**APPENDIX D**

**SAES-422  
Multistate Research Activity Accomplishments Report**

**Project Number:** NC-2040

**Project Title:** Metabolic Relationships in Supply of Nutrients for Lactating Cows

**Period covered:** November 1, 2016 to October 31, 2017

**Annual Meeting Dates:** October 23 – 24, 2017

**Date of This Report:** November 29, 2017

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**The following includes a summary of minutes of the annual meeting (A), summary of station reports (B), including list of publications (C), and impacts (D).**

1. **Summary of Minutes of Annual Meeting**

**Monday, October 23, 2017**

* Directory updated
* Dr. Steve Smith, USDA-NIFA, provided the following information:
  + Sonny Perdue was appointed Secretary of Agriculture. Sonny has Animal Agriculture background
  + President Trump and Sonny Perdue are both in support of research
  + NIFA is currently interviewing candidates for two National Program Leader positions in Animal Biosecurity and Veterinary Science (generally DVM, or strong animal health background)
  + NIFA recently filled two Program Specialist positions on the Agriculture and Food Research Initiative (AFRI) Coordination Team.
  + FY2017 RFAs have passed submission deadlines, some panels have met
  + NIFA is holding listening sessions in person and online. Steve will send information out, please participate
  + Stay tuned on future of challenge grants
* Rewrite (sub-committee: Tanya, Tim, Barry, Kevin)
  + Meet after USDA
    - kept the same objectives (each Tim, Barry, and Kevin took an objective; Tanya overseeing)
    - October 1st was the deadline to submit objectives; each of us received an invitation to rejoin the group, we need respond by October 31st
    - rewrite due December 1st
    - An email will go out requesting a description of what each of us are working on under each objective (past 5 years, next 5 years). Also emphasize any collaborations across research stations. Send by Nov 15th
    - Outputs
      * Discussed and agreed that we will propose a nutrition symposium for ADSA each year, chaired by Mike this year. We will put in a proposal for a multi-year symposium to fund one international speaker each year. Steve indicated it can be reviewed off-panel.
* Station reports

**Tuesday, October 24, 2017**

* Announcements for positions, individuals will send announcements out to the email list serve
* Election of Officers
  + Incoming Chair: Heather White
  + Incoming Secretary: Antonio Faciola
* Selection of Meeting Location
  + Considered other locations, if you have suggestions send to Antonio for 2019
  + For 2018, will pair with Discover conference
    - Will have our meeting Nov 1 to 2
    - White will seek pricing from Holiday Inn (previous venue) and venue for Discover conference (Eaglewood Resort and Spa)
    - Will contact Larry Miller and Molly Kelly to see if tagging on with Discover conference hotel contract is possible
* Station Reports
* Meeting adjourned at noon

**B. Summary of Station Reports**

University of California at Davis, Department of Animal Science

Station Researchers: James Fadel, Matthias Hess, and Ermias Kebreab

**STUDIES:**

***1. Artificial rumen system.***

System was established. We are in the process of determining some key parameters to facilitate comparison of results between different laboratories/working groups. Results should be available at next meeting.

***2. Effect of various feed additives.***

*In-vitro* system is being utilized to measure and determine changes of physicochemical parameters. Response of physicochemical and biological parameters after addition of feed additives will be evaluated to determine of particular feed additives has the potential to improve nutritional supply to lactating cows. Results should be available for next meeting.

***3. Linear program for water used for production of dairy feeds.***

This research is at the developmental stage and next year there should be more details to present.

***4. A meta analysis of casein infusion studies.***

Increased MP supply, achieved through postruminal casein infusion, was an important explanatory variable either by itself for some production responses (e.g., milk yield) or in interaction with MP supply or MP balance for other responses (e.g., MTPY). Meta-regressions showed that the relationship between ΔMP and the response of MTPY was highest when MP supply was low or MP balance was negative. The relationships between ΔMP and responses of plasma AA concentrations were influenced

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by the plane of nutrition (MP supply or MP balance), the stage of lactation (early vs. mid/late), the type of forage (grass/legume- vs. corn silage-based diets), and the ratio of microbial protein in MP supply. Including the variations of MTPY responses to increased protein supply according to dietary and cow characteristics can improve models currently used to balance dairy rations for protein and AA.

***5. Energetic costs of excess dietary protein***

The quantification of energetic costs associated with excreting N when dairy cows are fed protein in excess of requirement can assist in refining the nutrient requirements of dairy cattle. These results suggest N fed above requirement increases HP and decreases RE and MGE. It is not clear why the relationships of ExN with MGE and RE appear to be of greater magnitude when estimated separately than when MGE and RE are combined into the composite dependent variable of EB. This apparent inconsistency in covariate estimates could be a result of the changing relationship of RE and MGE throughout the course of the lactation cycle. More investigation into this topic is needed to understand the biological mechanisms driving these relationships.

***6. Bayesian mechanistic modeling***

A large amount of uncertainty about the principles that direct ruminant digestion and metabolism, including everything from rumen fermentation to genetic variation, exists. As long as this uncertainty remains, it is the modeler’s responsibility to provide a cogent analysis of the contribution of individual sources and collectively evaluate the degree of confidence to model predictions. Conventional deterministic mechanistic models do not provide error estimates and thus fail to provide an assessment of the risk associated with decisions based on model results. Bayesian mechanistic models capture the inherent variability of the biological system under study and provide an assessment of the error associated with complex model results.

