons (amplicon sequencing). However, due to the lack of available genomes for many important but uncultured rumen microbiota, there is still a large gap in accurate prediction of rumen microbial functional profiles based on the existing single species genomic information. Therefore, metagenomics and metatranscriptomics represent useful tools to globally catalog microbial gene/transcript profiles that reflect overall metabolic functions of the rumen microbiota. In addition, ruminal epithelial metabolic functions including nutrient absorption, cell proliferation, and energy metabolism influence the rumen environment via altering the ruminal pH and short chain fatty acid concentrations. However, research on rumen functionality as a whole (epithelial tissue and the rumen digesta) is lacking. To gain better understanding of the rumen function, the metagenomics-, metatranscriptomics-, and transcriptomics-based research to study host-microbial interactions in the rumen will be reviewed and the pros and cons of these methods will be discussed.

Key Words: omics, rumen, host-microbial interactions

456 Genetics of gut health robustness in dairy cows. J. C. Plaizier*[1], E. Khafipour[1], G. B. Penner[2], and M. A. Steele[3], [1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*University of Saskatchewan, Saskatoon SK, Canada,* [3]*University of Alberta, Edmonton, AB, Canada.*

A healthy digestive tract in dairy cows is critical for their production, health and welfare. An unhealthy digestive tract reduces feed intake, milk production, and milk quality, and may predispose cattle to laminitis, inflammation, diarrhea, and increased pathogen shedding. Excessive grain feeding reduces gut health by increasing the acidity, osmolarity, and endotoxin content of digesta, as well as by reducing the absorptive and barrier functions of gut epithelia, the utilization of nutrients, and the functionality of gut microbiota. Moreover, it affects the composition of digesta, and the sites of nutrient digestion. A healthy digestive tract in cows, therefore, requires healthy digesta, microbiota, and epithelia. The effects of excessive grain intake on gut health vary among cows. Hence, cows vary in their susceptibility to gut health disorders. Understanding the reasons behind this variation enables better management of gut health of dairy cows. Reasons behind the inconsistent impacts of high grain feeding on gut health include variations in the functionality of epithelia, the functionality of gut microbiota, and feeding behavior. Variation in the absorption of volatile fatty acids (VFA) in the rumen has a genetic component. Variation in the composition of gut microbiota is caused by a multitude of factors, including host genetics and environmental exposures. Feeding behavior and feed intake are also influenced by cow genetics, and medium values of heritabilities of feeding behavior traits, have been calculated. A challenge in estimating breeding values for "gut health robustness" is assessing phenotypes for this trait. This assessment may need to involve a standardized grain challenge followed by the monitoring of several gut health parameters to be effective. This approach is complicated by the invasiveness and inaccuracies of monitoring rumen pH. Metagenomics and metatranscriptomics of microbiota are probably too complex and costly to be used for routine diagnosis, but abundances of key taxa may contribute to the diagnosis. A combination of parameters, such as feed intake, fat yield and fatty acid profile, and markers of inflammation, such as acute phase proteins and endotoxins may be required. The presentation wil focus on genetics of gut health robustness in dairy cows.

Key Words: gut health, grain, genetics

457 Characterization of the bovine milk protein profile using proteomic techniques. S. L. Greenwood*, *The University of Vermont, Burlington, VT.*

Identification and characterization of the comprehensive bovine milk proteome has historically been limited due to the dichotomy of protein abundances within milk. The high abundance of a select few proteins, including caseins, α-lactalbumin, β-lactoglobulin, and serum albumin, has hindered intensive identification and characterization of the vast array of low abundance proteins within milk due to limitations in separation techniques and protein labeling capacity. In more recent years, the development and advancement of proteomics techniques has yielded valuable tools for characterization of the protein profile in bovine milk. More extensive fractionation and enrichment techniques, including the use of combinations of precipitation techniques, immunosorption, gel electrophoresis, chromatography, ultracentrifugation, and hexapeptide-based binding enrichment, have allowed for better isolation of lower abundance proteins for further downstream LC-MS/MS approaches. The different milk subfractions isolated during these processes can also be analyzed as individual entities to assess the protein profile unique to the different fractions, for instance, investigation of the skim milk-associated proteome versus the milk fat globule membrane-associated proteome. Updates to high-throughput methods, equipment, and software have also allowed for greater interpretation and visualization of the data. For instance, labeling techniques have enabled analysis of multiplexed samples and more accurate comparison of specific protein abundances and quantities across samples, while integration of gene ontology analysis has allowed for a more in-depth and visual representation of potential relationships between identified proteins. Inclusively, these developments in proteomic techniques have allowed for a rapid increase in the number of milk-associated proteins identified and a better grasp of the relationships and potential functionality of the proteins within the milk proteome.

Key Words: casein, whey, LC-MS/MS

458 Using high-throughput molecular biology techniques to study early conceptus development in dairy cows. E. S. Ribeiro*[1], J. E. P. Santos[2], F. Peñagaricano[2], E. Ticiani[1], M. R. Carvalho[1], and J. F. W. Sprícigo[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Pregnancy losses are substantial in dairy cattle and threaten reproductive efficiency. A significant portion of these losses occur during early stages of conceptus development, including the elongation phase. Elongation is a prerequisite for maternal recognition, implantation, and survival of pregnancy, and entails remarkable expansion of extraembryonic tissues along the uterine lumen in a short window of development. In a process not fully understood, the uterine histotroph increases the rate of proliferation and migration of conceptus cells and drives elongation. Using microarray technology, our research group investigated the transcriptome of bovine conceptus cells at early stages of elongation. The results demonstrated remarkable changes in biology of conceptus cells at the onset of elongation, which seem to characterize a cellular reprogramming event required for successful elongation. Functional analyses of the data revealed that substantial part of these changes are related to the metabolism of conceptus cells and, more specifically, indicated a greater utilization of lipids for synthesis of biomass, energy, cell signaling, and regulation of gene expression. Using liquid chromatography and mass spectroscopy, we investigated the composition of the histotroph and observed that lipids are in fact a constitutive part of the histotroph at the onset of elongation. Moreover, important differences in the histotroph composition were observed between pregnant and nonpregnant cows. These high-throughput platforms were also used to compare conceptuses and histotroph among groups of cows with distinct differences fertility to identify potential deviations from the norm that might be causing early pregnancy losses. We are now investigating the detailed composition of fatty acids and derivatives in the histotroph throughout the estrous cycle and early pregnancy, searching for SNPs located within regulatory regions of candidate genes believed to be important for elongation, and investigating their association with fertility traits in dairy cattle. Our long-term goal is not only to unravel the biology of conceptus elongation but also to develop strategies to minimize pregnancy losses in dairy cows.

Key Words: cow, pregnancy, omics

459 Metabolomics in dairy research: characterization of metabotype in healthy and disease states. J. Doelman*[1], L. N. Leal[1], M. A. Steele[2], and J. Martin-Tereso[1], [1]*Trouw Nutrition B.V., Amersfoort, the Netherlands,* [2]*University of Alberta, Edmonton, AB, Canada.*

Inclusion of metabolomic analysis in animal experiments offers unique perspective into how treatment responses are manifested as the final products of biological processes. Metabolomics offers a rapid, non-invasive approach to uncover specific metabolic fingerprints that are closely related to phenotype. The metabolome itself is a complex mixture of thousands of metabolites that are identified using high-throughput metabolomics platforms based on nuclear magnetic resonance (NMR) or mass spectrometry (MS). These platforms facilitate evaluation and integrated analysis of nutritional or therapeutic strategies, metabolic profiling of disease states and the identification of predictive biomarkers for the risk of disease. In dairy research, the use of metabolomics is becoming increasingly popular. The transition period in dairy production systems is associated with increased risk of metabolic diseases, particularly ketosis, metritis, mastitis, and retained placenta. Recent studies have sought to establish metabolic biomarkers, while metabolomic profiling reveals commonality among several diseases. Similarly, temporal profiling indicates metabolites associated with the development of ketosis change with disease progression, which may aid in identification of at-risk individuals. Identification of a health metabotype through the transition period has also been investigated as an innovative approach to extend the productive lifespan of the lactating dairy cow. Our own research has focused on the identifying the effects of nutritional programming on metabotype development in dairy heifers from neonates to mature, lactating cows. We found that an enhanced calf milk replacer feeding scheme in dairy heifers in early life appears to establish a lipid oxidative metabotype that is associated with elevated milk fat yield, maintenance of body condition and improved survival in first lactation. Lipid-associated metabolites are commonly identified in metabolomic evaluations in dairy cattle studies, indicating opportunity to develop nutritional and management strategies around these biomarkers. Used in conjunction with other measurements and technologies, metabolomics offers a fast and reliable tool for researchers to more fully elucidate metabolic adaptations toward understanding complex biological systems.

Key Words: metabolomics, dairy, nutrition

460 The genomic architecture of inbreeding: How homozygosity affects health and performance in dairy cattle. C. F. Baes*[1], F. Miglior[1,2], B. Makanjuola[1], C. Vogelzang[1], F. Schenkel[1], J. T. Howard[3], C. Maltecca[4], and G. Marras[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Canadian Dairy Network, Guelph, ON, Canada,* [3]*Department of Animal Science, University of Nebraska, Lincoln, NE,* [4]*Department of Animal Science, North Carolina State University, Raleigh, NC.*

Inbreeding depression is a growing concern in livestock production because it detrimentally affects fitness, health and welfare of animals. Genomic information can be analyzed to better capture Mendelian sampling, thereby enabling more accurate estimation of inbreeding. Continuous stretches of homozygous genotypes (runs of homozygosity, ROH) have been shown to provide a better estimate of autozygosity at the genomic level than conventional measures based on pedigree information alone. The objective of this study was to identify ROH associated with traits of economic importance in the Canadian Holstein population. Genotypes and phenotypic records were extracted from the Canadian Dairy Network database for all Holstein cows that had their first calving after January 1st, 2000. The different traits analyzed included milk production traits and various other traits of economic importance. Haplotypes contained within a ROH were identified and associated with the relevant traits using a 3-step algorithm. Genomic information and identification of ROH allow for the examination of the impact of homozygosity on recorded phenotypes on a genome-wide level, but also at specific regions of the genome. Multiple haplotypes when contained within a ROH were identified that had a consistent unfavorable effect both within and across groups of traits. Further investigation of these haplotypes and better understanding of their effects will be useful for a better understanding of the detrimental effects of homozygosity on fitness, health, and welfare of dairy cattle.

Key Words: genomic inbreeding, runs of homozygosity, autozygosity

461 Survey of relationship between bulk tank milk fat and true protein and de novo fatty acid content in Holstein dairy herds in the United States. D. M. Barbano*[1], M. E. Carabeau[3], H. M. Dann[2], and R. J. Grant[2], [1]*Cornell University, Ihtaca, NY,* [2]*W. H.Miner Agricultural Research Institute, Chazy, NY,* [3]*Poulin Grain Inc., Newport, VT.*

Our objective was to determine the relationship between bulk tank or tanker milk fat and true protein concentration and the concentration of de novo (DN: C4 to C14), mixed origin (MO: C16 and C16:1), and preformed (PF: > C18) fatty acids (FA) expressed as g per 100 g milk. Bulk tank or tanker milk samples from 167 Holstein farms (50 to >10,000 cows/farm) were collected from a wide geographic area within the US and analyzed for fat, true protein, and milk FA composition determined using mid-infrared red (MIR) milk analysis with a Delta model FTA MIR milk analyzer. Bulk tank and tanker milk samples were preserved with Microtab II at 1 tablet per 90 mL milk, refrigerated, and shipped overnight on ice for testing. Dairy farms included both total mixed ration and grazing feeding systems. Herd average milk production per cow ranged from 22 to 47 kg per cow per d while concentrations of fat and true protein were from 3.0 to 4.3% and 2.75 to 3.35%, respectively. Mean milk fat concentration (Y) increased significantly ($P < 0.05$) with increased DN FA concentration (X) ($Y = 2.241\ X + 1.794$; $R^2 = 0.62$) and increased MO FA concentration (X) ($Y = 1.894\ X + 1.184$; $R^2 = 0.77$) when expressed as g FA/100 g milk. Bulk tank milk fat concentration increased with increasing PF FA concentration, but the relationship was not as strong ($Y = 1.30\ X + 1.89$; $R^2 = 0.37$) as for DN and MO FA. Bulk tank milk true protein concentration increased with increasing DN FA concentration ($Y = 0.714\ X + 2.453$; $R^2 = 0.30$). The relationships over a wide geographic area of the US and more diverse farm management practices were similar to those found in a previous study of Holstein herds in northeastern US. Using data from bulk tank or tanker milk, FA composition may be useful in understanding rumen fermentation driven changes in milk fat and protein concentration.

Key Words: milk fat, milk true protein, de novo fatty acids

462 Simultaneous analysis of of three adulterants in raw milk using Fourier-transform infrared spectroscopy. D. C. S. Z. Ribeiro[1], W. L. F. Tavares[1], J. S. Lima[1], M. O. Leite[1], M. M. O. P. Cerqueira[1], L. F. Ferreira[1], J. P. A. Haddad[1], J. P. M. Heringer[1], and L. M. Fonseca*[1,2], [1]*Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [2]*FAPEMIG; CNPq PQ, Belo Horizonte, MG, Brazil.*

Fraudulent addition of foreign substances is a usual form of milk adulteration in some countries, usually to disguise poor quality parameters or even other illegal practices, such as water addition to the milk. For example, formaldehyde and hydrogen peroxide are illegaly used to reduce microbial countings, while bicarbonate is used to neutralize lactic acid formed during microbial growth. To date, not much is known about the influence of these foreign substances upon the raw milk quality evaluation using Fourier-transform infrared spectroscopy (FTIR) and flow cytometry methods. The objective of this work was to analyze the raw milk composition readings obtained by FTIR spectroscopy. Raw milk was adulterated with formaldehyde (25; 50; 100 ppm), hydrogen peroxide (100; 500; 1000 ppm) and sodium bicarbonate (300; 500; 1000 ppm) in vials containing bronopol as preservative, and stored at 2 temperatures (7°C and 25°C). Components (fat, protein, lactose, total solids, solids nonfat, MUN and SCC analyses; CombiScope FTIR, Delta Instruments), and total bacteria counting (TBC) (BactoScan FC, Foss Electric) were performed after storage (0, 3, 24, 48, 72, and 168 h). Multiple linear regression model was used for statistical analysis. The addition of these adulterants resulted in significant changes ($P \leq 0.05$) for all dependent variables. Formaldehyde addition resulted in slightly increased readings for all components, except for casein, SCC, TBC and freezing point, which decreased. Hydrogen peroxide addition resulted in higher results for evaluated milk parameters, except for lactose, casein, SCC and TBC, which decreased. Finally, sodium bicarbonate addition slightly increased fat, protein, lactose, TS, SNF, TBC and decreased casein, MUN, SCC, and freezing point results. These alterations were more impacting for MUN and TBC. It is concluded that components analyzed by FTIR method may be affected by foreign substances illegally added to the raw milk. However, through chemometric techniques, these abnormal spectrum readings have the potential to be used for FTIR monitoring of milk adulteration.

Key Words: raw milk, fraud, FTIR

463 Mid-infrared prediction of protein fractions in milk-based beverages and microfiltration retentates of skim milk. L. Di Marzo* and D. M. Barbano, *Cornell University, Ithaca, NY.*

The concentration of casein and serum protein and the relative proportion of casein to true protein in milk-based beverages and microfiltration (MF) retentates will influence their sensory and functional properties. Therefore, control of protein concentration and the ratio of casein and serum protein may be important commercially. Our objective was to develop partial least square (PLS) models using mid-infrared (MIR) spectra to predict true protein (TP), casein (CN), serum protein (SP), and CN as percentage of TP (CN%TP) content of microfiltration (MF) retentates and unflavored milk-based beverages. A total of 625 milk formulations varying in fat (2.5, 3.2, 3.9, 4.6, 5.3%), TP (2.5, 3.1, 3.7, 4.3, 4.9%), CN%TP (71, 75, 79, 83, 87%), and anhydrous lactose (3.5, 4.0, 4.5, 5.0, 5.5%) were produced using different combinations of skim milk ultrafiltration permeate, serum protein isolate, microfiltration retentate, cream, lactose monohydrate, and distilled water. MIR spectra were collected for each formulation, and in addition all formulations were analyzed in duplicate by Kjeldahl for total nitrogen, non-protein nitrogen, and non-casein nitrogen. Separate PLS models were developed for prediction of TP, CN, SP concentration (g/ 100 g of beverage) and CN%TP using the spectral ranges: (3,000 to 2,750, 1,800 to 1,700, and 1,580 to 1,000 cm^{-1}). The relative predictive differences (RPD) for TP, CN, SP, and CN%TP PLS models were 62.70, 31.45, 14.10, and 3.79, respectively, the standard errors of cross validation (SECV) were 0.0139, 0.0220, 0.0183, and 1.2405%, respectively, and R-squared for the models were 0.999, 0.999, 0.995, and 0.930, respectively. PLS models with RPD values <8 are not accurate enough for analytical purposes. The standard deviation of the difference (SDD) between reference and PLS predicted CN%TP was 1.236%. A more accurate prediction of CN%TP was achieved by using the PLS predicted CN and TP to calculate CN%TP (SDD = 0.558%).

Key Words: mid-infrared, casein, microfiltration

464 Measurement of anhydrous lactose content of milk: Within- and between-laboratory method performance. M. Portnoy* and D. M. Barbano, *Cornell University, Ithaca, NY.*

Historically, lactose has been reported as a calculated as residual difference of solids minus fat, protein, and a constant ash. This method generally over estimates milk lactose content by 0.2 to 0.3% lactose. Our objective was to document the within and between laboratory performance of the spectrophotometric enzymatic method (Association of Official Analytical Chemists method 2006.06) for determination of the lactose content of milk used in the USDA Federal Milk Market Order laboratories. Monthly, from January through December 2017, 6 or 7 laboratories tested 14 milk samples in duplicate and reported anhydrous lactose results. The mean relative repeatability (within lab) standard deviation (RSD_r) was 0.198 + 0.021% and relative reproducibility (between lab) standard deviation (RSD_R) was 0.352 + 0.053% for anhydrous lactose measurement in 2017 at a mean anhydrous lactose concentration of about 4.55% (mass/mass). The analytical performance of the lactose analysis method was comparable to Kjeldahl true protein analysis of milk with a mean RSD_r of 0.161 + 0.014% and RSD_R of 0.470 + 0.062% for Kjeldahl true protein measurement in 2017 at a mean true protein concentration of about 3.15% (mass/mass). To achieve better accuracy of analytical results for milk payment, dairy plant accounting for milk components, and calculation of more accurate milk energy output by dairy cows for nutrition management, the USDA Federal Milk Market Orders started reporting lactose content of milk on an anhydrous mass/mass basis starting in January 2017 and will no longer report lactose by difference.