University of California at Davis School of Vet Med

Station Researcher: Heidi A Rossow

**Project Title: Metabolic Relationships in Supply of Nutrients for Lactating Cows**

**Objective 1:** To quantify supply, availability, and interaction of nutrients and bioactive compounds utilized for efficient milk production while reducing environmental impact.

**Objective 2:** To identify and quantify molecular, cellular, and organismal signals that regulate partitioning and efficient conversion of nutrients to milk.

**Objective 3:** To use this knowledge of feed properties and metabolic and molecular quantitative relationships to challenge and refine precision feeding systems for dairy cattle.

**Progress of work**

Objective 1

1. We completed an experiment examining the effect of a fibrolytic enzyme on milk yield and body weight changes during a lactation on 1 commercial dairy herd. The second herd will be completed in January 2018.

2. Trial examining incidence of sub clinical and clinical ketosis and changes in milk yield and body weight in transition cows fed a glucose precursor mixed into a TMR completed Sept 10, 2017.

3. Calf trial examining weight gains and rumen development using blood BHBA levels in calves fed a phytogenic supplement in the starter mix completed.

4. Development of a benchmarking system to identify good feed management practices in post wean heifers - data collection completed.

Objective 2, 3

1. Quantifying mitochondrial enzyme activities to find markers that identify future high producing cows with longevity in the herd.

**The effects of blood composition and age on PBMC mitochondrial enzyme activity in pre-wean dairy calves. A Niesen, HA Rossow**

Mitochondrial enzyme activities were measured in 20 Holstein and 20 Jersey calves at 3-6 d, 10-15 d and 54-60 d of age to examine changes with age, breed and immune challenge. Blood cell profile was measured using a Hemavet 950FS at each time point to determine if the calf was fighting an immune challenge and calves were weighed at the beginning and end of the experiment to examine changes in MRC enzymes with growth. Citrate synthase activity was analyzed separately and also used as a scaling factor for mitochondrial number for all other complexes.

During the prewean period, mitochondria number (citrate synthase activity) decreased from 4 d to approximately 2 weeks of age but did not change at weaning (60 d). Complex V activity increased with age and was higher in higher ADG calves. As calves get older and grow, they should need more ATP and the availability of more ATP should increase their ADG. Similarly to citrate synthase, lower basophil levels are associated with increased complex V activity implying that calves with lower gains were fighting an allergen. Of all the enzyme complexes measured, complex 5 responded the most to changes in gain and age. Results from this experiment show that enzyme activities change in response to immune state, age and ability to grow. This method of measuring mitochondrial function also appears to be more responsive to changes in gain and physiological state of the calf than measurement of oxygen consumption.

**Do high and low producing dairy cows exhibit differences in mitochondrial enzyme activities during early lactation. A Niesen, HA Rossow**

The following experiment was conducted at the Purina Animal Nutrition Center, Gray Summit, MO. 56 Holstein cows (70±11 DIM) were assigned to one of four groups: primiparous high or low milk production and multiparous high or low milk production. Group assignments for each milk production parameter were made after data were collected by averaging each milk production parameter for primiparous cows and 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 early lactation and processed for crude mitochondrial extracts from PBMCs. Mitochondrial function of the extracts were 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 3 times within a week of blood collection at milking and analyzed for major components using a MilkoScan FT2 by FOSS (Mulgrave, Australia). Data was analyzed using the Mixed procedure of SAS (Version 9.4, SAS Inst. Inc.,Cary, NC) for high or low groups for each production parameter with cow as the experimental unit of interest and dependent variables parity production group and days in milk as a covariate. There were no interactions between production level group (high or low) and parity group. All milk production parameters followed similar patterns of significance relative to differences in mitochondrial enzyme activities with level of production and parity group. Citrate synthase activity was higher in primiparous cows than multiparous cows. Proton efficiency, which represents usage of H ions to produce ATP, and Complex V enzyme activities, have tendencies to be higher in multiparous than primiparous cows. Complex I activity was lower in low producing cows and not affected by parity indicating that this enzyme activity may be a marker of ability to produce milk. Previous data also indicates that complex I is not affected by age. These results indicate that there are differences in mitochondrial enzyme activities for complex I, between low and high producing cows, and differences in citrate synthase activity by parity group and primiparous vs.multiparous cows for energy corrected milk, milk fat yield and total solids yield. There is also a tendency (P ≤ 0.1) for differences in complex V activity and proton efficiency by parity group. Therefore although we did not follow these cows from a young age, it supports the assumption that we can differentiate between future high and low producing dairy cows using mitochondrial enzyme activity.

Cornell University

Station Researcher: Yves Boisclair

Objective 2: To identify and quantify molecular, cellular, and organismal signals that regulate

partitioning and efficient conversion of nutrients to milk.

Specific study 1: Factors regulating plasma adiponectin in transition dairy cows.

Investigators: Christopher S. Krumm, Sarah L. Giesy, Luciano S. Caixeta, W. Ronald Butler, Helga Sauerwein, Jin Wook Kim and Yves R. Boisclair.