Key Words: anhydrous lactose, method performance

465 The relationship between seasonal variation in bulk tank milk fat and true protein and milk fatty acid composition for Holstein herds. D. M. Barbano*[1], C. Melilli[1], M. E. Carabeau[3], H. M. Dann[2], and R. J. Grant[2], [1]*Cornell University, Ithaca, NY,* [2]*W. H. Miner Agricultural Research Institute, Chazy, NY,* [3]*Poulin Grain Inc., Newport, VT.*

Our objective was to determine if there is a relationship between seasonal variation in bulk tank milk fat and protein concentration and the concentration of de novo (DN: C4 to C15), mixed origin (MO: C16 and C16:1), and preformed (PF: > C18) fatty acids (FA) expressed as g per 100 g milk. Bulk tank or tanker milk samples from 46 Holstein farms was analyzed for fat, true protein, and milk fatty acid composition determined using mid-infrared red (MIR) milk analysis with a Delta model FTA MIR milk analyzer. Milk from each farm was analyzed between 6 and 25 times per month for the period of January 2014 through December 2017 as part of the routine milk payment testing at the St Albans Cooperative, St Albans, Vermont. A monthly average of the milk composition was calculated for 46 farms. The seasonal variation in fat and true protein content for the group of farms had the typical seasonal pattern with fat and true protein concentration being high in the winter months and low in the summer months. Seasonal variation of fat and true protein concentration (g/100 g milk) had a similar temporal pattern as DN + MO FA concentration (g/100 g milk), while this temporal pattern in relation to fat and true protein was not observed with variation in PF FA concentration. Mean milk fat concentration (Y) increased significantly ($P < 0.05$) with increasing DN + MO FA concentration expressed as g FA/100 g milk (X) (R-squared 0.60) and true protein concentration (X) (R-squared = 0.59). Bulk tank milk fat (R-squared 0.22) was increased significantly ($P < 0.05$), while no significant relationship ($P > 0.05$) between PF FA and true protein was observed. The typical decrease in milk fat and protein in the summer months may be related to factors that cause changes in rumen fermentation of carbohydrates and production of rumen volatile fatty acids (VFA) as reflected by lower de novo FA content of milk fat. Identification of seasonal factors influencing rumen VFA may lead to dairy cow feeding and management strategies that minimize seasonal variation in bulk milk composition for dairy product manufacture and allow more efficient production of dairy products.

Key Words: milk fat, milk true protein, de novo fatty acids

466 Vibrations during yogurt fermentation—Impact on particle formation and further texture defects. A. O. Körzendörfer*[1], P. Temme[2], E. Schlücker[2], J. Hinrichs[1], and S. Nöbel[1], [1]*Institute of Food Science and Biotechnology, University of Hohenheim, Stuttgart, BW, Germany,* [2]*Department of Chemical and Biological Engineering, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany.*

The quality of stirred yogurt is determined by texture attributes like appearance, flow behavior, and mouthfeel. In dairy industry, machines like pumps used for the commercial production generate vibrations that can spread to the fermentation tanks. During acidification, such vibrations disturb the gelation of milk proteins by causing texture defects including lumpiness and syneresis. Removal of visible particles by mechanical post-processing will lead to structure losses and lower viscosities. To study the effect of vibrations on yogurt structure systematically, an experimental setup was developed consisting of a vibration exciter (shaker) to generate defined vibrational states and accelerometers for monitoring. Tactile and audible frequencies as occurring in dairies up to 5,000 Hz can be generated. A novel method based on image analysis was established to quantify large particles ($d > 0.9$ mm) and evaluate textures objectively. Skim milk (3.4% protein) was heated to 95°C for 5 min and fermented in containers ($m = 900$ g) until pH 4.6. During acidification, containers were put on a table mounted on the shaker and vibrated from pH 5.6 to 5.2 (approximately 20 min). At a frequency of 30 Hz, amplitudes were set to different vibration accelerations up to 25 m/s^2. After fermentation, set gels were examined first and then processed into stirred yogurt for further analyses. Vibration treatments resulted in set gels with increased whey separation and firmness ($P < 0.001$). Resultant stirred yogurts showed a positive correlation between amplitude and particle number. Vibrations increased particle numbers from 37 ± 3 (control) up to 144 ± 12 (25 m/s^2, $P < 0.001$) particles per 100 g. Yogurts exhibiting a high particle number showed an inhomogeneous texture and a reduced water-holding capacity. Furthermore, the presence of large particles resulted in reduced apparent viscosities ($P < 0.001$). We concluded that vibrations increase the collision probability of aggregating milk proteins entailing a coarser network structure and stirred products with unfavorable texture properties. Manufacturers should consider vibrations as a further cause for quality defects.

Key Words: graininess, fermented milk product, yogurt structure

467 Development of a continuous cavitation-assisted thermal treatment for skim milk concentrate: Process characterization and microbial efficiency. J.Y. Sim*, S. I. Martinez-Monteagudo, and S. Anand, *Dairy and Food Science, South Dakota State University, Brookings, SD.*

Most of the thermoduric organisms and their spores are capable of surviving pasteurization (75°C/15 s), and they can enter downstream processes. Combinations of high temperatures and short holding times (130–150°C/2–10 s) are effective strategies to reduce the levels of thermoduric bacteria. However, the intense application of heat often

leads to over-processing, affecting quality and nutritional content. Strategies leading to reduce the impact of thermal processing have become a topic of industrial interest. A promising technology under development at our research group consists in combining the energy released by hydrodynamic cavitation with thermal treatment to reduce the levels of thermoduric sporeformers. This study summarizes our efforts in developing a continuous cavitation-assisted thermal treatment for the reduction of thermoduric sporeformers in skim milk concentrate (SMC). The experiments were conducted using a pilot scale cavitator coupled with a custom fabricated thermal unit. The SMC temperature in different processing steps was monitored during experiments. The increased in the temperature (delta T) due to cavitation increased with the SMC total solids (TS), yielding values of 31.35 ± 2.7, 35.15 ± 1.6, and 42.85 ± 1.4°C at 11, 25, and 36%, respectively. The experimental delta T data were modeled using a polynomial equation showing a satisfactory correlation ($R^2 = 0.98$). The microbial efficiency was evaluated in SMC (36% TS) inoculated with vegetative cells of *Bacillus coagulans* (ATCC 12245). The inoculated samples (4.67 ± 0.18 log) were treated by cavitation (60 Hz and 50 L h^{-1}), thermal treatment (75°C/15 s), and combined cavitation-thermal treatment. The 4.67 log of vegetative cell was reduced to 1.17 log by cavitation-thermal treatment, while thermal treatment reduced to 1.90 log. Contrary, individual cavitation did not produce any significant reduction. The outcomes of this study present opportunities for utilizing cavitation-assisted thermal treatment for inactivating thermoduric sporeformers and potentially the spores by a single pass.

Key Words: cavitation, skim milk concentrate

468 Controlling milk oxidation during high intensity retail LED light storage requires light-blocking and oxygen-barrier packaging properties. A. Wang*[1], C. H. Dadmun[2], R. M. Hand[3], and S. E. Duncan[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*College of Charleston, Charleston, NC,* [3]*Michigan State University, East Lansing, MI.*

Lighting in dairy retail cases have largely transitioned from fluorescent lighting to light-emitting diode lights (LED) to reduce energy consumption. However, LED light intensities in excess of 5000 lx are evident in retail cases, creating high potential for rapid and detrimental oxidation and destroying milk freshness. In this study, we investigated the interaction between packaging material, LED light intensity, and lighting exposure time on limiting milk oxidation. We compared 7 packaging conditions including traditional packaging [glass, translucent high density polyethylene (HDPE)], experimental packaging [white pigmented HDPE (4.9% TiO_2), white pigmented polyethylene terephthalate (PET, 4% TiO_2)], and light-exposed (clear PET) and light-protected control (foil-wrapped HDPE and PET) against 2 LED light intensities (1052 ± 484 lx and 5691 ± 512 lx) after 4 and 24 h. Higher LED light intensity (>5000 lx) and longer lighting exposure time (>24 h) significantly decreased ($P < 0.05$) dissolved oxygen and riboflavin concentration, and increased ($P < 0.05$) TBARS (final oxidation products) value in milk packaged with traditional and light-exposed packaging. Within 4 h of light exposure, white HDPE and white PET effectively protected milk freshness at both light intensities. After 24 h of light exposure, white HDPE failed to protect milk freshness while white PET successfully slowed down the milk oxidation rate at both light intensities based on TBARS and electronic nose analysis. To understand the effect of LED light intensity on consumer's choice of milk packaging, 72 frequent milk consumers were asked to select milk packaging from 2 retail cases with different light intensities. Among them, 50.9% of consumers preferred milk packaging displayed in low light intensity retail case while 49.1% of consumers preferred high light intensity. Light-blocking TiO_2-added PET provides better milk protection in high light intensity retail conditions, protecting milk nutrients and flavor, by limiting riboflavin activation and oxygen availability.

Key Words: milk, light intensity, packaging

469 Reconstitution of MFGM phospholipids in liposomes—Physical and chemical characterization. J. Ortega-Anaya*, I. García-Cano, D. Rocha-Mendoza, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

The MFGM is a very complex component in milk that provides phospholipids, including sphingomyelin, which are of great interest in human nutrition and have demonstrated biological activity in intestinal cells and more recently in early brain development. It is structured in a specific highly organized architecture in raw milk. After processing, we assume that this architecture/structure in the MFGM is a good model for developing functional dairy foods. MFGM and its components possess an interesting technological function as an ingredient. However, there is little information regarding the microstructure, physical and chemical features of highly pure MFGM phospholipid powders after hydration. In this work, we aimed to reconstitute and characterize MFGM phospholipids into liposomes (800 nm diameter) to investigate structural features upon hydration by confocal fluorescence microscopy. We also determined their physicochemical parameters such as z-potential by dynamic light scattering. Additionally, we conducted stability studies calculating the changes in the Tm value by fluorescence-based thermal shifts using SYPRO Orange dye as the fluorescent probe. A specific mixture of milk phospholipids kindly donated by Fonterra Co-Op, NZ was used to produce our vesicles by extrusion of a thin film. We have found that MFGM reconstituted liposomes (1.5 mg/mL in PBS buffer pH 7.4) associate into unique vesicles generating a binary mixture of liposomes (250 and 800 nm diameter) with a charge in colloidal solution of −21.5 mV indicating that negatively charged phospholipids are assembled predominantly in the surface of the liposomes.

Key Words: milk fat globule membrane (MFGM), liposomes, milk phospholipids

Dairy Foods V: Processing: Utilization of Whey

470 Use of acid whey protein as an ingredient in nonfat set-style yogurt. B. Wherry*[1], D. Barbano[2], and M. A. Drake[1], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Acid whey, resulting from the production of soft cheeses, is a disposal problem for the dairy industry. Few uses have been found for acid whey because of its high ash content, low pH, and high organic content, which requires costly waste treatment. In 2015, about 400 million pounds of cottage cheese curd was produced in the United States. This process results in acid whey disposal of over 18 million pounds of whey protein annually. The objective of this study was to explore the potential of recovery of whey protein from cottage cheese acid whey for use in yogurt applications. Cottage cheese acid whey and Cheddar cheese whey were produced from standard cottage cheese and Cheddar cheese make procedures, respectively. The whey was separated and pasteurized by high temperature short time (HTST) pasteurization and stored at 4°C. Food grade ammonium hydroxide was used to neutralize the acid whey to a pH of 6.4. The whey was heated to 50°C and concentrated using an ultrafiltration system equipped with 11 polyethersulfone cartridge membrane filters (10,000 kDa cutoff) to 25% total solids, 80% being protein. Skim milk was concentrated to 6% total protein. Nonfat, unflavored set-style yogurt made with acid whey protein (AWP) was compared with yogurt made with additional sweet whey protein (SWP) and yogurt made from skim milk concentrate (SM). Yogurt mix was standardized to protein, lactose, and fat of 6.00, 6.50, and 0.10% respectively, with 37% of the protein coming from the whey. Yogurt was fermented in 6-ounce plastic cups at 43°C to pH 4.6 and stored at 4°C. The experiment was replicated in triplicate. The yogurts were measured over a period of 8 weeks for pH, titratable acidity, whey separation, color, and gel strength. Trained panel profiling was conducted on d 0, 14, 28, and 56. Yogurt made with AWP had similar color and titratable acidity to those made with SWP ($P > 0.05$). Yogurt with AWP had higher values of syneresis, lower gel strength, higher sour taste and lower firmness and viscosity compared with yogurts made with SWP ($P < 0.05$). Both yogurts with AWP and SWP were distinct in sensory characteristics from the control yogurt made from SM and from each other. These results indicate that AWP can be used as an ingredient in yogurt.

Key Words: acid whey, yogurt, flavor

471 Calcium precipitation as a pretreatment for improving the membrane filtration behavior of acid whey from Greek-style yogurt. A. G. Ortiz Quezada*, A. C. Asaf, C. I. Moraru, and G. L. Sacks, *Cornell University, Ithaca, NY.*

Acid whey from Greek-style yogurt (GSY) is characterized by a high mineral content and low pH, which cause challenges for both its utilization as an ingredient or further processing. For example, the high concentration of calcium phosphate in GSY acid whey can lead to fouling during membrane processing, including reverse osmosis (RO) for concentration or ultrafiltration (UF) for fractionation. In this work, we sought to improve the filterability of GSY acid whey by developing an optimized pretreatment before membrane filtration. The pretreatment approach was to precipitate Ca by addition of phosphate salts. GSY acid whey (average pH = 4.4) was sourced from a NY State dairy. The effects of pH (6 to 10), calcium-to-phosphorus (Ca/P) ratio (0.3 to 1.3), temperature (40 to 70°C), and holding time (10 to 60 min) on Ca removal from acid whey were evaluated using response surface methodology. A central composite orthogonal design was employed, with 30 experiments per replicate. The amount of Ca removed by precipitation ranged from 62 to 97%. The most important parameters affecting Ca reduction were pH, Ca/P ratio, and the pH × Ca/P interaction, all of them with a P-value <0.01. Temperature and time did not have a significant effect on Ca reduction (P-value >0.05). Optimal conditions for Ca removal were found at pH ≥9 and a Ca/P ratio of 0.55. Under these conditions, Ca and Mg were reduced by 97% and 85%, respectively. This work will help identify a method for improving the processing behavior of acid whey, which will benefit the dairy industry and will reduce the environmental impact of this dairy byproduct.

Key Words: acid whey, filterability, Ca precipitation

472 Suitability of biomass produced by anaerobic digestion of manure and whey as a renewable peat moss substitute and economic assessment. D. J. McMahon*[1], D. Fallon[1], D.V. Bailey[3], N. Yousef[1], and C. Hansen[2], [1]*Western Dairy Center, Utah State University, Logan, UT,* [2]*Nutrition, Dietetics and Food Sciences Department, Utah State University, Logan, UT,* [3]*Applied Economics Department, Utah State University, Logan, UT.*

Previous studies have shown that use of an anaerobic digester for treatment of manure and whey mixtures has not been profitable for farmstead cheese operations in the United States because of the large capital expenses and low energy prices. Our objective was to determine the feasibility of using the biomass produced by anaerobic digestion as a substitute for peat moss (a non-renewable resource) that is used extensively in gardening and potting mixes. Cow manure and a manure-whey mixture containing 15% cheese whey were anaerobically digested in a 60 L induced bed reactor to steady state with a hydraulic retention time (HRT) of 10 d at 35°C. The effluent was collected and sent for microbial profiling as a soil amendment. Mean reduction in total fungi and protozoa (flagellates, amoebae and ciliates) from their initial levels of 3×10^3 mg/mL, 7×10^4/g, 3×10^6/g and 4×10^2/g was 99.9, 99.2, 99.8, and 96.8%, respectively. Reduction in COD was 87.5% and and 92.1% for manure and manure-whey mixture, respectively. The effluent was then air-dried and analyzed for its water holding capacity and suitability for use as a potting mix by measuring total porosity, and air capacity in comparison to a commercial potting mix. Both digested manure and manure-whey effluents were significantly ($P < 0.05$) higher in all 3 categories. The digester biomass had almost double the total porosity (66.7%) and water holding capacity (61.0%) of the potting mix (38.1%, 35.9%). Based on computer modeling of anaerobic digestion it was determined that maximum biomass production could be achieved if the HRT was reduced to 3.3 d. For a 210-cow in which manure is mixed with 25% whey this would produce 3.93 $yard^3$/d of biomass and 12,300 ft^3/d of methane. Based upon this scenario, net present value (NPV) and internal rate of return (IRR) at 12% discount rate over 20 years was dependent on the wholesale price of the biomass and the renewable energy credits (REC) for electricity generated. At a REC of \$0.01/kWh and \$0.95/ft^3 for biomass, the NPV is −\$18,205 and IRR is 7.64%. This increases to NPV of \$114,926 and IRR of 34.7% if the biomass is sold at \$1.45/ft^3 in comparison for peat moss that is sold at \$2.45/ft^3.

Key Words: anaerobic digestion, manure, whey

473 Whey tonics: One-step fermentation of acid whey to acetic acid. M. R. Lawton* and S. D. Alcaine, *Department of Food Science, Cornell University, Ithaca, NY.*

Effective utilization of acid whey remains a challenge for the Greek yogurt industry. The most common uses of acid whey are (1) application to agricultural fields, (2) incorporation into animal feed, or (3) as a feed source for anaerobic digesters. Acid whey contains several components with the potential for value-added consumer products. Wellness tonics or drinkable vinegars, which are high in acetic acid, are becoming popular among consumers for their health benefits. One of the main components in acid whey, lactose, can be converted to acetic acid through microbial fermentation. Vinegar has a 2-step fermentation process: anaerobic fermentation by yeast to convert sugar to ethanol, and aerobic fermentation by *Acetobacter* to convert ethanol to acetic acid. *Brettanomyces clausennii*, now popular in craft beers, is unique in its genus for its ability to use lactose. Under anaerobic conditions, this yeast can convert lactose to ethanol, and under aerobic conditions to acetic acid. Fermentation by *B. clausennii* has the potential to simplify vinegar production, allowing production of novel, dairy-based acetic acid beverages. The objective of this study is to initially characterize aerobic fermentation of lactose in acid whey to acetic acid by *B. clausennii*. Acid whey (250 mL) was inoculated with *B. clausennii* to obtain a concentration of ~10^5 cfu/mL. The fermentations were incubated at 30°C with shaking. Samples were taken every 24 h for 3 d. Levels of acetic acid were measured with an enzymatic assay kit. The experiment was repeated 3 times and the samples were analyzed in duplicate. A Student's *t*-test was used to determine significant differences in acetic acid levels in the treatment between d 0 and d 3. After 3 d the acetic acid concentration in the fermentation significantly ($P < 0.05$) increased from 0.09 ± 0.01 g/L to 2.70 ± 0.27 g/L. The control acetic acid levels were 0.09 ± 0.00 g/L on d 0 and 0.10 ± 0.01 g/L on d 3. These results show that there is potential to create a one-step fermentation process of acid whey to vinegar with *B. clausennii*. Further studies will include optimization of fermentation conditions and sensory evaluation.

Key Words: acid whey, byproduct, value-added

474 Carotenoids from dairy waste: evaluation of astaxanthin produced by *Haematococcus pluvialis* fed de-proteinized whey permeate. M. A. Brandt*[1], C. E. Tyl[1], J. G. Stepanek[2], M. L. Julius[2], and T. C. Schoenfuss[1], [1]*University of Minnesota, St. Paul, MN,* [2]*St. Cloud State University, St. Cloud, MN.*

Haematococcus pluvialis algae can be used to produce the valuable carotenoid pigment astaxanthin. Low value de-proteinized whey permeate (DWP) contains a high concentration of lactose, nitrogen, and ash that algae can use for growth. This project evaluated feeding DWP to *H. pluvialis* to produce astaxanthin. Astaxanthin produced by *H. pluvialis* is mainly present in esterified forms, rather than as free-astaxanthin, complicating quantification. A goal of this study was to develop an extraction and de-esterification method to more accurately quantify astaxanthin. Three solvent extraction methods were evaluated for the amount of oil extracted from freeze-dried *H. pluvialis* biomass. Acetone with grinding in a glass tissue grinder; 2:1 chloroform:methanol with sonication; and 1:1 petroleum ether:diethyl ether with 26% HCl pretreatment; The oil extraction efficiency was 8.9% (SD = 0.97), 14.5% (SD = 0.73), and 33.0% (SD = 0.21), respectively (n = 6). To de-esterify the astaxanthin to allow quantification by HPLC-UV with a free-astaxanthin standard, cholesterol esterase and chemical hydrolysis by KOH were compared. HPLC evaluation determined the cholesterol esterase hydrolysis method was more effective and allowed for quantification of the astaxanthin isomers. Two liters of *H. pluvialis* stock culture (ca. 25% peak density) were grown in 20 L carboys for 24 h at 4000 lx light under the following 3 feeding conditions, in duplicate: standard algal growth medium; standard algal growth medium + 10 g/L DWP; 10 g/L DWP. Biomass was removed and freeze-dried. Algal oil was extracted using the developed method. Carotenoids were analyzed by HPLC-UV/VIS and MS/MS. Total astaxanthin dry weight was 3.33 µg, 4.40 µg, and 3.61 µg per treatment, respectively. All free-astaxanthin isomers were included in this quantification, pending identity confirmation via MS. These results indicate that *H. pluvialis* can metabolize DWP, and that DWP alone provides sufficient nutrients for *H. pluvialis* growth and production of astaxanthin. This is promising for dairy processors, who can utilize this application for DWP as a novel revenue stream.

Key Words: astaxanthin, algae, permeate

475 The fermentation and distillation of sweet and acid whey and comparison of volatile compounds present in each distillate using headspace solid-phase microextraction (HS-SPME)-GC/MS. D. Risner, E. Tomasino, D. Batty, P. Hughes*, and L. Meunier-Goddik, *Oregon State University, Corvallis, OR.*

Disposal of whey is an economic and environmental challenge for artisanal creameries and acid whey producers. One possible solution is to ferment and distill whey to produce an alcoholic beverage that may be sold at a premium. The objectives of our research were to evaluate the fermentation and distillation of acid and sweet whey and to compare aroma profiles of the distillates. Acid whey was obtained from cottage cheese production and sweet whey from cheddar production. Volatile compounds from the distillates were extracted using headspace solid-phase microextraction and identified via gas chromatography- mass spectrometry (GCMS). Several differences in volatile composition were identified. Methyl ketones: 2-heptanone, 2-pentadecanone, 2-tridecanone, and 2-undecanone were found to only be present in artisanal creamery's distillate, whereas geraniol was found only to be present in the acid whey distillate. Significant differences in other compounds peak area were also identified. This research demonstrates that distilled spirits can be obtained from acid whey as well as sweet whey. Differences in aroma compounds likely have an effect upon the organoleptic qualities of spirits produced from whey.

Key Words: artisan vodka sustainability

476 Fetal exposure to thermal stress has long-term effects on mammary morphology and function in dairy cattle. A. L. Skibiel*, B. Dado-Senn, T. F. Fabris, D. R. Silva, G. E. Dahl, and J. Laporta, *University of Florida, Gainesville, FL.*

Cows experiencing heat stress in utero produce less milk in their first lactation relative to their normothermic herdmates. However, it is unclear if heat stress in utero affects development of the mammary gland (MG), which may affect future milk yield. We hypothesized that exposure of the fetus to heat stress in utero alters mammary microstructure and cell processes that determine mammary cell number later in life. Heifers (in-utero CL [IUCL], n = 10; in-utero HT [IUHT], n = 9), were born to dams housed in shaded barns either with fans and soakers or without cooling devices during late gestation (dry period, ~46 d), respectively. During their first lactation, heifer milk yield was recorded from calving to 84 d in milk (DIM). MG were biopsied at 21 and 42 DIM. Sectioned tissues were stained with Masson's trichrome to visualize morphology. Apoptosis and proliferation of mammary cells were determined through immunohistochemistry (TUNEL and Ki67, respectively). Alveoli luminal area was measured, alveoli were counted, MG connective tissue was quantified, and TUNEL and Ki67 positive and negative mammary epithelial and stromal cells were counted in Image J. Data were analyzed by repeated measures ANOVA or generalized linear mixed models using SAS. IUCL had lower colostrum yield (3.7 vs. 5.5 ± 0.4 kg/d, $P = 0.01$) but higher milk yield (31.5 vs. 30.2 ± 0.4 kg/d; $P = 0.05$) relative to IUHT. Alveoli number was similar between groups, but MG of IUCL tended to have larger alveoli than IUHT (4,390 vs. 3,577 ± 348 μm^2, $P = 0.11$). The MG of IUCL tended to have less connective tissue in the stromal compartment relative to IUHT (86,727 vs. 15,8329 ± 27,616 μm^2, $P = 0.08$). IUCL tended to have a greater percent of proliferating cells in MG, driven by a significant difference in percent stromal cells proliferating (2.0 vs. 1.0 ± 0.4%, $P = 0.05$). There was no difference between groups in percent mammary epithelial cells or stromal cells undergoing apoptosis ($P > 0.05$). These results suggest that fetal exposure to heat stress in utero adversely affects mammary development in the first lactation, with consequences for milk yield.

Key Words: intrauterine environment, fetal programming, mammary microstructure

477 In vitro histone manipulation of bovine mammary epithelial cells through methionine supplementation. F. Rosa* and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Recently the effects of methionine (Met) supplementation in milk performance of dairy cows through gene expression regulation have become more evident. Histone methylation (HM) can affect gene expression and consequently milk biosynthesis. Therefore, we evaluated the effect of Met on histone methylation in bovine mammary epithelial alveolar cells (MacT) incubated at increased concentrations of L-methionine. Prior to transfection cells were cultivated in high glucose Dulbecco modified Eagle's medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and Fungizone antimycotic. The plasmids used in this study were the pcDNA3-K9 and pcDNA3-K27 (Addgene) for analysis of HM through fluorescence resonance energy transfer (FRET) technology. Cells were seeded 24 h before transfection at 30,000 cells/well in a 96-well plate. Cells were transfected with Lipofectamine 3000 as the transfection reagent at 0.3 uL/well and at 50 ng/well of plasmid in a reduced serum medium (OptiMEM) deprived of FBS. Transfected cells were treated for 24h in triplicates with 0, 125, 250, and 500 μM L-methionine. An inverted fluorescent microscope for live imagining (EVOS FL Auto) equipped with a motorized scanning stage, and an environment-controlled chamber at 37°C and 5.0% of CO_2 was used to take 4 pictures/well at 4x magnification 0, 12, and 24h post-treatment. Transfection efficiency, viability, and quantification of HM were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS and significance was declared at $P \leq 0.05$. Least squares means separation was corrected using Tukey's test. Overall HM in K9 increased ($P \leq 0.05$) in cells incubated with Met as early as 12h post-treatment, and this effect remained until 24h. The HM in K27 by Met seemed to be less effective, in fact, HM tended ($P = 0.09$) to be lower in cells incubated with 500 uM of Met than control. To expand on these effects, global DNA methylation and gene expression analysis will be performed. Our results indicate that Met treatment can affect the HM status of histone tail residues differently, and this can result in profound changes in gene expression regulation. The extent of dietary Met in HM at the mammary gland level and consequently milk synthesis remains unknown.

Key Words: methionine, milk genomics

478 mTORC1 regulates de novo lipid synthesis in bovine mammary epithelial cells. M.-A. Guesthier*, J. Huang, and S. A. Burgos, *Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, Québec, Canada.*

Although metabolic pathways for milk synthesis are well understood, less is known about the mechanisms that control these processes. The mechanistic target of rapamycin complex 1 (mTORC1) is an evolutionary conserved nutrient-sensing pathway that plays a central role in the regulation of nutrient metabolism. Previous work has implicated mTORC1 in the control of protein synthesis in bovine mammary epithelial cells (BMEC), but whether it is involved in regulation of other milk components like lipids is not known. The objective of this study was to assess the role of mTORC1 on de novo lipid synthesis in BMEC. Primary BMEC were isolated from lactating mammary tissue of 3 independent cows and induced to differentiate by addition of lactogenic hormones (5 µg/mL insulin, 5 µg/mL prolactin and 5 µg/mL hydrocortisone). Data were analyzed using a randomized complete block design using PROC MIXED in SAS. Treatment differences were considered significant when $P < 0.05$. To assess the role of mTORC1 on lipid synthesis, BMEC were treated with a vehicle (control) or 100 nM rapamycin, a specific inhibitor of mTORC1, for 16 h. Phosphorylation of the mTORC1 targets eukaryotic initiation factor 4E binding-protein 1 (4EBP1) Thr70, ribosomal protein S6 (rpS6) Ser240/44, and S6 kinase 1 (S6K1) Thr389 were reduced ($P < 0.05$) in BMEC treated with rapamycin, compared with control cells, confirming mTORC1 inhibition. To determine the effect on mTORC1 activity on de novo lipid synthesis, we measured ^{3}H-acetate incorporation into total cellular lipids by liquid scintillation counting. We found that rapamycin-treated cells showed a decrease ($P < 0.05$) in total lipid synthesis which was reduced by 24% in treated BMEC. To identify potential mechanisms by which mTORC1 regulates lipogenesis in BMEC, we measured the mRNA abundance of enzymes involved in lipogenic pathways by real-time qPCR. *SREBF1* gene expression that encodes for sterol regulatory element binding protein 1, a master lipogenic transcription factor, was decreased ($P < 0.05$) by 20% in rapamycin treated BMEC. Rapamycin also reduced

expression of the key lipogenic genes *FASN*, *FABP3* and *DGAT1*, but not of *SCD1* or *ACACA*. In conclusion, these results demonstrate an important role for mTORC1 in regulation of de novo lipid synthesis in BMEC. *Funded by grants from NSERC to S.A.B. and a scholarship from Op+Lait-FRQNT to M.-A.G.*

Key Words: bovine, mechanistic target of rapamycin complex 1 (mTORC1), lipid

479 Citrate and choline in milk are biomarkers of mammary inflammation in heat stressed and LPS challenged dairy goats. A. Contreras-Jodar*[1], S. Love[1], N. Mehaba[1], G. Caja[1], and A. A. K. Salama[1,2], [1]*Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*South Dakota State University, Brookings, SD.*

As a follow up of a previous experiment done in dairy goats (Love et al., 2016; ADSA Annual Meeting) to elucidate the response to an *E. coli* endotoxin (LPS) challenge, milk data by nuclear magnetic resonance (^{1}H-NMR) from Murciano-Granadina dairy does in mid-lactation (n = 8; 2.2 ± 0.1 L/d) and submitted to 12–12 h photoperiod and thermoneutral (TN; THI_{NRC} = 65–59; n = 4) or heat stress (HS; THI_{NRC} = 83–75; n = 4) conditions (day-night, respectively), were reprocessed. THI (thermohigrometric) values were calculated according to NRC (1971). On d-12 of experiment, TN and HS does were infused with 2 mL of LPS (10 µg) in one udder-half, at random, whereas the other udder-half was with saline. Milk samples were collected post-LPS challenge (h 0, 4, 6, 12 and 24) and analyzed by ^{1}H-NMR spectroscopy. Data analyses were done by R v.3.2.3 and included PCA (principal component analysis) and PLS-DA (partial least square-discriminant analysis) assessment, with cross validation leave-one-out, to detect milk biomarkers. Biomarkers were identified by cow's milk data (Sundekilde et al., 2013). Milk citrate increased in HS does (R^2 = 0.96; Q^2 = 0.24) indicating a shift in macrophages mitochondrial function (i.e., transporting mitochondrial citrate to cytosol to produce inflammatory mediators such as PGE_2, ROS and NO). When HS does were challenged with LPS, there were dramatic increases of choline, *N*-acetylcarbohydrates (i.e., *N*-acetylactosamine and *N*-acetyglucosamine) and L-lactate, as well as, a strong decrease of lactose in milk (R^2 = 0.79; Q^2 = 0.49). Expected benefits of choline (mainly synthesized from Met) were the modulation of the immune function by mean the neurotransmitter acetylcholine and the protective role of N-acetylcarbohydrates against the adherence of pathogenic bacteria to the mammary epithelium. L-Lactate reflected the activation of the immune system by mitochondrial-oxidative shift to cytosolic (glycolytic) pathway. In conclusion, the metabolomic profile of goat's milk was markedly affected by the environmental conditions and the udder health status. Milk biomarkers indicated the occurrence of inflammatory stages in the mammary gland under HS and LPS stressing conditions, being citrate and choline, respectively, the most affected metabolites. *Supported by MINECO Spain (Projects AGL-2013-44061-R and RTA2015-0035-CO3-02*.

Key Words: metabolomics, goat mastitis, heat stress

480 The effect of night restricted feeding on the molecular circadian clock of the mammary gland. I. J. Salfer* and K. J. Harvatine, *The Pennsylvania State University, University Park, PA.*

Circadian rhythms are generated within tissues through a molecular clock made of a set of transcription factors that oscillate in a 24-h manner. These rhythms can be entrained both by photoperiod and feeding time. Dairy cows display daily rhythms of milk synthesis that are altered by feeding time, but the role of molecular clocks in these rhythms is poorly understood. To determine the impact of feeding on the mammary molecular clock, 11 mid-lactation (168 ± 50 DIM) multiparous Holstein cows were used in a crossover design (5 or 6 cows per treatment) with two 25-d periods. Treatments included (1) ad libitum feed available for the entire day (AL), or (2) night-restricted feeding, where feed availability was limited to 16 h/d from 2000 h to 1200 h (NR). All cows were housed in the same 19:5 light:dark cycle. Milk samples were collected at 0700 h and 1900 h on d 11 and d 17 of each period and analyzed for fat and protein concentration. Mammary tissue was collected from all cows via needle biopsy to represent 4 times across the day (0400, 1000, 1600, and 2200 h). The expression of clock genes *Bmal1*, *Clock*, *Cry1*, *Per1*, *Per2,* and *Rev-erbα* was determined at each time point using Real-Time RT-PCR. Cosinor rhythmometry was performed using SAS Proc Mixed to determine if expression of clock genes fit a 24 h rhythm and if the amplitude and acrophase (time at peak) differed between treatments. The NR treatment reduced dry matter intake (1.9 kg), yield of milk (3.0 kg), fat (160 g) and protein (113 g), and milk protein concentration (0.06%; all $P < 0.05$), while milk fat concentration was not affected ($P = 0.91$). Daily rhythms of *Bmal1*, *Clock*, and *Per1* were not observed in either treatment. *Cry1* fit a 24 h rhythm in both treatments ($P < 0.05$), with an increased amplitude and an acrophase occurring 8.5 h later in NR compared with AL. *Rev-erbα* fit a daily rhythm in the NR treatment ($P = 0.03$) and tended to fit a rhythm in the AL treatment ($P = 0.05$). The acrophase of *Rev-erbα* was shifted 6.5 h later in NR compared with AL, but the amplitude did not differ by treatment. *Per2* did not express a daily rhythm in the AL group, but a rhythm was induced in NR cows ($P = 0.02$). These results indicate that key components of the mammary molecular clock are influenced by feeding time.