In transition dairy cows, plasma levels of the insulin-sensitizing hormone adiponectin fall to a nadir at parturition and recover in early lactation. The transition period is also characterized by rapid changes in metabolic and hormonal factors implicated in other species as positive regulators of adiponectin production (i.e., negative energy balance, lipid mobilization) and others as negative regulators [i.e., reduced leptin and insulin and increased growth hormone and non-esterified fatty acids (FA)]. To assess the role of onset of negative energy balance and lipid mobilization after parturition, dairy cows were either milked thrice daily (lactating) or never milked (non-lactating) for up to 4 weeks after parturition. Plasma adiponectin was 21% higher across time in non-lactating than lactating cows. Moreover, nonlactating cows recovered plasma adiponectin at similar rates as lactating cows even though they failed to lose body condition. Next, we assessed the ability of individual hormones to alter plasma adiponectin in

transition dairy cows. In the first experiment, dairy cows received a constant 96 h intravenous infusion of either saline or recombinant human leptin starting on d 8 of lactation. In the second experiment, dairy cows were studied in late pregnancy (LP, starting on prepartum d -31) and again in early lactation (EL, starting on d 7 postpartum) during a 66 h period of basal sampling followed by 48 h of hyperinsulinemic-euglycemia. In the third experiment, cows were studied either in LP (starting on d -40 prepartum) or EL (starting on d 7 postpartum) during a 3 h period of basal sampling followed by 5 d of bovine somatotropin treatment. Plasma adiponectin was reduced by an average of 21% in EL relative to LP in these experiments but neither leptin, insulin, or GH treatment affected adiponectin in LP or EL.Finally, the possibility that plasma FA repress plasma adiponectin was evaluated by intravenous infusion of a lipid emulsion in non-pregnant, non-lactating cows in the absence or presence of glucagon for 16 consecutive hours. The intralipid infusion increased plasma FA concentration from 102 to over 570 μM within 3 h but had no effect on plasma adiponectin irrespective of presence or absence of glucagon. Overall these data suggest that energy balance around parturition may regulate plasma adiponectin but

do not support roles for lipid mobilization or sustained changes in the plasma concentration of leptin, insulin, growth hormone or FA.

Specific study 2: Regulation of plasma FGF21 in transition dairy cows.

Investigators: Luciano S. Caixeta, Sarah L. Giesy, Christopher S. Krumm, James W. Perfield II,

Anthony Butterfield, Katie M. Schoenberg, Donald C. Beitz and Y.R. Boisclair.

Modern dairy cows meet the energy demand of early lactation by calling on hormonally-driven

mechanisms to increase the use of lipid reserves. In this context, we recently reported that fibroblast growth factor-21 (FGF21), a hormone required for efficient use of lipid reserves in rodents, is upregulated in periparturient dairy cows. Increased plasma FGF21 in early lactation coincides with elevated circulating concentrations of glucagon (GCG) and non-esterified fatty acids (NEFA). To assess the relative contribution of these factors in regulating FGF21, two experiments were performed in energy sufficient, non-pregnant, non-lactating dairy cows. In the first study, cows were injected with saline or GCG every 8 h over 72 h. GCG increased hepatic FGF21 mRNA by an average of 5-fold over matched controls but had no effect on plasma FGF21. In the second study, cows were infused and injected with saline, infused with intralipid and injected with saline or infused with intralipid and injected with GCG. Infusions and injections were respectively administered IV over 16 hours and SC every 8 h. Intralipid infusion increased plasma NEFA from 92 to 550 μM within 3 h and increased plasma FGF21 from 1.3 ng/ml to >11 ng/ml 6 h later; FGF21 mRNA increased by 34-fold in liver but remained invariant in adipose tissue. GCG injections during the intralipid infusion had no additional effects on plasma NEFA, liver FGF21 mRNA or plasma FGF21. These data implicate plasma NEFA as

a key factor triggering hepatic production and increased circulating concentrations of FGF21 in early lactation.

University of Delaware

Station Researcher: Tanya Gressley

**OBJECTIVE 1:** To quantify supply, availability, and interaction of nutrients and bioactive compounds utilized for efficient milk production while reducing environmental impact.

**Evaluate bioavailability and animal performance response to rumen protected amino acid products.** Our lab conduced 2 studies to assess the bioavailability of rumen protected sources of methionine, lysine, and histidine. These studies evaluated products in development and results are being used to further refine those products. Ultimately, provision of rumen protected but bioavailable forms of rumen protected amino acids will allow for precision feeding to meet amino acid needs of cows while reducing the environmental impact of feeding. We also conducted one lactation trial evaluating the ability of rumen protected lysine to replace blood meal in the ration. Reduced reliance upon blood meal to meet amino acid requirements will reduce the need for animal based protein products and will result in less variable amino acid supply.