Key Words: circadian rhythm, lactation, food entrainment

481 Evaluation of increasing serotonin concentrations and effect on calcium metabolism in mid- to late-lactation dairy cows. M. K. Connelly*, S. R. Weaver, H. Fricke, J. Kuehnl, M. Klister, and L. L. Hernandez, *University of Wisconsin-Madison, Madison, WI.*

Subclinical hypocalcemia (SCH) predisposes cows to other peripartu-rient disorders. Mitigation strategies are crucial to prevent dairy cows from succumbing to SCH. Our lab has previously demonstrated that pre-partum infusions of 5-hydroxytryptophan (5-HTP), the precursor to serotonin, modulates calcium metabolism in transition period dairy cows, yet the precise mechanism in the mammary gland is unknown. The objective of this study was to determine if intravenous (IV) infusion of 5-HTP in mid-late lactation dairy cows regulated calcium metabolism in the mammary gland. This study utilized a randomized complete block design with 12 multiparous Holstein cows, blocked by parity, receiving either IV 1.5 mg/kg 5-HTP (n = 6) or saline (n = 6) for 3 consecutive days. Baseline blood samples were taken before treatment and blood and mammary tissue was collected at 0, 8, and 24 h post final 5-HTP administration. Mammary tissues were analyzed using qPCR to evaluate mRNA expression of calcium sensing receptor (CaSR), parathyroid hormone related-protein (PTHrP), plasma membrane calcium ATPase2 (PMCA2), calcium release-activated calcium channel protein 1 (ORAI1), and secretory pathways calcium-ATPase 1 (SPCA1), and whole blood samples were analyzed for serotonin and total calcium concentrations. Milk yield was recorded daily. Serotonin concentrations were increased ($P < 0.0001$) in 5-HTP treated cows. Interestingly, there was no difference in CaSR, PTHrP, PMCA2, ORAI1, and SPCA1 expression between 5-HTP and saline treated cows ($P = 0.51$, $P = 0.92$, $P = 0.43$, $P = 0.16$, and $P = 0.08$, respectively). However, total blood calcium decreased in 5-HTP compared with saline treated cows after each IV infusion ($P = 0.0074$, $P = 0.0901$, $P = 0.0008$, respectively).

Milk yield was not different ($P = 0.57$) between treatments. This data suggests serotonin's modulation of calcium homeostasis may be dependent upon physiological stage of lactation in the dairy cow. Further experiments should be conducted to determine the impact of stage of lactation on serotonin's manipulation of calcium metabolism.

Key Words: serotonin, lactation, calcium

482 Dry period heat stress impacts mammary protein metabolism in the subsequent lactation. B. Dado-Senn*[1], A. L. Skibiel[1], E. Meyer[2], S. I. Arriola Apelo[2], and J. Laporta[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

Dry period (DP) heat stress impairs milk production in the subsequent lactation, decreasing milk protein yields. We hypothesized that DP heat stress will impact milk protein synthesis in the subsequent lactation, potentially through altered amino acid (AA) transport and impaired mechanistic target of rapamycin (mTOR) signaling in the mammary gland. Holstein cows were enrolled into heat-stressed (HT, shade, n = 12) or cooled (CL, shade, fans and soakers, n = 12) treatments for a 46-d DP (THI ≥ 68). After calving, all cows were managed together and cooled. Milk yield and components were recorded (AfiFarm) daily until 84 d in milk (DIM). Milk samples were collected at 14, 42, and 84 DIM to analyze milk protein profile. Mammary gland biopsies (n = 6/treatment) were collected at peak lactation (42 DIM). Western blotting was used to determine expression and phosphorylation of mTOR signaling pathway proteins (ULK1, p70 S6K, rpS6, and 4E-BP1), and qRT-PCR to measure gene expression of mTOR targets and AA transporters (*SLC1A1, 1A5, 3A2, 7A1, 7A5,* and *36A1*). Data were analyzed by general linear mixed models in SAS. Compared with CL cows, milk protein yield and percentage was reduced in HT cows by 0.2 kg/d and 0.11%, respectively (0.95 vs. 1.15 kg/d ± 0.24, $P < 0.01$; 2.79 vs. 2.90 ± 0.02%, $P < 0.01$), but milk protein profiles did not differ. At 42 DIM, gene expression of mammary AA transporters *SLC1A1* and *SLC3A2* was upregulated in HT cows ($P = 0.02$, $P = 0.05$, respectively). Expression, rather than phosphorylation, of mTORC1 targets was altered in HT cows. Proteins stimulated by mTORC1 phosphorylation to sustain mRNA translation for milk protein synthesis, p70 S6K and its substrate RPS6, were downregulated in HT cows ($P = 0.01$, both). Proteins suppressed by mTOR phosphorylation and involved in autophagy (ULK1) or repression of mRNA translation (4E-BP1) were upregulated in HT cows ($P = 0.01$). Gene expression of RPS6 and p70 S6K did not correlate with protein expression, as they tended to be upregulated in HT cows ($P < 0.10$). In conclusion, DP heat stress reduced milk protein percentage and yield, possibly through inhibited mTOR signaling and altered AA transporter expression.

Key Words: mTOR, amino acid, heat stress

Ruminant Nutrition Platform Session II: Protein and Amino Acid Nutrition

483 Effects of infused leucine and isoleucine or methionine, lysine, and histidine on cow performance. P. S. Yoder*[1,2], X. Huang[1], and M. D. Hanigan[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*Perdue AgriBusiness, Salisbury, MD.*

Leucine and to a lesser extent, isoleucine, possess signaling properties promoting cellular anabolic metabolism via mammalian target of rapamycin, but in vivo evidence is limited. In contrast, methionine, lysine, and histidine are well documented to limit milk protein synthesis for some diets. The objective of this study was to evaluate production responses from supplementing 2 groups of amino acids in a 2 × 2 factorial design. Eight cows (102 ± 19 DIM) were assigned to 4 jugular infusion treatments consisting of saline (SAL), methionine plus lysine plus histidine (MKH), isoleucine plus leucine (IL), or MKH plus IL in a replicated 4 × 4 Latin square design. Periods were 18 d in length comprising 8 d of rest followed by 10 d of jugular amino acid infusion. Daily infusion amounts were 21 g of methionine, 38 g of lysine, 20 g of histidine, 50 g of leucine and 22 g of isoleucine. The SAL diet had observed daily deficits of 539 g of metabolizable protein, 20 g of methionine, 48 g of lysine, 7 g of histidine, 89 g of leucine, and 25 g of isoleucine according to CNCPS v6.55 and 498 g of metabolizable protein by NRC (2001). Cows were ad libitum fed a common diet consisting of 15.2% crude protein, and 1.61 Mcal/kg NE_L on a dry basis. Dry matter intake increased for MKH by 0.5 kg/d ($P = 0.05$). Milk yield increased 2.3 kg/d ($P = 0.02$) by infused IL and no change was observed for MKH ($P = 0.39$). Milk protein concentration increased by 0.13 percentage units for MKH ($P < 0.001$) whereas yield increased for both MKH and IL by 88 g/d ($P < 0.01$) and 57 g/d ($P = 0.02$) respectively. The milk protein yield increase for MKH^+IL was 145 g/d ($P < 0.01$) versus SAL. Energy corrected milk tended to increase for IL by 1.6 kg/d ($P = 0.13$). No differences were observed for fat yield or concentrations, milk urea nitrogen, feed efficiency, body weight change, or milk N efficiency, that latter being 38.6%. Increases in milk protein yield were observed from 2 groups of amino acids independently and additively which contradicts the single limiting amino acid theory that a single nutrient will limit milk protein production.

Key Words: amino acids, milk protein synthesis

484 Effects of varying extracellular amino acid concentration on amino acid transport in mammary epithelial cells. P. S. Yoder*[1,2], J. J. Castro[3], T. Ruiz-Cortes[4], and M. D. Hanigan[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*Perdue AgriBusiness, Salisbury, MD,* [3]*Dairy Visions LLC, Chandler, AZ,* [4]*Universidad de Antioquia, Medellin, Antioquia, Colombia.*

Understanding mechanisms and quantitative description of cellular responses to changing nutrient supply is critical for predicting milk component yields. Our objective was to evaluate cellular uptake and kinetic behavior of individual amino acids (AA) simultaneously. Bovine mammary epithelial cells were grown to confluency and fed media with an AA profile and concentration similar to dairy cows for 24 h. Treatments were 4 AA concentrations, 0.36, 2.30, 4.28, and 6.24 m*M*, which represents 16, 100, 186, and 271% of typical plasma AA concentrations in dairy cows. Twenty-four plates of cells (100 × 20 mm) were assigned to each treatment. Cells were first subjected to treatment media enriched with ^{15}N labeled AA for 24 h and then incubated with treatment media enriched with ^{13}C labeled AA for 0, 15, 60, 300, 900, 1800, and 3600 s. Intracellular free AA, intracellular protein-bound AA, and extracellular media AA were analyzed for concentrations and isotopic enrichment using gas chromatography mass spectrometry. Additionally, the total protein, weight, and count of cells were measured. A dynamic 9-pool model was constructed representing extracellular, intracellular, and turnover pools for individual AA and respective isotopes and then solved via numerical integration. Fluxes were extracellular to intracellular entry, intracellular efflux, protein turnover, transamination, oxidation, and synthesis. As an example, cellular influx and efflux ranged from 0.9 to 11.1 and 0 to 9.5 nmol/min for Leu whereas Ala ranged from 1.6 to 50.0 and 0.9 to 56.9 nmol/min for influx and efflux. The average root mean square prediction error as percent of the mean for the 9 pools was 14.6 and 10.6 percent for Leu and Ala respectively. When AA influx was standardized to total pool of the respective AA, Michaelis-Menten kinetics were observed with a K_m of 72 and 345 μmol for Leu and Ala respectively. The described model provides insight on individual AA transport kinetics when subjected to varying extracellular concentrations, which might be useful for better understanding and future modeling of AA uptake by the udder.

Key Words: amino acids, transport

485 Lactational performance of dairy cows in response to supplementing *N*-acetyl-L-methionine as a source of rumen-protected methionine. F. X. Amaro*[1], K. G. Arriola[1], Y. Jiang[1], D. Kim[1], A. P. Cervantes[1], V. P. Silva[1], M. C. N. Agarussi[1], J. T. Silva[1], A. T. Adesogan[1], L. F. Ferraretto[1], C. R. Staples[1], J.-S. Eun2,3, J. S. Park[3], J. O. Moon[3], D. Vyas[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT,* [3]*Biotechnology Research Institute, CJ CheilJedang, Suwon, South Korea.*

Adequate supply of essential and limiting amino acids such as methionine is critical for maintaining milk production by high-producing dairy cows. The objective was to assess the effects of supplementing incremental amounts of *N*-acetyl-L-methionine (NALM), a source of rumen-protected methionine, on DMI, milk production, and dairy efficiency in lactating dairy cows. Sixty multiparous Holstein dairy cows in early lactation (27 ± 4.3 d-in-milk) were fed for 16 wk in a randomized complete block design. Animals were grouped based on actual milk yield, and calving date. Dietary treatments included (1) control (no NALM); (2) 15 g/d NALM; (3) 30 g/d NALM; and (4) 45 g/d NALM. Diets were formulated to meet or exceed nutritional requirements of lactating dairy cows producing at least 42 kg/d of milk and to under supply metabolizable methionine (control) or supply increasing amounts with increasing NALM supplementation. Data were analyzed using the GLIMMIX procedure of SAS, using covariate in the model for all variables tested. Linear and quadratic effects with NALM were tested. Supplementation of NALM did not affect DMI ($P = 0.37$) regardless of the dose, whereas milk yield tended to be greater ($P = 0.07$) with intermediate levels of NALM (15 and 30 g/d) compared with the control. Milk true protein concentration was not influenced by supplementing NALM at 15 and 30 g/d, but these inclusion levels increased milk protein yield relative to the control ($P = 0.04$) possibly due to the increase in milk yield. Dairy efficiency (milk yield/DMI) was greatest at 30 g/d NALM ($P = 0.03$) and was unaffected by supplementing NALM at 15 or 45 g/d compared with the control. Supplementation of NALM at 30 g/d

Table 1 (Abstr. 486). Effects of dietary supplementation with RPA on milk production by dairy cows (mean ± SEM; *P = 0.016)

Treatment group	No. of cows	Milk yield (MY)		Change in MY (kg/cow/7d): d 7 − d 0	CV of MY change (%)
		d 0	d 7		
Non-handling control	14	27.7 ± 2.3	31.5 ± 3.4	3.77 ± 3.22^{NS}	321
Isonitrogenous control (Ala)	13	26.3 ± 3.5	30.9 ± 3.0	4.58 ± 3.36^{NS}	264
Unprotected Arg product	14	26.0 ± 3.3	31.3 ± 2.3	5.36 ± 3.59^{NS}	259
Rumen-protected Arg product	13	26.2 ± 2.6	33.6 ± 2.1	7.35 ± 2.70*	132

resulted in greatest milk yield, milk protein yield, and dairy efficiency when fed to early to mid-lactating dairy cows. However, the greatest amount of NALM supplementation (45 g/d) led to no beneficial effects on lactational performance. Overall results in the current study suggest that NALM supplementation can improve productive performance of dairy cows when added at 30 g/d.

Key Words: dairy efficiency, milk production, *N*-acetyl-L-methionine

486 Dietary supplementation with a rumen-protected L-arginine product enhances milk production by dairy cows. A. B. Keith, M. C. Satterfield, F. W. Bazer, and G. Wu*, *Texas A&M University, College Station, TX.*

This proof-of-concept study was conducted on HAW Farms (Belen, NM) to test the hypothesis that L-arginine (Arg) can enhance milk production by lactating cows. A rumen-protected Arg product (RPA, which contained a by-pass matrix) and an unprotected Arg product (UPA) that had the same composition as RPA were manufactured by Biotechnology Services & Consulting Inc. (Coppell, TX). Before parturition, 54 healthy Holstein cows (parities 1 to 4), weighing 550–600 kg, were assigned randomly to receive either no dietary supplementation (non-handling control) or dietary supplementation of 500 g RPA, 500 g UPA or an isonitrogenous amount of L-alanine (Ala) per day beginning on d 1 to 4 after parturition (the initial day of supplementation = d 0 of the trial). Each cow was fed twice daily a typical silage- and alfalfa hay-based lactation diet containing 17% crude protein (25 kg DM/d) to meet the NRC-recommended requirements of nutrients, and had free access to drinking water. A supplement was administered to cows twice daily (equally divided doses at 8 a.m. and 6 p.m.) by gavaging immediately after consumption of their regular meals. On d 0 and 7 of the trial, milk yields of cows were determined using an auto-milking system. On either day, the composition of nutrients did not differ ($P > 0.05$) among the 4 groups of cows. Changes in milk yields between d 7 and 0 (analyzed by the paired *t*-test) were significant ($P = 0.016$) for the RPA group but not significant ($P > 0.05$; NS) for the other groups (Table 1). Interestingly, variation in milk yield change was much less for RPA-supplemented cows, compared with the other groups of cows. These results indicate that cows fed RPA had more consistent lactation performances and produced more milk.

Key Words: arginine, lactation, milk

487 Efficiency of utilization of amino acid increased with energy supply at low and high metabolizable protein supply in dairy cows. C. Omphalius*[1,2], H. Lapierre[3], L. Bahloul[2], and S. Lemosquet[1], [1]*PEGASE, INRA, Agrocampus-Ouest, Rennes, France,* [2]*Adisseo France S.A.S, Antony, France,* [3]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Implementing the concept of a variable efficiency of use of individual AA (AA_{ef}) in feeding systems requires knowledge on the effect of net energy of lactation (E) in addition to the effect of metabolizable protein supply (P expressed as PDI, INRA). Therefore, AA_{ef} were determined at 2 levels of E (LE: 24.8 vs. HE: 32.6 Mcal/d of NEL) and 2 levels of P (LP: 1,261 vs. HP: 2,227 g/d of PDI) using a 4 × 4 Latin square with 4 cows and 2-wk periods. Individual AA_{ef} was calculated as the sum of milk protein yield (MPY), endogenous fecal (EFP) and scurf

Table 1 (Abstr. 487).

In mol/12h of N	Diet				SEM	*P*-value		
	LELP	HELP	LEHP	HEHP		E	P	E × P
$AA_{S\text{-}EU}$	7.4	7.1	13.6	13.8	0.5	0.76	<0.01	0.18
MPY	3.7	4.2	4.1	5.0	0.2	<0.01	<0.01	0.15
EFP	1.1	1.4	1.0	1.5	0.1	<0.01	0.68	0.02
Uptake								
EAA	2.3	2.6	3.0	3.3	0.1	0.02	<0.01	0.92
NEAA	1.3	1.5	1.2	1.4	0.2	0.10	0.40	0.81
AA_{eff}								
EAA	0.74	0.92	0.44	0.54	0.01	<0.01	<0.01	<0.01
NEAA	0.68	0.84	0.40	0.50	0.01	<0.01	<0.01	<0.01
MU: milk output								
EAA	1.30	1.26	1.51	1.38	0.04	0.06	0.01	0.28
Group 1[1]	1.08	1.02	1.01	1.03	0.03	0.44	0.31	0.15
Group 2[2]	1.23	1.28	1.50	1.34	0.08	0.45	0.06	0.18

[1]His, Met, Phe, Tyr, and Trp.

[2]Lys, Ile, Leu, and Val.

proteins (INRA, 2018) multiplied by their respective AA profile (Lapierre et al., 2016; CNC: 205) and divided by the AA supply minus the AA endogenous urinary losses (AA_{S-EU}). Mammary net uptake (MU) of AA (6 samples in 12 h) was determined on d 13 of each period. Data were analyzed by MIXED procedure (SAS) with cow as random effect. Increasing E and P supply increased MPY, essential AA (EAA) MU and only increasing E supply tended to increase non-EAA (NEAA) MU. Increasing E supply was partially achieved by increased DMI (14.5 vs. 19.1 kg/d) increasing EFP. Therefore, the AA_{ef} increased with increasing E supply but at a higher extent in LP diets. The gain in AA_{ef} with increasing E was mainly explained through a change in the partition of AA toward MPY and EFP at whole body level and of MU AA toward MPY as suggested by the lower EAA MU: milk output ratio. The decreased AA_{ef} with increased P supply was related to a decreased NEAA and EAA MU relative to the AA_{S-EU} and to an increased MU: milk output ratio of group 2 AA (Lys, Ile, Leu and Val), both suggesting a higher AA catabolism (Table 1).