**Impact of different buffers on measures of post-ruminal fermentation.** We used an abomasal starch infusion model to increase post-ruminal fermentation and evaluated the ability of different buffers to impact fecal measures of hindgut fermentation. In a 5 x 5 Latin square design, ten multiparous cows were randomly assigned to treatments of a high starch diet and supplemented with fed sodium bicarbonate (FSB), calcium carbonate (FCC), or calcium carbonate and magnesium oxide (FCCM), or abomasally infused encapsulated sodium bicarbonate (ISB). All cows were infused twice daily with 1 g/kg body weight corn starch suspended in 1.5 L of tap water. Rumen fluid and fecal samples were collected on day 7 of each period at 4-hr intervals beginning at 6:30 am and ending at 2:30 am on the following day for measurement of pH, volatile fatty acids (VFA), and lipopolysaccharide (LPS). Feed samples were collected on day 7 of each period after the 6:30 am sampling. Milk samples were collected on day 7 during the morning and afternoon. Treatment did not affect rumen pH, but fecal pH was higher in the FCCM group (pH 6.64; P < 0.001) than in the CON group (pH 6.47; P<0.001). Time affected total rumen VFA, but not rumen lactate, acetate, propionate, isobutyrate, valerate, isovalerate, or total VFA (P > 0.10). There were no effects of treatment on fecal lactate, butyrate, isobutyrate, valerate, or isovalerate (P > 0.10); however treatment affected acetate (P = 0.04) and propionate (P= 0.03) and tended to affect total VFA (P = 0.07). Total VFA were greater for FCC and FCCM compared to CON (P = 0.03 and 0.007, respectively). Similarly, acetate was greater for FCC and FCCM compared to CON (P = 0.02 and 0.003, respectively), and propionate was greater for FCC and FCCM compared to CON (P = 0.01 and 0.005, respectively). In addition, fecal acetate was lower in FSB compared to FCCM (P = 0.05). The contrast of CON vs. (ISB + FCC + FCCM) was also significant for total VFA, acetate, and propionate, due to lower VFA for CON vs. the proposed post-ruminal buffers. Fecal dry matter was affected by time (P < 0.001), due to the lowest dry matter at 0 h (13.0%), intermediate dry matter at 4, 12, and 16 h (13.4 to 13.8%), and greatest dry matter at 8 and 20 h (14.4 and 14.5%, respectively, however treatment did not affect fecal dry matter. The data suggest that FCC and FCCM have postruminal buffering capability, but we still need to complete analyses of fecal LPS and nutrient digestibility.

**Impact of different levels of abomasal starch infusion on measures of systemic inflammation and intestinal fermentation.** Six ruminally cannulated Holstein cows in mid to late lactation were used in a replicated 3×3 Latin square with 21 day periods. The first 14 days of each period were used for adaptation and during the last week of each period cows were abomasally infused with 0, 1, or 3 g/kg bodyweight corn starch per day. Rumen pH was continuously measured during days 12 to 14 and 19 to 21 of each period using indwelling pH meters. Samples of feces and rumen fluid will be collected every 6 hours on days 14 and 21 of each period, with the first sample occurring at the time of feeding (0 h). Feces and rumen samples were used to evaluate pH and VFA concentration. Additionally, feces will be evaluated for LPS and composite fecal samples were used to measure nutrient digestibility. Blood samples were collected from the coccygeal (tail) vein at the time of feeding (0 h) and 6 hours later on days 14 and 21 of each period for measurement of acute phase proteins and leukocyte profile. The experiment was recently completed and analyses and results are pending.

University of Florida

Station Researcher: Timothy Hackmann

 Objective 2: To quantify metabolic interactions among nutrients that alter synthesis of milk.

**Progress of work**

*1. Using fluorescent compounds to identify carbohydrates (substrates) consumed by mixed rumen bacteria.*

Junyi Tao, Halima Sultana, John P. Driver, Corwin D. Nelson, and Timothy J. Hackmann

Most rumen bacteria are uncultured, making it hard to identify their niche and what feed carbohydrates (substrates) they consume. Here we suggest using fluorescent compounds to identify what carbohydrates bacteria consume. We suggest that fluorescent compounds would enable us to literally see which bacteria are consuming which carbohydrates. When coupled with cell sorting and DNA sequencing, this approach would enable us to formally identify these bacteria by the carbohydrates consumed. In this study, we apply this approach to a fluorescent glucose compound (2-NBDG). Using microscopy, we found mixed rumen bacteria readily took up 2-NBDG (Fig. 1B). Using fluorimetry, we found had a maximum velocity (*Vmax*) of 0.180 (0.05 SEM) nmol mg protein−1 min−1 and Michaelis constant (*Km*) of 6.37 (SEM 4.86) μM (n = 3). Flow cytometry showed that >10% of cells were positive for 2-NBDG uptake (Fig. 1C), and sorting led to cells of >95% purity (Fig. 1E). Work is ongoing to 1) sequence sorted cells for identification, and 2) synthesize and test uptake of other glucose compounds. Our work supports that 2-NBDG, in combination with other compounds, could be used to identify which bacteria consume carbohydrates, helping define what role uncultured bacteria play in the host.

*2. Genomes of rumen bacteria encode atypical pathways for fermenting hexoses to short-chain fatty acids*

Timothy J. Hackmann, David K. Ngugi, Jeffrey L. Firkins, and Junyi Tao

Bacteria have been thought to follow only a few well-recognized biochemical pathways when fermenting glucose or other hexoses. These pathways have been chiseled in the stone of textbooks for decades, with most sources rendering them as they appear in the classic 1986 text by Gottschalk. Still, it is unclear how broadly these pathways apply, given that they were established and delineated biochemically with only a few model organisms. Here we show that well-recognized pathways often cannot explain fermentation products formed by bacteria. In the most extensive analysis of its kind, we reconstructed pathways for glucose fermentation from genomes of 48 species and subspecies of bacteria from one environment (the rumen). In total, 44% of these bacteria had atypical pathways, including several that are completely unprecedented for bacteria or any organism. In detail, 8% of bacteria had an atypical pathway for acetate formation; 21% for propionate or succinate formation; 6% for butyrate formation; and 33% had an atypical or incomplete Embden-Meyerhof-Parnas pathway (see Fig. 2). This study shows that reconstruction of metabolic pathways—a common goal of omics studies—could be incorrect if well-recognized pathways are used for reference. Further, it calls for renewed efforts to delineate fermentation pathways biochemically.