Key Words: amino acid, efficiency, mammary gland

488 Leucine and lysine alter inflammatory response of immune cells from growing cattle. M. Garcia*, K. A. Pearl, E. C. Titgemeyer, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

Bovine neutrophils alter their inflammatory response upon amino acid supplementation, yet research evaluating effects of specific amino acids is scarce. We examined effects of lysine (Lys) and leucine (Leu) on inflammatory responses of neutrophils (PMN) and mononuclear cells (PBMC) from growing Holstein steers (173 ± 3.7 kg BW). Seven steers were used in a 6 × 6 Latin square design with a factorial arrangement of 2 levels of Lys (0 and 6 g/d) and 3 levels of Leu (0, 15, and 30 g/d). Steers were fed a basal diet and infused with all required nutrients except Lys. Treatments were delivered by abomasal infusion. Blood was collected on d 7 of each 7-d period. A hematology analyzer was used to measure blood cell composition, and immune cells were isolated using density gradients. To harvest media for cytokine measures, isolated cells were treated with lipopolysaccharide (LPS, 0 or 1 μg/mL) for 2 (PMN) or 24 h (PBMC); PBMC were treated with concanavalin-A (10 μg/mL) to enhance proliferation. PMN were exposed to dihydrorhodamine and labeled *E. coli* for 50 min to assess oxidative burst and phagocytosis. Period and steer were considered random effects, and data were transformed (square root or log10) to attain normality. Lys reduced lymphocyte numbers (6.40 vs. 6.61 ± 0.52 × 10^3/μL, $P = 0.04$). Leu (30 g/d) almost doubled eosinophil numbers but only for non-Lys fed steers (0.36, 0.31, and 0.63 ± 0.12 × 10^3/μL, $P = 0.02$). PMN phagocytic and oxidative burst responses were not impacted by treatment. In the presence of LPS, TNF-α produced by PMN (1.7, 8.5, 17.1 ± 6.9 pg/mL, $P = 0.08$) and IFN-γ produced by PBMC (384, 431, 523 ± 87 pg/mL, $P = 0.01$) increased or tended to increase linearly as Leu increased. In the absence of LPS, TNF-α produced by PBMC tended to increase quadratically as Leu increased (424, 630, 431 ± 99 pg/mL, $P = 0.08$). Regardless of LPS, Lys tended to increase production of IFN-γ by PBMC (353 vs. 400 ± 83 pg/mL, $P = 0.10$). These findings point to a role for dietary Lys and Leu in regulating immune responses.

Key Words: amino acid, immunity, steer

489 Alterations in amino acid transporters and the mTOR pathway in adipose tissue of Holstein cows during the periparturient period in response to methionine supply. Y. Liang*[1], F. Batistel[1], C. Parys[2], and J. J. Loor[1], [1]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, HanauWolfgang, Germany.*

Recent studies in non-ruminants have revealed that mammalian target of rapamycin (mTOR) signaling pathway plays a critical role in the regulation of adipose tissue function. The objective of the current study was to investigate whether increasing the methionine supply would alter mRNA expression of amino acid transporters and mTOR pathway components in adipose tissue during the periparturient period. Sixty multiparous Holstein cows were used in a block design and assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH, Germany). Mepron was fed from −28 to 30 d relative to parturition at 0.09% and 0.10% of the diet dry matter during the prepartum and postpartum period, respectively. Blood sampled from the coccygeal vessel at −14, 7, and 30 d relative to calving date was used for amino acid analysis. Adipose tissue obtained from each group on d −10, 10 and 30 relative to calving date was used for RT-PCR analysis. The statistical model included the random effect of block and fixed effect of treatment, time and its interaction. A treatment × time was observed for the plasma concentrations of Asn, Cys, and Gly due to higher responses in MET-supplemented cows ($P < 0.05$). Compared with control, MET-fed cows had greater concentration of Met, Lys, Thr, Leu, Val, and Phe in plasma ($P < 0.05$). Among the 7 amino acid transporters measured, MET-supply increased the mRNA expression of Gln (*SLC38A1*), Glu (*SLC1A1*), L-type amino acid (Met, Leu, Val, Phe; *SLC3A2*), small zwitterionic α-amino acid (*SLC36A1*), and neutral amino acid (*SLC1A5*) transporters in adipose tissue ($P < 0.05$). Expression of *AKT1*, *RPS6KB1*, and *EIF4EBP1* were also upregulated in MET-supplemented cows in adipose tissue ($P < 0.05$), suggesting enhanced insulin signaling and activation of the mTOR pathway. Overall, the data indicate that enhanced methionine supply during the periparturient period enhances circulating amino acid concentrations and the availability and uptake of amino acids by adipose tissue.

Key Words: amino acid, dairy cow, mTOR

490 Methionine supply during the periparturient period alters glutathione metabolism in adipose tissue of Holstein cows. Y. Liang*[1], F. Batistel[1], C. Parys[2], and J. Loor[1], [1]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, Urbana, IL,* [2]*Evonik Nutrition & Care GmbH, HanauWolfgang, Germany.*

The periparturient period is characterized by increased oxidative stress status. Glutathione is one of the major intracellular antioxidants and is important for alleviating oxidative stress. Nuclear factor (erythroid-derived 2)-like factor 2 (NFE2L2) is a transcription factor that induces antioxidant-responsive genes. The hypothesis was that enhanced methionine supply has a positive effect on molecular pathways associated with glutathione synthesis and activation of NFE2L2 in adipose tissue. Sixty multiparous Holstein cows were used in a block design and assigned to a control diet or the control plus ethylcellulose rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH, Germany). Mepron was fed from −28 to 30 d relative to parturition at 0.09% and 0.10% of diet dry matter during the prepartum and postpartum period, respectively. Adipose tissue obtained on d −10, 10, and 30 relative to calving date was used for RT-PCR analysis. Blood was sampled at −14, 7, and 30 d relative to calving date. The statistical model included the random effect of block and fixed effect of treatment, time and its interaction. Compared with control, MET cows had lower plasma concentrations of reactive oxygen metabolites ($P = 0.08$) and higher

concentrations of methionine ($P = 0.09$) and glutathione ($P = 0.02$). Among enzymes associated with the methionine cycle and transsulfuration, expression of cystathionine-β-synthase in adipose tissue was upregulated in MET cows ($P = 0.06$). Expression of glutamate-cysteine ligase, glutathione reductase, and transaldolase 1 in adipose tissue also was upregulated in MET cows ($P < 0.05$), suggesting a positive effect of enhanced methionine supply on intracellular reduced glutathione availability. The upregulation of NFE2L2 in MET cows also is suggestive of a link between adipose tissue methionine metabolism, glutathione synthesis, and the antioxidant response ($P = 0.06$). No interactions with time were observed for gene expression in adipose tissue. Overall, the data indicate that enhanced methionine supply during the periparturient period can benefit the availability of reduced glutathione and activation of antioxidant-responsive genes in adipose tissue.

Key Words: oxidative stress, NFE2L2, transition cow

491 Predictions of rumen outflow of amino acids in dairy cattle. A.J. Myers*[1], H. Lapierre[2], R.R. White[1], H. Tran[3], P. J. Kononoff[3], R. Martineau[2], W. P. Weiss[4], and M. D. Hanigan[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*Agricultural and Agri-Food Canada, Sherbrooke, QC, Canada,* [3]*Department of Dairy Science, University of Nebraska, Lincoln, NE,* [4]*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

The objective of this work was to update and evaluate predictions of essential AA (EAA) outflows from the rumen. The model was constructed based on previously derived equations for ruminally undegraded (RUP), microbial (MiP) and endogenous (EndP) protein rumen outflows, and revised estimates of the EAA composition of each protein fraction. Corrections were included to account for incomplete recovery of EAA during 24-h acid hydrolysis. The predicted protein and EAA rumen outflows were evaluated against a data set of observed values from the literature. Initial evaluations indicated a mean bias for nonammonia, nonmicrobial N flow ([RUP + EndP] / 6.25) of 12 g N/d. Root mean squared errors (RMSE) of EAA predictions ranged from 29.9 to 43.7% of observed mean values. Concordance correlation coefficients (CCC) ranged from 0.29 to 0.48. Except for Leu, all EAA rumen outflows were overpredicted by 4.0 to 35 g/d. In addition, significant slope bias was present for all EAA except Met and Leu. It is unclear if the observed bias in EAA flows reflects problems in the prediction equations. Under the assumption that the mean and slope biases may be due to problems in the prediction equations, regression equations were derived to adjust predicted EAA flows. The residuals of the EAA flows were regressed on each of the 3 protein flows. Residuals were correlated with each of the 3 protein flows for all EAA. After applying these new linear regressions, RMSE for EAA predictions ranged from 25.0 to 32.9% of observed mean flows. The mean bias was removed for all predicted EAA flows; however, a small slope bias was introduced for Lys, and Thr. The CCC ranged from 0.43 to 0.57. For the purposes of predicting post-ruminal EAA flows and based on fit statistics, adjusting the mean and slope biases using the adjustments from the protein EAA flows yielded marginally better results than predicting EAA flows without adjustments. Future work should focus on identifying the cause of the observed prediction bias.

Key Words: mechanistic model, amino acid, rumen outflow

492 Predicting milk protein production from amino acid supply. M. D. Hanigan*[1], H. Lapierre[2], R. Martineau[2], and A. M. Myers[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*Agriculture and Agri-Food Canada, Lennoxville, QB, Canada.*

Efficient diet design requires accurate predictions of the relationship between nutrient inputs and milk output. The NRC (2001) model has been shown to be biased in predicting overall milk production and production responses to varying dietary protein intake and exhibits relatively low precision (~25% RMSE). The objective of this work was to test revised representations of the relationship between nutrient supply and milk protein production. Data used were from 237 published studies containing 724 treatment means. Microbial protein outflows were predicted from Roman-Garcia et al. (2016), and RUP flows from White et al. (2017). The AA composition of protein flows was from Sok et al. (2017). Digestibility of the RUP AA was from Paz-Manzano et al. (2014). Two model forms were tested: Milk Protein = DEI + EAA_i + EAA_i^2 + NEAA + St + FA + Milk Fat % + DIM (1); and Milk Protein = DEI + EAA_i + $(EAA_i/DEI)^2$ + NEAA + St + FA + Milk Fat % + DIM (2) where DEI represented digestible energy intake, EAA the digested supply (g/d) of the ith essential AA, NEAA the digested total supply of other AA, and St and FA dietary starch and fatty acid contents (% of DM). Essential AA included all except Trp. All combinations of EAA terms were tested with the presence of squared terms requiring linear term presence. The best submodel for eqn. (1) contained terms for DEI, Arg, Leu, Lys, Met, Thr, NEAA, St, DIM, and milk fat with an AICc of 8191 and RMSE of 12.5%. The best submodel for eqn. (2) contained the same terms plus Phe with an AICc of 8173 and RMSE of 11.9%. Exclusion of His, Ile, and Val from both models indicates they are always provided in excess, or there is inadequate independent variance to define responses to those AA. The results indicate that expressing declining efficiency of AA use for milk protein production as a square of the ratio of AA to DEI is superior to the square of AA. These equations demonstrate that responses to individual EAA and energy are additive and our models must reflect that if improved accuracy and precision are to be achieved in diet formulation.

Key Words: essential amino acids, milk protein, model

493 A new model to predict microbial protein synthesis in the rumen. L. E. Moraes*[1], R. R. White[2], and J. L. Firkins[1], [1]*The Ohio State University, Columbus, OH,* [2]*Virginia Tech, Blacksburg, VA.*

Feeding systems worldwide use metabolizable protein as the unifying unit for computing protein availability in feeds and the protein requirements for various physiological functions. Metabolizable protein is the sum of microbial true protein synthesized in the rumen from RDP and endogenous sources and RUP. In practical feeding situations, these quantities are seldom measured, so mathematical models are needed to predict the amount of ruminally synthesized microbial protein. The objective of this study was to develop a model to predict microbial protein production in the rumen using a more mechanistic and generalizable approach by representing the supply of both RDP and rumen-degraded carbohydrates. A bisubstrate Michaelis-Menten functional form was used to associate the amounts of rumen-degraded NDF (RDNDF) and starch (RDST) with 2 Michaelis-Menten constants and the amount of RDP with the asymptotic response (Vmax). The mechanistic biological reasoning was that, from a mass balance standpoint, the amount of RDP determines the limiting microbial protein produced while the amount of degraded carbohydrates are associated with the affinity (Km) of substrate used for carbon precursors in cellular growth (derivation of cell constituents for dividing cells). The equation is an efficiency derivation with actual microbial N, and actual RDNDF and RDST derived using diet-level prediction factors (White et al., 2017; J. Dairy Sci. 100: 3611–3627). The model was fitted with 583 treatment means from 154 publications using a Bayesian nonlinear hierarchical modeling approach. The fitted model was Microbial N (g/d) = (117.0 + 0.071 × RDP)/[(1 + 0.043/

RDNDF)(1 + 0.051/RDST)] where RDP is in g/d and both RNDF and RDST are in kg/d. The Bayesian hierarchical approach allowed successful introduction of between study variability in all model parameters. Model evaluation suggested low slope and mean bias (<2% of MSE). Likewise, a 5-fold cross-validation suggested good predictive ability (Concordance Correlation Coefficient, CCC = 0.52) and relatively low root mean square prediction error (29% of mean) when compared against models from the literature.

Key Words: microbial protein, rumen degradable protein, carbohydrate

494 Diets to maximize milk protein secretion: Is the single limiting amino acid model the whole story? L. E. Armentano*, *University of Wisconsin, Madison, WI.*

This talk will challenge the idea of defining the dietary protein requirements for lactating cows based solely on the limiting amino acid concept. One form of the 'limiting amino acid model' is that milk protein yield potential is set, but it can be reduced below this potential by a deficiency in a single essential amino acid. A corollary of this is that the total dietary crude or metabolizable protein (MP) can be minimized to the point that the requirement for this first limiting amino acid is met. This model certainly includes the possibility of sequential second and third limiting amino acids, which become limiting as the original first limiting amino acid reaches its requirement. One problem with this model in the ruminant animal is that a large portion of the MP is derived from rumen microbial reproduction. Unlike animal protein, a community of microbial species has a malleable genetic blueprint and may respond to the amino acid content of the rumen-degraded protein in a complex way. Another limitation of the limiting amino acid model is that potential milk protein yield may be influenced by many dietary (and other) factors. Insulin is known to increase milk protein yield in cases where milk protein yield is limited by MP or where milk protein secretion has been increased due to additional MP. Removing crude protein from the diet and replacing it with other dietary constituents could affect circulating insulin and modulate milk protein secretion independently of the limiting amino acid. One identified mechanism for insulin is through activation of mTOR, a regulatory protein in mammary secretory cells. This same mTOR is simultaneously regulated by essential amino acids, but the essential amino acids that have the largest effect (e.g., leucine, isoleucine), are different than the those normally suggested as most limiting (methionine, lysine and histidine). Optimizing milk protein secretion and nitrogen efficiency must consider the amino acid composition of MP and animal needs, but careful experimentation and quantitative model development must consider alternatives beyond only the concept of one or a few limiting amino acids.

Key Words: insulin, mTOR, microbial

495 Impacts of short-term sodium salicylate administration and infrequent milking on early lactation cow energetics and milk yield through mid-lactation. M. Garcia*, C. Ylioja, L. K. Mamedova, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

We hypothesized that at the onset of lactation, inflammation may contribute to an adaptive temporary insulin resistance to protect against hypoglycemia, but infrequent milking may reduce nutrient demands to the extent that this adaptation is unnecessary. Thirty-three multiparous Holstein cows were used to evaluate whether sodium salicylate (SS) and milking frequency (MF) alter energy status and milk yield. Cows were randomly assigned to frequent (3 × /d) or infrequent milking (1 × /d), and SS (2.3 g/L in drinking water) or control treatments in a 2 × 2 factorial design for 5 d, beginning ~24 h postpartum. Cows were fed a standard TMR to meet nutrient requirements. For measures of digestibility, TMR, refusals, and fecal samples were collected from 3 to 5 DIM. All variables, except digestibility, were analyzed as repeated measures. SS did not interact with MF for any of the variables measured ($P > 0.10$). MF did not alter water or DM intakes, total-tract NDF or DM digestibilities during treatment ($P > 0.10$). One × /d-cows produced less milk (25.0 vs. 33.1 ± 1.3 kg/d, $P < 0.01$) and energy corrected milk (ECM, 35.0 vs. 40.4 ± 1.6 kg/d, $P = 0.02$). Fat content was greater in 1 × /d-cows (5.3 vs. 4.2 ± 0.3%, $P = 0.01$) but protein and lactose content did not differ ($P > 0.10$). Three × /d-cows were in a more negative energy balance (−13.0 vs. −8.6 ± 1.2 Mcal/d of NE_L, $P < 0.01$), produced more ECM per unit of DM intake (2.9 vs. 2.4 ± 0.1, $P < 0.01$), and had lesser milk urea N concentrations (7.8 vs. 10.1 ± 0.7 mg/dL, $P = 0.04$). When 1 × /d-cows were switched to 3 × /d at d 6, their milk yield rapidly returned to that of 3 × /d-cows and yields were similar from wk 2 to 17 of lactation (55.8 vs. 56.8 ± 1.3 kg/d for 1 × and 3 × /d, respectively, $P = 0.59$). SS-treated cows produced more milk on wk 9 (62.0 vs. 55.1 ± 2.1 kg/d, $P = 0.03$) but the average 17-weeks milk yield did not differ (55.8 vs. 56.8 kg/d for control and SS, respectively, $P = 0.59$). No other impacts of SS on these outcomes were observed. Although milking frequency had expected impacts on energetics, we found no evidence that SS influences this response.

Key Words: Inflammation, infrequent milking, transition cows

496 Mitochondrial function of cryopreserved liver biopsies during early and late lactation of dairy cows. M. Garcia-Roche*[1,2], A. Casal[2], M. Carriquiry[2], C. Quijano[1], and A. Cassina[1], [1]*Centro de Investigaciones Biomédicas - Departamento de Bioquímica, Facultad de Medicina, Montevideo, Montevideo, Uruguay,* [2]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Montevideo, Montevideo, Uruguay.*

Early lactation is an energy demanding period for dairy cows that threatens both animal health and milk yield. Metabolic changes occur in this period, affecting the liver, a key organ in gluconeogenesis and lipoprotein metabolism. We studied the impact of early lactation on hepatic mitochondrial function of dairy cows. Twenty-four Holstein-Friesian multiparous cows (664 ± 65 kg BW, 3.0 ± 0.4 BCS, spring calving) were assigned in a randomized block design to either (G0) total mixed ration (TMR) fed ad libitum (70% forage: 30% concentrate) or (G1) grazing plus supplementation from 0 to 180 d postpartum (DPP). The G1 cows grazed on *Festuca arundinacea* or *Medicago sativa* in one (6h) or 2 sessions (18h) depending on heat stress conditions 20–30 kgDM/cow/day) and were offered TMR (15 kgDM/cow/day) or supplemented with 5.4 kgDM of a commercial concentrate, respectively. From 180 to 250 DPP, all cows grazed *Festuca arundinacea* (10h; 30 kgDM/cow/day) and were offered TMR (16 kgDM/cow/day). Liver biopsies were collected at 35 and 250 DPP and cryopreserved using sequential freezing in modified UW medium using 10% DMSO. Mitochondrial function was assessed measuring oxygen consumption rates using complex I and II substrates. Mitochondrial content was determined measuring citrate synthase (CS) activity. Data were analyzed as repeated measures in a mixed model including feeding strategy, DPP and their interaction. Maximum respiratory rate and oligomycin-sensitive respiration were unchanged in G0 (6.7 ± 1 vs. 7.0 ± 1, $P = 0.6$; 2.9 ± 0.7 vs. 2.5 ± 0.7 $pmolO_2$/min/mg, $P = 0.5$) during both early and late lactation and were lower in early lactation in G1 (2.8 ± 1 vs. 7.5 ± 1, $P < 0.0001$; 1.2 ± 0.7 vs. 2.7 ± 0.7 $pmolO_2$/min/mg, $P = 0.03$). However, no differences between groups were observed in CS activity (47 ± 4 vs. 43 ± 3, $P = 0.5$; and 43 ± 5 vs. 48 ± 3 mU/mg, $P = 0.3$; for G0 and G1 respectively suggesting that mitochondrial content did not vary. Our results indicate that mitochondrial function is impaired during early lactation in cows in the grazing system and not in the TMR system, which could be related to differences in plane of nutrition.

Key Words: hepatic mitochondrial function, pasture vs. TMR, cryopreservation

497 Independent effects of metabolizable protein and heat stress affected milk production and plasma free fatty acid and insulin concentrations in dairy cows. J. D. Kaufman*, H. R. Bailey, and A. G. Ríus, *University of Tennessee, Knoxville, TN.*

Environmental and nutritional factors influence productivity of dairy cows during lactation, possibly via similar physiological mechanisms. The objective of this study was to test the effects of heat stress (HS) and metabolizable protein (MP) on milk production and plasma metabolites. Thirty-six multiparous Holstein cows (219 ± 27 d in milk) were assigned to a factorial arrangement of treatments in a completely randomized design. Treatments were imposed for 21 d and consisted of HS or cooling with fans and sprinklers (CO) and high or low MP diets [17.9% (HMP) or 14.2% (LMP) crude protein on a DM basis]. Cows were exposed to prevailing weather of July and August in Tennessee. By design, CO cows were feed restricted to match intake of HS cows receiving the LMP treatment. Body temperature was monitored, and milk and blood samples were collected and analyzed. Treatment effects were tested using the Mixed procedure in SAS (LSM ± SEM). No interactions were detected, thus main effect differences are reported hereafter. Compared with CO, HS increased ($P \leq 0.03$) rectal (38.4 vs. 39.0 ± 0.09°C) and vaginal temperatures (38.6 vs. 39.3 ± 0.09°C) and respiration rate (48.6 vs. 76.2 ± 2.2 breaths/min). Relative to CO, HS decreased ($P < 0.01$) yields of energy-corrected milk (37.5 vs. 33.9 ± 0.8 kg/d), protein (0.99 vs. 0.93 ± 0.02 kg/d), and fat (1.47 vs. 1.30 ± 0.04 kg/d) but did not affect circulating insulin (21.4 vs. 21.7 ± 3.02 µU/mL). Metabolizable protein did not affect energy-corrected milk yield. Low MP increased ($P \leq 0.05$) milk fat yield (1.36 vs. 1.41 ± 0.04 kg/d) and plasma free fatty acid concentrations (159 vs. 204 ± 21.6 µEq/L) but decreased ($P < 0.01$) circulating insulin (28.3 vs. 14.9 ± 3.0 µU/mL). In summary, HS did not affect circulating insulin and reduced synthesis of protein, fat, and energy-corrected milk. Low MP reduced circulating insulin, increased circulating free fatty acids, and did not affect energy-corrected milk

yield. Hormonal and metabolite adaptations in LMP cows may have promoted the use of nutrients to support milk synthesis.

Key Words: crude protein, heat stress, milk production

498 Oxidative stress in periparturient dairy cows and its relationship with negative energy balance markers. E. Mariani[1], G. Invernizzi*[1], G. Savoini[1], A. Baldi[1], and I. Politis[2], [1]*Department of Health, Animal Science and Food Safety, Universita degli Studi di Milano, Milan, Italy,* [2]*Department of Animal Science and Aquaculture, Agricultural University of Athens, Athens, Greece.*

Living organism's defensive systems are very sensitive and complex mechanisms. Endogenous production of reactive oxygen species (ROS) is one of the main way these systems can achieve their goals. On the other side, ROS can be dangerous also for the living cells and the right balance between pro-oxidant and antioxidant is essential to avoid oxidative stress. Peripartum is typically a very stressful period of life in producing animals and dairy cows can experience a negative redox status at this time. The aim of the trial was to study the relationship between negative energy balance markers such as NEFA and BHBA and oxidative stress in transition dairy cows. Thirty Holstein Friesian dairy cows over 2 commercial farms located in Milan area were involved in the trial. Blood samples were collected at dry-off, at calving and at 30 DIM. ROS and serum antioxidant capacity (SAC) were measured on samples and the Oxidative Stress index (OSi) was calculated. On the same samples NEFA, BHBA and α-tocopherol concentrations were determined. Statistical analysis was performed with MIXED and CORR procedures of SAS. NEFA and BHBA increased around parturition compared with dry off levels ($P < 0.05$). At 30 DIM, NEFA decreased to concentrations similar to pre-partum, BHBA concentration instead remained high. α-tocopherol blood levels decreased at calving and increased at 30 DIM at higher values than dry-off (3.12 vs.. 3.59 μg/mL, $P < 0.05$). ROS results were not significant over the time and SAC values showed a tendency ($P = 0.05$) to have lower concentration at calving compared with dry-off. OSi results were significantly higher at calving compared with pre-calving (0.167 vs.. 0.214, $P < 0.05$) and 30 DIM indexes (0.214 vs.. 0.178, $P < 0.05$). There was a positive correlation between NEFA (0.44, $P < 0.05$) and BHBA (0.28, $P < 0.05$) serum levels and OSi. The results observed, consolidate the goodness of OSi in detecting oxidative stress in transition cows.

Key Words: oxidative stress, transition dairy cow, negative energy balance

499 Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy. J. E. Santos*[1], H. M. Golder[2], E. Block[3], and I. J. Lean[2], [1]*University of Florida, Gainesville, FL,* [2]*Scibus, Camden, NSW, Australia,* [3]*Arm & Hammer Animal Nutrition, Princeton, NJ.*

Objectives were to use meta-analytic methods to determine the effects of changes in DCAD prepartum on productive performance and health of dairy cows. The literature was systematically reviewed and 42 experiments that manipulated the mineral composition prepartum, including 134 treatment means and 1,803 cows, were included in the meta-analysis. Five experiments with 15 treatment means reported responses for 151 nulliparous cows. Data collected included mineral composition of diets, parity, breed, days fed prepartum diets, and means and respective SEM for urine pH, DMI, BW, BCS, productive performance, concentrations of minerals and metabolites in blood, and incidence of diseases. Random effects meta-analyses were conducted weighting by the inverse of SEM squared to account for the precision of each experiment. Models include the effects of DCAD, parity group prepartum, and other covariates that showed significance in univariable analysis. Final models were selected based on parsimony, biological relevance, and model fit. Reducing the DCAD reduced DMI prepartum but improved postpartum intake. Interactions between DCAD and parity group were observed for yields of milk, FCM, fat, and protein because reducing the DCAD improved those responses in parous cows; however, reducing the DCAD either had no effect on yields of milk and protein, or reduced the yield of FCM and fat in nulliparous cows. The model predicted that reducing the DCAD from +200 to −100 mEq/kg would increase DMI postpartum 1.0 kg/d and milk yield 1.7 kg/d. Concentrations of Ca in blood on the day of calving and postpartum increased with a reduction in DCAD, which explained the reduction in risk of milk fever observed in parous cows fed diets with negative DCAD. As the DCAD decreased, the risk of retained placenta and metritis also decreased, resulting in fewer disease events per cow in both nulliparous and parous cows. These results support the recommendation that parous cows should be fed diets with negative DCAD, but data available did not allow for detection of the ideal negative DCAD that optimizes production and minimizes health problems in parous cows.

Key Words: DCAD, health, transition cow

500 Hyperlipidemia promotes hepatic ceramide accumulation during steatosis. J. E. Rico, S. L. Giesy, Y. R. Boisclair, and J. W. McFadden*, *Cornell University, Ithaca, NY.*

Hyperlipidemia develops with hepatic steatosis and insulin antagonism in domestic ruminants. Enhanced hepatic fatty acid uptake contributes to de novo ceramide synthesis in non-ruminants. In turn, circulating ceramide antagonizes insulin sensitivity in skeletal muscle tissue. Our objective was to determine whether the induction of hyperlipidemia modifies hepatic ceramide concentrations and the mRNA expression of ceramide synthases (CerS). Six non-pregnant, nonlactating Holstein dairy cows (682 kg ± 22), were used in a crossover design with treatments consisting of i.v. infusion (100 mL/h) of either saline (control) or triacylglycerol (TG) emulsion (Intralipid 20%; Frasenius Kabi) for 16 consecutive hours. The feeding level was set at 120% of estimated energy requirement with meals offered every 2 h. Blood was collected at regular intervals and liver biopsied at 16 h. LC/MS was used to quantify ceramides, monohexosylceramides (GlcCer), and lactosylceramides (LacCer) in liver. Real-time PCR was utilized to evaluate CerS isoform expression. Data were analyzed using a mixed model (fixed effects of treatment and random effect of cow). As previously established, TG infusion increased hepatic TG, and plasma total fatty acid and ceramide levels by 16 h, relative to control ($P < 0.01$). Hepatic total ceramide and very long chain C22:0, C24:0, C26:0 ceramide concentrations were increased during TG infusion (~72%; $P < 0.05$). TG infusion also enhanced hepatic very long chain GlcCer and LacCer levels (e.g., C22:0 GlcCer; $P < 0.05$). However, hepatic C16:0 and C18:0 ceramide levels were not modified by treatment. In support of enhanced de novo synthesis of very long chain ceramides during TG infusion, C22:0 and C24:0 dihydroceramide concentrations were elevated 70–110% ($P < 0.05$). Moreover, C22:0 and C24:0 ceramide were positively correlated with their dihydroceramide precursors ($r \geq 0.88$, $P < 0.01$). Bovine liver primarily expressed CerS2 and CerS6, and TG selectively enhanced CerS2 mRNA by 60% ($P < 0.05$). We conclude that hyperlipidemia promotes the de novo synthesis and accumulation of very long chain ceramides in dairy cattle experiencing hepatic steatosis.

Key Words: ceramide, hepatic steatosis, hyperlipidemia

501 Diet starch content and fermentability affect markers of inflammatory response and oxidative stress during early lactation. R. I. Albornoz*[1], L. M. Sordillo[1], B. J. Bradford[2], L. K. Mamedova[2], and M. S. Allen[1], [1]*Michigan State University, East Lansing, MI,* [2]*Kansas State University, Manhattan, MI.*

Our objective was to evaluate effects of diet starch content and fermentability (SF) on inflammatory response and oxidative stress markers during the early postpartum (PP) period and its carryover effects. Fifty-two multiparous Holstein cows were used in a randomized block design experiment with a 2 × 2 factorial arrangement of treatments. Diets were formulated to 22% (LS) or 28% (HS) starch with dry ground corn (DGC) or high moisture corn (HMC) as the primary starch source. Treatments were fed from 1 to 23 d PP and then switched to a common diet until 72 d PP to measure carryover (CO) effects. Diets were formulated to 22% forage NDF and 17% CP for the treatment period (TP) and 20% forage NDF, 17% CP and 29% starch for the CO period. Blood was collected before feeding (0730 h) once a week during TP and every second week during the CO period. Blood plasma was analyzed by colorimetry for concentrations of haptoglobin (HAP), albumin and reactive oxygen species (ROS), with LPS-binding protein (LBP) and TNF-α evaluated during the TP only. Data were analyzed separately for TP and CO using a Mixed Model including treatment interactions with time. During the TP, treatments interacted to affect concentrations of TNF-α, HAP and LBP ($P < 0.07$), with HMC increasing their concentrations for HS (9.29 vs. 8.42 pg/mL, 0.54 vs. 0.41 mg/mL and 5.85 vs. 4.67 µg/mL, respectively) and decreasing their concentrations for LS (5.88 vs. 11.3 pg/mL, 0.29 vs. 0.44 mg/mL and 4.41 vs. 6.02 µg/mL, respectively) compared with DGC. Effects of treatments diminished over time for LBP and HAP with no differences by the end of the TP. Opposite treatment interaction was observed for albumin, with HMC tending to decrease albumin for HS (3.25 vs. 3.34 g/dL) and increase albumin for LS (3.38 vs. 3.29 g/mL; $P = 0.13$) compared with DGC. HMC tended to increase ROS compared with DGC (57.5 vs. 52.0 RFU/µL; $P = 0.07$). Treatment effects diminished for all variables during the CO period. Results during the TP suggest that feeding LS-DGC and HS-HMC elicited a more pronounced inflammatory response, with ROS increased by HMC treatments.

Key Words: diet starch, inflammation, early lactation

502 Lactation performance and energetic metabolism of transition cows fed rumen protected glucose. C. S. McCarthy*[1], B. C. Dooley[1], E. H. Branstad[1], A. J. Kramer[1], E. A. Horst[1], E. J. Mayorga[1], M. Al-Qaisi[1], M. A. Abeyta[1], G. Perez-Hernandez[2], B. M. Goetz[1], A. R. Castillo[3], M. R. Knobbe[4], C. A. Macgregor[4], J. P. Russi[5], J. A. D. R. N. Appuhamy[1], L. H. Baumgard[1], and H. A. Ramirez Ramirez[1], [1]*Iowa State University, Ames, IA,* [2]*Universidad Autónoma Chapingo, Chapingo, México,* [3]*University of California, Cooperative Extension, Merced, CA,* [4]*Grain States Soya, Inc., West Point, NE,* [5]*Rusitec Argentina, General Villegas, Buenos Aires, Argentina.*

Carbohydrate supply and availability in the small intestine may limit milk production in transition dairy cows. Thus, experimental objectives were to evaluate the effects of rumen protected glucose (RPG) on milk production and post-absorptive metabolism in transition dairy cows. Fifty-two multiparous cows were blocked by previous 305ME and randomly assigned to one of 2 iso-energetic and iso-nitrogenous treatments: (1) control diet (CON, n = 26) or (2) a diet containing RPG (RPG, pre-fresh 8.4% RPG DM basis, post-fresh 9.5% RPG DM basis; n = 26). Cows received their respective dietary treatments from d −21 to 28 relative to calving via individual feeding gates. Weekly BW, BCS, milk samples and fecal pH were recorded until 28 DIM, whereas milk yield was recorded through 105 DIM. Blood samples were collected on d −7, 3, 7, 14, and 28 relative to calving. Data were analyzed using repeated measures in the MIXED procedure of SAS. Fecal pH was similar ($P = 0.87$) among treatments; however, compared with pre-calving, both treatments had a reduction in pH following calving (7.2 vs 6.6; $P < 0.01$). Milk yield was similar among treatments ($P = 0.37$) for 1 to 28 DIM (42.8 ± 1.2 kg/d), as well as through 105 DIM ($P = 0.92$; 47.3 ± 1.3 kg/d). Dry matter intake pre-calving (12.0 ± 0.7 kg/d) and post-calving (22.0 ± 0.7 kg/d) were unaffected by treatment ($P \geq 0.65$). Milk fat, protein and lactose were similar among treatments ($P \geq 0.40$; 4.36 ± 0.13%, 3.66 ± 0.11% and 4.73 ± 0.06%, respectively). Plasma concentration of BUN and glucose were similar ($P \geq 0.57$) among treatments; however, postpartum concentration of circulating BHBA tended ($P = 0.13$) to be 0.24 mmol/L lower with a concomitant reduction ($P < 0.01$) in NEFA concentration in the RPG-fed cows compared with CON (630 vs. 456 ± 68 µEq/L). Circulating insulin tended to be increased ($P = 0.06$; 27%) in RPG-fed cows compared with CON. Based upon circulating hormones and metabolites, it appears that treatment influenced energy partitioning pathways in the periparturient period such that RPG-fed cows mobilized less adipose tissue compared with the control cows while maintaining milk yield.

Key Words: bypass carbohydrates, ketosis, transition period

503 Elevated prepartum adiposity predisposes cows to hepatic steatosis with distinct postpartum lipidome remodeling. J. E. Rico*[1,2], A. N. Davis[1,2], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV.*

Obesity promotes hepatic steatosis via (1) enhanced fatty acid (FA) uptake and triacylglycerol (TAG) esterification, (2) increased partitioning of FA toward the synthesis of lipid mediators of metabolic disease such as ceramide and diacylglycerol (DAG), and (3) the inflammation-driven degradation of sphingomyelin (SM) and phosphatidylcholine (PC), which may compromise lipoprotein secretion. We aimed to determine whether elevated prepartum adiposity predisposes dairy cows to a modified hepatic lipidome reflecting metabolic impairment during the peripartum. Lean (BCS 3.0 ± 0.2; n = 13) and overconditioned (OVER; BCS 3.9 ± 0.3; n = 12) Holstein dairy cows were enrolled −28 d, and liver tissue was biopsied at d −12 and 10, relative to parturition. Untargeted lipidomics was performed using quadrupole time-of-flight mass spectrometry. Multivariate analysis of normalized, auto-scaled lipids included ANOVA and Pearson correlation coefficient procedures. Lipidomics revealed 406 hepatic lipids including 83 TAG, 106 monoalkyl-diacylglycerols (MADAG), 22 DAG, 32 ceramides, 30 SM, 33 PC, and 41 phosphatidylethanolamines (PE). Elevated postpartum plasma FA levels were accompanied by increased liver lipid content (7 versus 11%; $P < 0.05$) and plasma β-hydroxybutyrate (430 versus 506 µM; $P < 0.01$) in OVER. Omics revealed 98 complex lipids affected by adiposity ($P < 0.05$). Relative to lean, OVER displayed elevations in TAG, MADAG, DAG, and select ceramides, while showing stronger reductions in PC postpartum ($P < 0.05$). A total of 13 PC decreased in OVER, relative to lean (e.g., PC 36:6; $P < 0.05$). Several SM were lower in OVER (e.g., SM 40:3; $P < 0.05$), although the changes were not uniform across all SM moieties (e.g., SM 46:1 increased in OVER; $P < 0.05$). Total ceramides were positively correlated with total DAG and TAG (FDR <0.01) but inversely related to select PC and SM (FDR <0.01). Prepartum adiposity remodels the postpartum hepatic lipidome. Future research will need to determine whether the peripartal depletion of hepatic PC predisposes overconditioned cows to impaired lipoprotein secretion and fatty liver disease.