Station Researcher: Antonio Faciola

Project 1: Assessing potentially digestible NDF and energy content of canola meal from twelve Canadian crushing plants over four production years

E. M. Paula, J. L.P. Daniel, L. G. Silva, H. H. A. Costa, and Antonio. P. Faciola

The objective of this study was to assess NDF digestibility and energy content of canola meals (CM) produced in Canada over a 4-year period. Canola meal samples were collected from 12 Canadian crushing plants over 4-years (total = 48) and analyzed for chemical composition, potentially digestible NDF (pdNDF), total digestible nutrients at maintenance (TDN1x), and NEL simulating a cow consuming 3x maintenance (NEL3x). To estimate TDN1x and NEL3x, pdNDF was calculated as: 1) pdNDFOBS = (NDF - NDICP - iNDF), using observed CM iNDF values after 288-h in situ ruminal incubations; 2) pdNDFNRC = (NDF - NDICP - ADL) × {1- [ADL/(NDF - NDICP)]0.667}, according to NRC 2001; 3) pdNDFCNCPS = (NDF - NDICP - iNDF), according to the CNCPS that calculates iNDF as acid detergent lignin (ADL) × 2.4. Concentrations of NDF, NDICP, and ADL in all equations were given in % of DM. Truly digestible NDF was estimated multiplying the observed and predicted pdNDF by 0.75. Then TDN1x and NEL3x were calculated assuming a diet with 74% of TDN1x according to NRC 2001 equations. Regressions of predicted (NRC or CNCPS) vs. observed values were performed using Proc Reg of SAS (Table 1). pdNDFOBS, pdNDFNRC, and pdNDFCNCPS averaged 15, 8, and 2.4% of DM, respectively. The TDN1x average were 73, 67, and 64%, respectively. The NEL3x average were 1.88, 1.73, and 1.63 Mcal/kg, and ranged from 1.73 to 2.08; 1.51 to 1.94; and 1.4 to 1.87 Mcal/kg DM for NEL3xOBS, NEL3xNRC, and NEL3xCNCPS, respectively. Our results indicate that NEL3x from CM diets may be underestimated in current nutritional models due to underestimations in CM NDF digestibility. More accurate information on CM NDF digestibility may improve energy content estimation, thus improving diet formulation accuracy.

Project 2: Bovine mammary epithelial cell (MAC-T) phenotype impacts TNFα-mediated MAPK signaling and inflammation

L. G. Silva, B. S. Ferguson, L. L. Hernandez, A. P. Faciola

The objective was to determine if MAC-T phenotype would impact inflammatory signaling and inflammatory gene expression. MAC-T cells were cultured under basal (DMEM 10% fetal bovine serum and 10 μg/ml of insulin) or lactogenic conditions (basal media + 1 ug/mL ovine prolactin, 0.5 ug/mL hydrocortisone, and 10 mM sodium acetate) and mitogen-activated protein kinase (MAPK; ERK, JNK, and p38) phosphorylation and pro-inflammatory gene expression examined in response to tumor necrosis factor alpha (TNFα). Statistical analysis was assessed via ANOVA and Tukey’s post-hoc analysis at *P* ≤ 0.05. MAC-T cells were co-stimulated with increasing concentrations of TNFα (0, 10, 30, 100, 300, 1000 pM). Cell lysates were harvested 15 min post-TNFα stimulation and assessed for MAPK phosphorylation via immunoblotting. JNK and p38 phosphorylation increased in a dose-dependent manner; yet the magnitude of JNK and p38 signaling was greater under basal compared to lactogenic conditions. Cells were next stimulated in parallel with TNFα (300 pM) and lysates harvested over time (0, 15, 30, 100, 120, 180 min). JNK and p38 phosphorylation were robust and transient in MAC-T cells stimulated with TNFα over time. Similar to dose-response experiments, JNK and p38 signaling were significantly more robust in MAC-T cells under basal conditions. We next examined inflammatory gene expression in MAC-T cells cultured under basal or lactogenic conditions and co-stimulated in the presence or absence of TNFα (300 pM). RNA was isolated and PCR array performed to evaluate the expression of 83 inflammatory genes. Pro-inflammatory gene expression was increased in MAC-T cells in response to TNFα. Consistent with enhanced MAPK signaling; pro-inflammatory gene expression was significantly increased in MAC-T cells under basal compared to lactogenic conditions. Real-time qPCR was used to validate PCR array findings. Collectively, our data demonstrate that MAC-T cells cultured under basal conditions are more responsive to TNFα. These findings suggest that investigators consider the importance of MAC-T phenotype when designing future inflammation-related studies.

Project 3: Effects of replacing soybean meal with canola meals varying in rumen undegraded protein on ruminal fermentation in vitro

Hugo F. Monteiro, Eduardo M. Paula, João L. P. Daniel, Pedro D. B. Benedetti, Rebecka Bittner, Lorrayny G. Silva, Teshome Shenkoru and Antonio. P. Faciola