Key Words: adiposity, fatty liver, lipidome

504 Time course of changes in lactation performance, blood metabolites, inflammation and milk fatty acids during subacute ruminal acidosis induction and recovery in dairy cows. E. Sandri[1], Y. Couture[2], R. Gervais[3], J. Levesque[1], and D. Rico*[1], [1]*CRSAD, Deschamabult, QC, Canada,* [2]*Université de Montreal, Saint-Hyacinthe, QC, Canada,* [3]*Université Laval, Quebec, QC, Canada.*

Twelve ruminally cannulated cows (120 ± 52 DIM; 35.5 ± 8.9 kg of milk/d; mean ± SD) were randomly assigned to treatment in a Latin square design with 21-d periods. Treatments were (1) subacute ruminal acidosis (SARA) induction, (2) recovery, and (3) control. SARA was induced by feeding a diet containing 29% starch, 24% NDF, and 2.8% fatty acids (FA), whereas the recovery and control diets contained 20% starch, 31% NDF, and 2.3% FA. Experimental sampling took place on d 0, 3, 7, 10, 14, 17, and 21 of each period. Data were analyzed as repeated measures using the MIXED procedure of SAS. Dry matter intake and milk yield were increased during induction of SARA from d 10 to 21 and from d 14 to 21, respectively ($P < 0.05$). Milk fat concentration was reduced from d 3 to 14 of SARA induction, whereas lower milk fat yield was observed on d 3 only ($P < 0.05$). The acetate-to-propionate ratio was lower, whereas the concentrations of total volatile FA and lactate were greater during SARA as compared with the control period ($P < 0.05$). Plasma insulin and lipopolysacharide binding protein concentrations increased during SARA induction, whereas plasma nonesterifed FA and milk BHBA decreased ($P < 0.05$). The ratio of milk *t*10 to *t*11 18:1 increased during the SARA induction period ($P < 0.05$), but concentration of *t*10 18:1 remained below 0.5% of FA, whereas *t*10*c*12 18:2 was not detected. The ratio of *t*10-to-*t*11 C18:1 was positively correlated with the acidosis index (area under pH <5.8/DMI; r = 0.36; $P < 0.05$). Negative associations were observed between the acidosis index and the milk fat concentrations of *iso* 14:0, *iso* 15:0, *iso* 16:0, *iso* 17:0, and *anteiso* 17:0 (r = −0.23 to −0.30, $P < 0.05$). Plasma cytokines (IFNα, IFNγ, IL-13, IL-1α, IL-1 F5, IL-21, IP-10, MIG, MIP-1β, and TNFα) were not affected by treatment at any time point. From d3 to 21, most parameters were not different between the recovery and the control periods. Induction of SARA resulted in a rapid and transient reduction in milk fat synthesis, which was not strongly associated with ruminal biohydrogenation.

Key Words: acidosis, dairy cows, milk fatty acids

505 Injectable trace minerals (selenium, copper, zinc, and manganese) alleviates inflammation and oxidative stress during an aflatoxin challenge in lactating multiparous Holstein cows. R. T. Pate* and F. C. Cardoso, *Department of Animal Sciences, University of Illinois, Urbana, IL.*

Trace minerals are vital in oxidative stress; however, limited research is available on the effects of trace mineral supplementation during an aflatoxin (AF) challenge. The objective of the study was to determine the immunological effects of 2 subcutaneous supplementations of 15 mg/mL Cu, 5 mg/mL Se, 60 mg/mL Zn, and 10 mg/mL Mn (Multimin 90, Multimin North America, Fort Collins, CO) given at 1 mL/90.7 kg of BW in response to an AF challenge. Fifty-eight Holstein cows [BW (734 ± 60kg); DIM (191 ± 93)] were assigned to 1 of 3 treatments in a randomized complete block design. The experimental period (63 d) was divided into an adaptation phase (d 1 to 56) and a measurement phase (d 57 to 63). From d 57 to 59 cows received an AF challenge, which consisted of 100 µg of AFB1/kg of dietary DMI administered orally via balling gun. Treatments were: saline injection and no AF challenge (NEG), saline injection and AF challenge (POS), and trace mineral injection and AF challenge (MM). Injections were performed subcutaneously on d 1 and 29 at 1 mL/90.7 kg of average BW. Blood was sampled on d 0, 56, 60, and 63, and liver samples were taken on d 0 and 60. Two treatment orthogonal contrasts, CONT1 (NEG vs. POS) and CONT2 (POS vs. MM), were made. Milk urea nitrogen and BUN were higher for cows in POS (14.3 mg/dL and 16.5 mg/dL, respectively) than cows in MM (13.3 mg/dL and 15.8 mg/dL, respectively; $P = 0.03$ and 0.04, respectively). Liver concentrations of Se and Fe were higher for cows in MM (4.6 and 214.4 µg/kg liver DM, respectively) compared with cows in POS (4.0 and 190.8 µg/kg liver DM, respectively; $P = 0.04$ and 0.02, respectively). Cows in MM tended to have higher plasma glutathione peroxidase activity (30.2 nmol/min/mL) compared with cows in POS (24.2 nmol/min/mL; $P = 0.10$). An upregulation of liver *GPX1* was observed for cows in POS (1.07 ± 0.05) compared with cows in MM (0.95 ± 0.05; $P = 0.01$). In conclusion, subcutaneous supplementation of trace minerals induced a positive antioxidant response during an AF challenge.

Key Words: aflatoxin, trace mineral, blood urea nitrogen

506 Characterization of hepatic sphingomyelin during the peripartum. A. N. Davis*[1,2], J. E. Rico[1,2], and J. W. McFadden[1,2], [1]*Cornell University, Ithaca, NY,* [2]*West Virginia University, Morgantown, WV.*

Ceramide mediates insulin resistance in obese diabetics. In dairy cattle, the transition from gestation to lactation is characterized by the accrual of ceramide in liver, skeletal muscle, and circulation. Current evidence suggests that the de novo synthesis of ceramide from saturated fatty acids contributes to hepatic ceramide synthesis; however, sphingomyelin (SM) hydrolysis may also contribute to ceramide supply. Our objective was to characterize changes in hepatic SM during the peripartum in dairy cattle. Twenty-five pregnant, multiparous lean (BCS 3.0 ± 0.2) or overconditioned (OVER; BCS 3.9 ± 0.3) Holstein cows were enrolled 28 d before expected parturition. Blood samples were routinely collected. Liver biopsies and the hyperinsulinemic-euglycemic clamp were performed 2 wk before and after parturition. Sphingolipids were quantified using mass spectrometry. Data were analyzed using a mixed model with repeated measures. Similar to hepatic ceramides, C24:0 and C22:0 SM were the most abundant SM species. Although adiposity and time did not modify total SM levels, the hepatic concentrations of C16:0, C18:0, and C20:0 SM increased with time ($P < 0.01$). In contrast, hepatic C24:0, C24:1, and C26:0 decreased postpartum ($P < 0.01$). Total dihydro-SM (DHSM) increased with time ($P < 0.01$); albeit, C16:0, C24:0, and C26:0 DHSM decreased after parturition ($P < 0.01$). Hepatic C18:0 and C20:0 SM, and C20:0 DHSM levels were greater in OVER ($P < 0.01$), relative to lean. In contrast, very long-chain C26:0 and C26:1 SM, and C26:1 DHSM were lower in OVER ($P < 0.05$), relative to lean. Hepatic total DHSM and individual SM were positively correlated with liver lipid content (e.g., C16:0, C18:0, C20:0; $P < 0.01$), whereas C26:0 SM and DHSM were inversely associated with steatosis ($P < 0.01$). Similar relationships were observed between hepatic SM and circulating fatty acids. Prepartum, long-chain SM were positively associated with insulin sensitivity (clamp index; $P < 0.05$), whereas very long-chain SM were negatively related to insulin action ($P < 0.05$). The observed reciprocal response between long-chain and very long-chain SM mirror peripartal changes in hepatic and plasma ceramide.

Key Words: liver, peripartum, sphingomyelin

507 Determining immune-modulating components of *Saccharomyces cerevisiae* with RAW 264.7 murine macrophages. S. E. Sivinski*, R. A. Rusk, J. L. McGill, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

Feed components can modulate the immune system, but in vivo data are expensive and rare; thus, an effective screening tool for evaluating such nutrients is needed to enable informed selection of candidate immunomodulators for in vivo investigation. This study used RAW 264.7 murine macrophages as an innate immunity in vitro screening tool to identify immune-modulating properties of *S. cerevisiae* and some of its components. Treatments were 0.01, 0.1, or 1 mg/mL of whole *S. cerevisiae* cells (WC), mannan, Zymosan, or D-mannose at either pH 3 or 7. A pH of 3 was used to mimic the acidic conditions of the stomach to assess potential alterations of component activity. The cells were transfected with a vector that drove expression of an alkaline phosphatase reporter gene upon activation of NFκB. Cells (n = 6 wells/treatment) were incubated with treatments for 18 h. Cell supernatants were then incubated for 2 h with alkaline phosphatase (AP) substrate media (QUANTI-Blue) to assay enzyme activity. After normalizing values across plates using unstimulated and LPS (1 μg/mL) stimulated cells as negative and positive controls, intra- and inter-assay CVs were 3.9% and 15.8%. The effects of treatment, pH, and dose(treatment) were evaluated by mixed models. Treatment and dose(treatment) affected AP activity (both $P < 0.001$) whereas pH did not ($P = 0.31$). Mannan at 0.1 mg/mL and WC at 1 mg/mL tended to increase AP activity (both $P = 0.07$), while mannan and D-mannose at 1 mg/mL, WC at 0.1 mg/mL, and Zymosan at 1, 0.1, and 0.01 mg/mL increased AP activity (0.10 ± 0.02, 0.09 ± 0.02, 0.10 ± 0.02, 1.85 ± 0.04, 1.62 ± 0.04, and 0.78 ± 0.03 OD, respectively; all $P < 0.001$), compared with unstimulated cells. Zymosan increased AP activity vs. mannan, WC, and D-mannose (1.37 ± 0.02 vs. 0.05 ± 0.01, 0.05 ± 0.01, and 0.03 ± 0.01 OD, respectively; all $P < 0.001$), and WC stimulated a greater response than D-mannose (0.05 ± 0.01 vs. 0.03 ± 0.01 OD; $P = 0.03$). Overall, this innate immunity in vitro screening method was useful for quickly determining immune-modulating properties and concentrations of *S. cerevisiae* components.

Key Words: bioactive nutrient, nutritional immunology, in vitro screening method

508 Long-term effects of reduced-fat distillers grains with and without monensin on performance of dairy cows. D. L. Morris*[1], S. H. Kim[1], P. J. Kononoff[2], and C. Lee[1], [1]*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH,* [2]*Department of Animal Science, University of Nebraska, Lincoln, NE.*

This study investigated the long-term effects of high inclusion of reduced-fat corn distillers grains with solubles (RFDG) with and without monensin on intake, production, milk fatty acid profile, and plasma AA profile in lactating cows. The experiment was conducted for 11 wk (2-wk diet adaptation, 9-wk experimental period of data collection) with 36 Holstein cows (80 ± 31 DIM; mean ± SD at start of trial) in a randomized block design. Cows were blocked by parity and DIM and assigned to the following diets: (1) CON, control diet; (2) DG, CON with RFDG included at 28.8% (dry matter basis) replacing soybean meal, soyhulls, and supplemental fat; (3) DGMon, DG with monensin (Rumensin; Elanco Animal Health, Greenfield, IN) supplemented at a rate of 20 mg/kg of DM. Data were analyzed with a model that included the fixed effect of treatment, week, and interaction of diet and week, the random effect of cow within block, repeated measurements were included. Orthogonal contrasts were used to compare CON vs. DG and DGMon and DG vs. DGMon. Milk yield was not affected (40.3 vs. 40.8 kg/d; $P = 0.58$) by DG and DGMon compared with CON. However, dry matter intake (DMI; 24.9 vs. 26.4 kg/d), milk fat yield (1.12 vs. 1.55 kg/d), milk protein yield (1.24 vs. 1.32 kg/d), and energy-corrected milk yield (37.7 vs. 43.5 kg/d) decreased ($P < 0.01$) for DG and DGMon compared with CON. Feeding DGMon compared with DG did not affect ($P \geq 0.102$) DMI (24.4 vs. 25.4 kg/d) and milk yield (39.2 vs. 41.3 kg/d), but decreased ($P \leq 0.02$) milk fat yield (1.08 vs. 1.23 kg/d), milk protein yield (1.20 vs. 1.28 kg/d), and energy-corrected milk yield (36.0 vs. 39.4 kg/d). Interactions between treatment and wk ($P \leq 0.08$) for DMI, milk fat yield, and energy-corrected milk indicate production responses to DG and DGMon vs. CON were decreased over the experimental period. In the current study, feeding a high-DG diet did not sustain long-term DMI and production, and supplementing monensin to a high-DG diet further decreased DMI and production.

Key Words: reduced-fat distillers grains, monensin, performance

509 Effects of feeding corn stover pelleted with soybean meal or distillers grains on lactating dairy cow performance. B. C. Dooley*[1], C. S. McCarthy[1], E. H. Branstad[1], G. M. Bonetto[3], R. Zeeck[2], A. G. Carpenter[4], J. O. Sarturi[5], and H. A. Ramirez-Ramirez[1], [1]*Iowa State University, Ames, IA,* [2]*Pellet Technology USA, Gretna, NE,* [3]*Instituto Nacional de Tecnología Agropecuaria, Manfredi, Córdoba, Argentina,* [4]*University of Guelph, Ridgetown, ON, Canada,* [5]T*exas Tech University, Lubbock, TX.*

The objective of this study was to determine the effects of corn stover (CSP) pelleted with 1 of 2 protein sources, soybean meal (SBM) or distillers grains (DG), on lactation performance of dairy cows. To do so, 45 Holstein cows (30 primiparous, 15 multiparous; 118 ± 33 DIM and 604 ± 70 kg BW) were used in replicated 5 × 5 Latin squares with 28-d periods, using a 2 × 2 + 1 augmented factorial arrangement of treatments. Cows were individually fed their respective diets, which were formulated to contain 10 or 15% (DM basis) corn stover-based pellets processed with SBM (CS-SBM) or DG (CS-DG) as replacement of corn gluten pellets from the control diet. Treatments were (1) Control (CON); (2) 10% CS-SBM; (3) 15% CS-SBM; (4) 10% CS-DG; and (5) 15% CS-DG. Performance data were analyzed using the MIXED procedure of SAS. Relative to CON (26.9 ± 0.49 kg/d), all other diets decreased DMI ($P < 0.01$); within diets with CSP, feeding CS-SBM resulted in 0.57 ± 0.24 kg/d greater DMI ($P = 0.02$) compared with CS-DG, whereas 15% inclusion rate reduced DMI compared with 10% (21.9 vs 23.6 ± 0.44 kg/d; $P < 0.01$). The CON resulted 32.1 ± 0.69 kg milk/d whereas diets with 10% and 15% CSP were 2.5 and 3.8 ± 0.39 kg/d milk lower ($P < 0.01$), respectively. Compared with CS-DG, milk yield tended to be greater in CS-SBM by 0.46 ± 0.24 kg/d ($P = 0.06$); including 15% CSP reduced milk yield by 1.33 ± 0.24 kg/d ($P < 0.01$). However, 15% inclusion rate increased milk production efficiency (1.30 vs 1.26 ± 0.03; $P = 0.01$). Inclusion rate did not affect milk fat concentration but feeding CS-SBM resulted in greater milk fat concentration and yield compared with CS-DG (3.88 vs 3.71 ± 0.08%, 1.11 vs 1.05 ± 0.02 kg/d; $P < 0.01$). Milk protein was unaffected by protein source but MUN tended to be lower in CS-DG ($P = 0.08$). Despite differences in milk yield and composition, fat-corrected milk (FCM) efficiency was similar ($P \geq 0.19$) across all treatments at 1.30 kg FCM/kg DMI.

These results demonstrate that corn stover-based pellets with SBM or DG can replace pelleted corn gluten feed with limitations on DMI and milk yield but sustained FCM production efficiency.

Key Words: by-products, crop residue, corn stalks

510 Production effects of extruded soybean meal in early lactation cow diets. M. T. Harper*[1], J. Oh[1], A. Melgar[1], K. Nedelkov[2], S. Räisänen[1], X. Chen[3], C. M. M. R. Martins[4], M. Young[1], T. Ott[1], D. M. Kniffen[1], R. Fabin[5], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Trakia University, Stara Zagora, Bulgaria*, [3]*College of Pastoral Agriculture Science and Technology, Lanzhou University, Gansu, China*, [4]*School of Veterinary Medicine and Animal Science, University of Sao Paulo, Pirassununga, Brazil*, [5]*Fabin Bros. Farms, Indiana, PA*.