This study aimed to evaluate the effects of replacing soybean meal (SBM) with canola meals (CM) differing in RUP (% of CP) content (38% RUP, LCM or 50% RUP, HCM) on in vitro ruminal fermentation, including total gas and CH4 production. Two in vitro experiments were conducted. Both experiments had three 48 h incubations, totaling 18 observations per treatment. Experiment 1 tested 3 protein supplements as the sole ingredient (SBM, LCM, and HCM). Experiment 2 tested 3 TMR containing the protein supplements from experiment 1. Measurements were: pH, gas production (GP), degradability kinetics, metabolizable energy (ME), CH4 production, in vitro true OM digestibility (*iv-*tOMd), and VFA production. Data were analyzed using the Mixed procedure of SAS. Degradability kinetics were fitted using the NLIN procedure of SAS. Means were compared by orthogonal contrasts: SBM vs. (LCM + HCM) and LCM vs. HCM. Partial data is presented in Table 1. The SBM ingredient had greater *iv-*tOMd, VFA production, ME, total GP48 and CH4 (mM) when compared to both CM. When comparing both CM ingredients, HCM had shorter lag phase and lower branched-chain VFA production. However, ingredients did not differ in CH4 (g/kg dOM). In experiment 2, SBM diet had a trend to increase total CH4 production (mM), but diets did not differ in CH4 (g/kg dOM). The SBM diet also had a trend to increase valerate and *iso*-valerate concentration which may indicate more proline and leucine deamination. When comparing both CM diets, HCM increased final pH and had a trend to lower ME, while decreased total GP48.

Project 4: Effects of camelina cake supplementation at two dietary fat levels on ruminal fermentation and nutrient flow in a dual-flow continuous culture system

V. L. N. Brandao, X. Dai, L. G. Silva, E. M. Paula, T. Shenkoru, A. P. Faciola

This study aimed to assess whether camelina cake (CC) could partially replace canola meal at two dietary fat levels and its effects on ruminal fermentation and nutrient flow in a dual-flow continuous culture system. Diets were randomly assigned to eight fermenters in a 2x2 factorial arrangement of treatments (CC and fat level) in a 4x4 Latin square design with four 10-d experimental periods consisted of 7 d for diet adaptation and 3 d for sample collection. Treatments were: A) 7.7% CC inclusion at 5% EE (CC5); B) no CC at 5% EE (NCC5); C) 17.7% CC at 8% EE (CC8); and D) no CC at 8% EE (NCC8). Diets contained 55% orchard hay and 45% concentrate, and fermenters were fed 72 g of DM twice daily. On d 8, 9, and 10 of each period effluent samples were collected for analyses of digestibility, VFA, NH3, N-balance, microbial growth, amino acids and fatty acids flow. Statistical analysis was performed using the MIXED procedure of SAS. Partial data is presented in Table 1. Ruminal pH, NH3 total VFA and the flow of most AA were not affected by treatments; however, CC decreased acetate and increased propionate molar proportions. Ruminal digestibility of OM, NDF, ADF and CP were decreased by CC inclusion. Concentrations of isomers from C18:1, C18:2, C18:3 were affected by CC supplementation; overall CC inclusion increased isomers *cis* and decreased *trans*. Inclusion of CC decreased biohydrogenation. The shift from acetate to propionate, increased outflow of important FA and reduction on biohydrogenation observed when CC was included may be advantageous; however, dietary fat was deleterious to N flow.

Project 5: Camelina seed supplementation at two dietary fat levels change ruminal bacterial community composition in a dual-flow continuous culture system

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This experiment aimed to determine the effects of camelina seed (CS) supplementation at different dietary fat levels on ruminal bacterial community composition and how it relates to changes in ruminal fermentation in a dual-flow continuous culture system. Diets were randomly assigned to 8 fermenters (1,200-1,250 mL) in a 2 × 2 factorial arrangement of treatments in a replicated 4 × 4 Latin square with four 10-d experimental periods that consisted of 7 d for diet adaptation and 3 d for sample collection. Treatments were: 1) no CS at 5% ether extract (EE, NCS5); 2) no CS at 8% EE (NCS8); 3) 7.7% camelina seed at 5% EE (CS5); and 4) 17.7% CS at 8% EE (CS8). Megalac was used as a control to adjust EE levels. Diets contained 55% orchardgrass hay and 45% concentrate, and fermenters were equally fed a total of 72 g/d (DM basis) twice daily. The bacterial community was determined by sequencing the V4 region of the 16S rRNA gene using the Illumina MiSeq platform. Sequencing data were analyzed using mothur and statistical analyses were performed in R and SAS. The most abundant phyla across treatments were the *Bacteroidetes* and *Firmicutes,* accounting for 49% and 39% of the total sequences, respectively. The bacterial community composition in both liquid and solid fractions of the effluent digesta changed with CS supplementation but not by dietary EE. Including CS in the diets decreased the relative abundances of *Ruminococcus* spp*.*, *Fibrobacter* spp*.*, and *Butyrivibrio* spp. The most abundant genus across treatments, *Prevotella,* was reduced by high dietary EE levels, while *Megasphaera* and *Succinivibrio* were increased by CS supplementation in the liquid fraction. Correlatively, the concentration of acetate was decreased while propionate increased; C18:0 was decreased and polyunsaturated fatty acids, especially C18:2 n-6 and C18:3 n-3, were increased by CS supplementation. Based on the correlation analysis between genera and fermentation end products, this study revealed that CS supplementation could be energetically beneficial to dairy cows by increasing propionate-producing bacteria and suppressing ruminal bacteria associated with biohydrogenation. However, attention should be given to avoid the effects of CS supplementation on suppressing cellulolytic bacteria.

Project 6: Nutritional Evaluation of Forage Ephedra (*Ephedra nevadensis*) as an Alternative Forage Using a Dual-flow Continuous Culture System

Claudia Sampaio, Eduardo de Paula, Lorrayny da Silva, Virginia Brandao, Xiaoxia Dai, Teshome Shenkoru, Barry Perryman, and Antonio Faciola

Forage ephedra (*Ephedra nevadensis;* ephedra) is well adapted and grazed by sheep and cattle in the Western US; however, its nutritional value has not been well established. The objective of this study was to determine ruminal digestibility, ruminal microbial fermentation, and bacterial N synthesis of ephedra as compared to cheatgrass (*Bromus tectorum*; CG) and orchard grass hay (*Dactylis glomerata L*.; OGH). Diets were randomly assigned to 6 fermenters in a dual-flow continuous culture system in a 3 × 3 Latin square design with three 10-d experimental periods consisted of 7 d for diet adaptation and 3 d for sample collection. Fermenters were fed a total of 72 g of dry matter/d equally divided in 2 portions per day. Diets were: 1) 100% air-dried ephedra, 2) 100% air-dried CG and 3) 100% air-dried OGH. Liquid and solid dilution rates were adjusted daily to 10% and 5%/hour, respectively. A 500 mL sample was taken on 8, 9, and 10 d and analyzed for nutrient digestibility and microbial growth. Two 10 mL subsamples were filtered through two layers of cheesecloth and preserved with 0.2 mL of 50% sulfuric acid and 2 mL of metaphosforic acid for subsequent ruminal NH3–N and VFA analyses, respectively. Statistical analysis was performed using the PROC Glimmix procedure of SAS. Partial data are presented in Table 1. The NH3-N concentration was greater for CG followed by ephedra and lowest for OGH (*P < 0.01*). Ruminal pH was greater for ephedra (*P < 0.01*), while total VFA was the lowest (*P < 0.01*). Ephedra had greater acetate molar proportion, which resulted in the greatest acetate: propionate ratio (*P < 0.01*). Ephedra also had the lowest propionate molar proportion. Results from this study indicate that ephedra should not be used as the main forage in cattle diets due to its poor fermentation as evidenced by the lowest total VFA and propionate molar proportion.

Project 7: Mentoring graduate students as a young faculty: Challenges and opportunities

Antonio Faciola

The objective of this presentation is to highlight challenges and opportunities associated with effective mentoring as a young faculty. Effective mentoring, at any stage of one’s career, is challenging. However, effective mentoring while on the tenure-track is crucial for the success of the mentor and the mentee. The current academic environment is fast-paced, highly competitive, and expectations are higher than ever before. Young faculty are expected to excel in teaching, research, and service. High quality teaching and service are very important, but time consuming and often undervalued by administration. High quality research, evidenced by publications and extramural funding, is generally the most important criterion for tenure and promotion. This environment leaves little room for mentoring, and young faculty may feel the need to choose between mentoring or writing papers and grant proposals. Effective mentoring is widely recognized as important for student success; however, I would argue that it is also important for the success of young faculty members: whether one receives tenure is highly dependent on the productivity and success (or lack thereof) of his/her graduate students. Considering this, I have developed a mentor-mentee agreement that aims to: 1) provide students with clear information on what resources and support the lab can offer them, 2) explain what the lab will expect of them, and 3) give students an opportunity to share their previous experiences and future goals, so that a tailored mentoring plan can be made. The goal of this agreement is to achieve the best lab environment possible – one that fosters high productivity and student satisfaction by providing a healthy work environment where everyone feels valued and committed to the success of the group. The current academic environment may seem counteractive to effective mentoring; however, allowing time to clearly lay out expectations, tailoring development plans for each student, and following up on a regular basis can increase the likelihood of student success, which will contribute to young faculty success.

Iowa State University

Station Researcher: Hugo Ramírez Ramírez

*Objective 1: To quantify supply, availability, and interaction of nutrients and bioactive compounds utilized for efficient milk production while reducing environmental impact.*

A standard procedure for measurement of fecal pH in dairy cows does not currently exist. Consequently, sample preparation may influence the precision of this measurement; thus, limiting comparisons across literature reports. The objectives of this study were to determine if differences exist based on preparation method, and to determine variation across methods. Thirty fresh fecal samples were collected from lactating Holstein cows housed in the same pen and consuming the same diet. Five samples were collected at a time and prepared according to the following methods: 1) direct measurement (DIR) in which the pH probe was directly inserted into the fecal sample; 2) strained fecal fluid (STR) obtained by squeezing the fecal sample through four layers of cheesecloth. Three dilution rates (distilled water:feces) were also tested: 3) 0.5:1 dilution (D1), 4) 1:1 dilution (D2), and 5) 2:1 dilution (D3). Each sample was prepared using all methods, resulting in a total of 150 pH measurements. The UNIVARIATE and GLM procedures of SAS were used to test normality and homogeneity of variance, respectively. The Shapiro-Wilk test confirmed that data was normally distributed (P = 0.08). The Levene’s test showed heterogeneity of variance (P = 0.02), thus the SATTERTHWAITE approximation of degrees of freedom for denominator was used for the analysis of variance via the GLIMMIX procedure. Sample preparation method affected (P < 0.01) pH values, resulting in D3 having the highest pH of 6.91 ± 0.04, followed by D2 with a value of 6.79 ± 0.04. Measurements of pH by D1 and DIR were similar, and averaged 6.67 ± 0.04 (P = 0.17); whereas, STR had the lowest value of 6.60 ± 0.04. Descriptive statistics showed the standard deviation for the STR method was 0.173 and 0.174 for D2, while that of D1, D3 and DIR was 0.224, 0.226 and 0.296, respectively. These results demonstrate that pH measurements in strained fecal fluid or a 1:1 dilution rate have reduced variability when compared to direct measurements and other dilution rates.