The objective of this experiment was to evaluate the productive and reproductive effects of replacing solvent extracted soybean meal (SSBM) with extruded soybean meal (ESBM) in a total mixed ration for early lactation cows. Thirty-four Holstein cows (12 primiparous and 22 multiparous) were used in a randomized complete block design experiment with 17 cows per treatment. Feeding was ad libitum for 5 to 10% refusals. A fresh cow diet was fed the first 14 d DIM followed by a lactation diet from 15 to 60 DIM. The SSBM lactation diet consisted of (DM basis): 33% corn silage, 14% alfalfa haylage, 5% hay/straw, 12% SSBM and 36% concentrates. In the ESBM diet, ESBM replaced SSBM. Lactation SSBM and ESBM diets were 18.9 and 18.8% crude protein, 28.3 and 28.0% neutral detergent fiber, and 3.3 and 4.2% ether extract, respectively. Enteric CH_4 emission was measured using the GreenFeed system. Data were analyzed using the MIXED procedure of SAS. Milk yield (45.9 kg/d; SEM = 0.81), DM intake (22.4 kg/d; SEM = 1.18), and feed efficiency (FE; 2.06 kg/kg milk; SEM = 0.07) were not different between treatments ($P \geq 0.32$). Energy-corrected milk (ECM) yield (47.6 vs 44.9 kg/d; SEM = 1.11, $P = 0.09$) tended to be higher for SSBM but ECM FE was not different. Treatments did not affect milk urea N, or milk components except for milk true protein yield, which was greater (1.40 vs 1.31 kg/d; SEM 0.06, $P = 0.04$) for SSBM. Concentrations of milk fatty acid (FA) ≥ 18C and *trans* FA, specifically *trans* 10 C18:1, were increased ($P \leq 0.02$) in ESBM. Treatment did not affect blood cell counts or nonesterified FA and β-hydroxybutyrate concentrations. Milk progesterone concentrations did not reveal any differences in onset of 1st or 2nd luteal phases (LP) after calving, length of 1st LP, or interval between 1st and 2nd LP. Enteric CH_4 emission (394 g/d; SEM 19.1), CH_4 yield (14.8 g/DMI; SEM = 0.72) and CH_4 intensity (7.9 g/kg milk; SEM = 0.48) were not affected by treatment. ESBM did not improve lactational or reproductive performance of early lactation dairy cows and decreased ECM and milk protein yields, possibly due to higher unsaturated FA intake compared with SSBM.

Key Words: extruded soybean meal, dairy cow

511 Effect of source and processing of protein feedstuffs pelleted with treated corn stover in dairy cow diets. K. L. Ostendorf*[1] and K. F. Kalscheur[2], [1]*University of Wisconsin, Madison, WI,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Corn stover treated with alkaline and pelleted with protein may lower feed costs and partially replace a variety of concentrate feeds in dairy cow diets. The objective was to evaluate treated corn stover pelleted with different sources and processing of protein feedstuffs on lactation performance and rumen characteristics. Twenty lactating Holstein cows were enrolled in a multiple 5 × 5 Latin square design with 21-d periods and randomly assigned to 1 of 5 treatments: (1) control diet with no pellets (CON), (2) treated corn stover with extrusion processed soybean meal pellets, (3) treated corn stover with unprocessed soybean meal pellets, (4) treated corn stover with extrusion processed dried distillers grains with solubles pellets, and (5) treated corn stover with unprocessed dried distillers grains with solubles pellets. All diets were formulated to contain 53.5% forage and 46.5% concentrate (DM basis). For pellet diets, inclusion rate was maintained at 20% (DM basis) and replaced 40% ground corn, 32.5% canola meal, 25% soybean hulls, and 2.5% limestone. Data were analyzed using the MIXED procedure of SAS. Dry matter intake (DMI) was greater ($P < 0.001$) for cows fed CON diet (30.8 kg/d) than cows fed pellet diets (27.1, 26.4, 26.2, 26.5 kg/d). Cows fed CON diet were greater ($P < 0.001$) in milk yield compared with cows fed pellet diets (47.5 vs. 42.4, 42.7, 43.0, 42.8 kg/d). However, DMI and milk yield did not differ for cows fed the pellet diets ($P > 0.10$). Milk fat and lactose percentages were unaffected ($P > 0.10$) across all 5 treatments, while cows fed CON diet produced greater milk protein percentage than cows fed pellet diets ($P < 0.001$). Feed efficiency (energy-corrected milk/DMI) tended ($P = 0.08$) to increase for cows fed pellet diets compared with cows fed CON diet. There were no differences in total ruminal VFA for cows fed CON vs pellet diets ($P > 0.10$), however; protein processing decreased ruminal acetate and increased propionate percentages ($P < 0.01$). At 20% inclusion rate in the diet, there does not appear to be an advantage of one pellet over the other in terms of performance.

Key Words: treated corn stover, pellet, protein

512 Quantifying the effects of amino acid profile, energy supply, and diet nutrient composition on the requirement of metabolizable protein by lactating dairy cows. L. E. Moraes*[1], J. L. Firkins[1], H. Lapierre[2], E. Kebreab[3], and R. R. White[4], [1]*The Ohio State University, Columbus, OH,* [2]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada*, [3]*University of California, Davis, CA*, [4]*Virginia Tech, Blacksburg, VA*.

The objective of this study was to quantify and characterize the effects of amino acid profile, energy supply and covariates describing diet nutrient composition and cow's physiological status on the efficiency of utilizing metabolizable protein (MP) and on the combined MP requirement for maintenance and lactation. A Bayesian hierarchical modeling approach was used to model the total true protein export (PE, milk + scurf + metabolic fecal protein) as a function of the net MP supply (digestible microbial protein + digestible RUP – endogenous urinary protein) using 333 treatment means from 87 publications. The nonlinear model was constructed with a logistic function and heterogeneous error variances were modeled proportionally to the SE of the milk protein yield means. A regression of the predicted study-specific logistic parameters on the amino acid profile (expressed as % of total essential AA supply), DE intake (DEI), diet content of fatty acids, starch, cow's body weight and DIM was used to explain the between study variation and quantify the effects of these covariates. The combined MP requirement was defined as the MP needed to predict a given PE in the fitted model. Thus, an effect of any covariate in the functional parameters of the fitted model had a direct effect on the efficiency of utilizing MP as well as on the combined MP requirement. Lysine, Met, His, Val, Phe, and Thr significantly affected the efficiency and the MP requirement ($P < 0.05$). Likewise, metabolic body weight, DIM as well as diet concentrations of starch, fatty acids, and DEI all affected the protein export, efficiency, and the MP requirement ($P < 0.05$). The model allows assessment of impact of changing the AA profile, diet and varying cow's physiological status on

the combined MP requirement. For example, the effect of DEI was larger at greater MP supplies and protein exports. At a net MP supply of 3 kg/d, a 5 Mcal increase in DEI predicted an increase of 36 g in the protein export. Likewise, at a PE of 1 kg, a 5 Mcal increase in DEI reduced the requirement for net MP by 54 g.

Key Words: nonlinear, protein, requirement

513 Quantifying the variation in resilience to protein-deficient diets in lactating dairy cows. E. Liu* and M. J. VandeHaar, *Michigan State University, East Lansing, MI.*

Diets with less protein improve profitability unless the savings from feeding less protein are outweighed by lost milk revenue. Our objective was to quantify the variation among cows in their ability to maintain production (be resilient) when fed protein-deficient diets. Mid-lactation Holstein cows with initial energy-corrected milk (ECM) of 40 ± 9 kg/d (n = 149, in 5 blocks) were fed either a high protein diet (18% CP; HP) or low protein diet (14%CP; LP) in a crossover design with 2 treatment periods of at least 28 d/period. Both diets contained at least 9.8% rumen-degraded protein. HP and LP diets were similar for each block, but HP contained expeller soybean meal that was replaced by soybean hulls and ground corn in LP. HP contained 4% units more CP, 2% units less starch, and 2% units less NDF than LP. Cows were fed ad libitum. Fixed effects of block, parity, diet, period, cohort, and cohort by diet, and random effect of cow were included in the model to examine milk output and total capture of energy and protein (assuming body energy gain = 6 × BW gain/d and body protein gain = 0.14 × BW gain/d) between diets. Compared with LP, HP increased ECM (39.4 vs. 35.4 kg/d; $P < 0.01$), milk protein (1.28 vs. 1.14 kg/d; $P < 0.01$), BW gain (0.47 vs. 0.11 kg/d; $P < 0.01$), MUN (15.1 vs. 9.3 mg/dL; $P < 0.01$), captured energy (32.4 vs. 27.2 Mcal/d; $P < 0.01$), and captured protein (1.35 vs. 1.15 kg/d; $P < 0.01$). Decreases in milk and captured energy and protein for each cow when fed LP compared with HP were calculated and modeled by ECM per kg metabolic BW when fed HP, DIM, parity, cohort, block, and all interactions. The residual term from the prediction model was used to quantify variation among cows for resilience to LP. The R^2, means, and standard deviations (SD) of means and residuals were, respectively, (1) 0.74, 4.0, 3.2 and 1.6 for kg ECM; (2) 0.80, 0.14, 0.10, and 0.05 for kg milk protein; (3) 0.60, 5.0, 5.5 and 3.5 for Mcal captured energy; and (4) 0.67, 0.17, 0.16 and 0.09 for kg captured protein. We conclude that half of the variation remains for the residual term and suggest that genetics contributes to part of this variation.

Key Words: dairy cow, variation, protein-deficient diet

514 Feeding incremental amounts of rumen-protected histidine to lactating dairy cows. Y. Zang*[1], L. H. P. Silva[2], M. G. Khan[1], A. F. Brito[1], and M. Miura[3], [1]*University of New Hampshire, Durham, NH,* [2]*Federal University of Viçosa, MG, Brazil,* [3]*Ajinomoto Co. Inc., Kawasaki-shi, Japan.*

The dairy industry can benefit from the use of low crude protein (CP) diets due to improved N efficiency. However, His could become limiting in CP-deficient diets. Therefore, our objective was to evaluate the effect of rumen protected (RP)-His on production, and N and AA metabolism in dairy cows. Eight multiparous Holstein cows (130 ± 30 DIM, 42 ± 2 kg/d milk) were used in a replicated 4 × 4 Latin square design with 28-d experimental periods. Treatments included a basal diet composed (DM basis) of 50% corn silage, 15% haylage, and 35% concentrate supplemented with 0, 82, 164, and 246 g/d RP-His (Ajinomoto Inc.) and 11 g/d RP-Met (Smartamine M). Dietary His represented 2.06, 2.28, 2.52, and 2.75% of MP supply, respectively. Milk samples were collected in the last 2 wk of each period, with blood, urine (spot), and muscle samples in wk 4. Data were analyzed with the MIXED procedure of SAS, evaluating treatment effects with linear and quadratic contrasts. Treatments had no effect on DMI and milk composition. In contrast, yields of milk (30.8 to 32.9 kg/d; $P = 0.01$) and milk true protein (0.92 to 0.99 kg/d; $P < 0.01$) increased linearly, while milk fat yield (1.23 to 1.30 kg/d; $P = 0.06$) tended to increase linearly with RP-His. Plasma His (33.2 to 63.1 μ*M*; $P < 0.001$) and carnosine (29.3 to 33.5 μ*M*; $P = 0.03$), as well as muscle His (20.3 to 35.5 μ*M*; $P < 0.001$) showed linear responses to RP-His, whereas no treatment effects were detected for plasma Met and Lys. There were no treatment effects on muscle carnosine and anserine. Milk urea-N (11.7 to 12.9 mg/dL; $P = 0.03$) and urinary excretion of urea-N (23.7 to 27.0% of N intake; $P = 0.04$) increased linearly in response to RP-His. Treatments did not affect urinary excretion of uric acid, allantoin, and total purine derivatives. Overall, supplementation with RP-His to a CP-deficient diet improved milk and milk protein synthesis, likely as a result of increased plasma His. Increased milk urea-N and urinary excretion of urea-N suggest that plasma His may have exceeded the requirement with excess deaminated and converted to urea in the liver due to moderate milk production in our study.

Key Words: dairy cow, rumen-protected amino acid, histidine

515 Predicting energy-corrected milk and milk true protein yields using NorFor or the Nutritional Dynamics System version of the Cornell Model. G. A. Broderick*[1], M. Åkerlind[2], N. I. Nielsen[3], and P. Nordgren[2], [1]*Broderick Nutrition & Research LLC, Madison, WI,* [2]*Växa Sverige, Uppsala, Sweden*, [3]*SEGES, Aarhus N, Denmark*.

Table 1 (Abstr. 515). Linear regression parameters from fitting NorFor and NDS predictions to observed ECM and MTP yields

		Estimates							
Trait	Model	R^2	RMSE[1]	Intercept	SE[1]	Prob[2]	Slope	SE	Prob[3]
ECM	NorFor	0.914	0.787	6.3	3.4	0.078	0.80	0.09	<0.001
	NDS	0.855	2.168	−22.7	9.2	0.027	1.69	0.25	<0.001
MTP	NorFor	0.920	0.027	0.19	0.10	0.068	0.78	0.08	<0.001
	NDS	0.788	0.077	−0.36	0.28	0.226	1.30	0.23	<0.001

[1]Root mean square error and standard error.

[2]Probability that intercept is different from 0.

[3]Probability that slope is different from 1.

The NorFor nutrition model (http://www.norfor.info/) has been used extensively for formulating dairy rations in Scandinavia. The Nutritional Dynamics System (NDS) version of the Cornell model (http://www.rumen.it/en/ndspro) has been widely adopted by consulting nutritionists in North America. We initiated a comparison of how well NorFor and NDS predicted observed yields of energy-corrected milk (ECM) and milk true protein (MTP). Data from 5 published trials (21 diets) were entered into NorFor (version 2017.12.0.187) and NDS (version 3.9.5.01). Analyzed composition data for major feed ingredients were used to modify tabulated feed compositions in both models. NorFor reports ECM directly but total milk protein rather than MTP; MTP was computed by multiplying total milk protein by 0.95. The lower of the energy- or MP-allowable ECM and energy- or MP-allowable MTP reported by NDS were used in fitting. Data were from short-term Latin square trials; thus, yields were not corrected for BW change in either model. Predictions of ECM and MTP were regressed on observed data using SAS, including trial and diet(within trial) in the general linear model; regression parameters are in Table 1. Both models explained most of the observed variation; however, root mean square errors were smaller and coefficients of determination were greater when fitting NorFor data. Moreover, standard errors of intercepts and slopes were smaller for NorFor. Slopes from fitting ECM and MTP predictions from both models were different from 1, indicating significant slope bias. Further work using much larger databases is needed to test the relative effectiveness of the NorFor and NDS models.

Key Words: NorFor, Nutritional Dynamics System, Cornell model

516 Insulin is required for essential amino acid stimulation of mTORC1 signaling in mammary cells. V. L. Pszczolkowski*, M. M. Kurth, E. Meyer, and S. I. Arriola Apelo, *Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

Insulin stimulates milk protein synthesis through repression of tuberous sclerosis protein complex and recruitment of the mechanistic target of rapamycin complex 1 (mTORC1) to the lysosomal membrane, where mTORC1 can be activated by AA. We analyzed the necessity of insulin signaling for essential AA (EAA) stimulation of mTORC1 substrates in mammary epithelial cells. MAC-T cells (n = 3) were serum and insulin starved overnight and further starved for EAA for 4 h before incubating for 1 h in Dulbecco's modified Eagle's medium supplemented with insulin (100 n*M*) or vehicle, and 0, 0.05, 0.1,0.3, 1.0, and 3.0 m*M* of EAA at the profile of casein. Nonessential AA were maintained at 1.2 m*M*. Intracellular proteins were isolated and analyzed by Western blotting for total and phosphorylated forms of the mTORC1 substrate and protein synthesis regulator S6 kinase 1 (Thr 389), its downstream substrate ribosomal protein S6 (Ser 240/244), and the mTORC1 substrate and autophagy initiator factor ULK1 (Ser 757). Amino acid dose response parameters (linear and quadratic) were estimated for the phosphorylated to total ratio of each of the above-mentioned proteins, using the linear model and confidence interval functions in RStudio. Amino acid response parameters were estimated independently in the presence and absence of insulin. Intercepts were higher in the presence of insulin, from 70% for ULK1 to 10-fold for S6K1, indicating an AA-independent effect of insulin on mTORC1 activity. On the other hand, essential AA significantly stimulated mTORC1 activity only in the presence of insulin, where linear and quadratic parameters for the 3 target proteins were significantly different than zero ($P < 0.01$). In the absence of insulin, linear and quadratic parameter estimates were no different than zero ($P > 0.05$), but significantly different than insulin-treated parameter ($P < 0.05$). Our results indicate that EAA have insulin-dependent saturable effects on mTORC1 activity within physiological levels, suggesting that disruption of insulin signaling would negatively affect milk protein synthesis.

Key Words: insulin, amino acids, mechanistic target of rapamycin (mTOR)

517 Assessing bioavailability of amino acids from various feedstuffs in dairy cattle using a stable isotope–based approach. X. Huang*[1], K. A. Estes[2], P. S. Yoder[1], and M. D. Hanigan[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*Balchem Corp., New Hampton, NY.*

Improving N efficiency can be achieved in dairy cows by more precisely supplying amino acids (AA) relative to requirements which necessitates accurate supply estimates of individual amino acids from feedstuffs. This study was conducted to determine absorption of individual AA from various feedstuffs. Seven heifers (258 ± 28 kg BW) were assigned to 8 treatment sequences in a 7 × 8, incomplete, Latin square design. Treatments were a control base diet (BD) plus 10% (DM basis) corn silage (CS), grass hay (GH), alfalfa hay (AH), soybean hulls (SH), dried distiller grains (DDGS), wet brewer grains (BG), or corn grain (CG)). Each period was 10 d. The base diet contained a mix of corn silage, dry hay, and soybean meal. The crude protein content for AH, BD, BG, CG, CS, DDGS, GH, and SH diets were 22.5, 23.1, 23.3, 21.8, 21.8, 23.5, 22.0, and 22.1% respectively. Blood AA entry rates were estimated for each AA by fitting a 4-pool dynamic model to blood ^{13}C AA enrichment over the course of a 2h ^{13}C algal AA infusion. Dry matter intake tended to be lower for BG diet ($P = 0.072$) and N intake was lower for CG, CS and GH diets ($P < 0.01$). Apparent total-tract N digestibility was 64.7 ± 4.6%, without significant difference among treatments. The plasma entry rates of Ile, Leu, Lys, Met, Phe, Thr, and Val were 5.61, 5.99, 5.90, 5.74, 5.61, 5.83, 5.76, and 5.87 mmol/min for AH, BD, BG, CG, CS, DDGS, GH, and SH, respectively, and no significant difference was detected among treatments. For nonessential AA (Ala, Asp, Pro, Tyr, Glu, Gly), the plasma entry rates were 14.00, 14.84, 15.18, 15.25, 14.31, 17.51, 13.56, and 14.20 mmol/min for AH, BD, BG, CG, CS, DDGS, GH, and SH respectively, and DDGS had trend to be higher ($P = 0.075$). Given the derived AA plasma entry rates, the individual AA supply by ingredient can be determined by regression in future work.

Key Words: amino acids, bioavailability, dairy cattle

Author Index

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