Kansas State University

Station Researcher: Barry Bradford

*Objective 1: To quantify supply, availability, and interaction of nutrients and bioactive compounds utilized for efficient milk production while reducing environmental impact.*

**Effects of dietary supplementation of Scutellaria baicalensis extract during early lactation on milk production of dairy cattle**

**Investigators:** K. E. Olagaray\*, M. J. Brouk\*, F. Robert†, E. Dupuis†, and B. J. Bradford\*

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**Experimental Procedures**

Multiparous Holstein cows (*n* = 122) on a commercial farm were used in a randomized block design to determine the effect of short term (5-day) and long term (60-day) postpartum administration of *Scutellaria baicalensis* extract (**SBE**) on 305-day milk yield and early lactation milk markers of inflammation and metabolic function. Cows were blocked by parity (2 and 3+), calving date, and risk factors (high risk block: calving difficulty score ≥ 3 or twins), then randomly assigned within block to one of three treatments. Upon calving, cows were moved into a fresh pen where they had free access to an automatic milking system (**AMS**; Austronaut A3, Lely Ltd., Maassluis, the Netherlands), but were encouraged through the AMS if their voluntary attendance was less than 3 visits that day. Cows were managed per site SOP.

Cows were fed a partial mixed ration (**PMR**) twice daily and were provided with pelleted concentrate feed in the AMS. *S. baicalensis* extract (Groupe CCPA, Janze, France) was combined with the dairy’s standard robot feed formulation and pelleted. The control and treatment pelleted feeds were stored in two feed bins that independently supplied the milking robots. Treatments were 1) control (*n* = 39), 2) short term (5-day) administration after calving of the Scutellaria pellet (*n* = 42; **SBE5**), and 3) long term (60-day) administration after calving of the Scutellaria pellets (*n* = 40; **SBE60**). Treatments began within 24 hours after calving. All cows received the control pellet, with the amount based on stage of lactation and milk production. Treatment cows were allocated 1.8 kg of the treatment pellet (delivering 100 g test material/day) in place of an equal amount of control pellet across all milkings for either 5 or 60 days. Pellet allocation was based solely on DIM during the first 50 days of lactation, then from day 51 until 2 weeks prior to dry off, total pellet allocation was based on a feed table which incorporated milk production. The feeding program distributed the target amount of treatment feed across the average number of daily milkings per cow. Due to the nature of AMS, voluntary deviations from a cow’s average number of milkings resulted in slight excesses or shortfall in actual provision of pellet compared to the targeted allocation, and instances in which not all the feed allocated for that particular milking was dispensed were recorded as rest feed. Reported pellet intake is the difference between total pellet allowance and rest feed. The PMR, control pellets, and treatment pellets were sampled every 2 weeks and composited by month for nutrient analysis by Dairy One Forage Laboratory (Ithaca, NY). Nutrient analyses are reported as averages across the study for the PMR in Table 1 and the pelleted feeds in Table 2.

Milk samples were collected on days 1, 3, and once during days 5-12 of lactation, followed by weekly sampling for the remainder of the 120-day collection period. Milk samples collected during the first 2 weeks of lactation were used for both biomarker analysis (haptoglobin and β-hydroxybutyrate [**BHBA**]) and component analysis; subsequent samples were used only for composition analysis. Milk composition was analyzed by MQT labs (Kansas City, MO).

Cows were scored every 2 weeks for body condition on a 5-point scale [1 = extremely thin to 5 = extremely obese from week -3 to week 17 relative to calving. Daily milk production, DIM, number of milkings per day, programmed feed daily allocated and feed provided for both pelleted feeds, and rumination data were recorded on an individual cow basis and collected using the management software, Time for Cows (**T4C**). Culling data were reported in PC Dart by the farm staff.

Milk yield, milk composition, milking frequency, pellet offered, rumination time, and body condition score (**BCS**) data were summarized by week relative to calving for statistical analysis. Milk yield, milk composition, milking frequency, and total pellet intake were analyzed separately for the 60-day treatment period (weeks 1-9) and the carryover period. Statistical analysis was performed using SAS (version 9.4, SAS Institute., Cary, NC) to model the fixed effects of treatment, week, parity and two-way interactions of these variables, as well as the random effects of barn and cow. Repeated measures within cow were modeled with autoregressive and heterogeneous autoregressive covariance structures, and the one with the least Bayesian information criterion was selected for each dependent variable. Repeated measures within cow for BCS and milk haptoglobin were modeled with spatial power covariance structures because of unequal spacing of time points.

**Results and Discussion**

***Treatment provision and total pellet offered***

Test material delivered for the first 5 DIM was not different between SBE5 and SBE60 (*P* = 0.41; 80.78 and 83.08 ± 0.34 g/day, respectively). There was an effect of DIM (*P* < 0.001) as cows adapted to the AMS; however, there was no treatment × DIM interaction (*P* = 0.94). Mean test mFaterial provision for SBE60 ranged between 92.15 and 97.82 g/d during weeks 1-9 of lactation. Pellet feeding records (T4C) confirmed that no treatment feed was allocated to control cows nor to SBE5 cows after day 5 of lactation. Total pellet offered over the first 63 DIM (Table 3) differed by treatment and week, and had a treatment × week interaction (all *P* < 0.001; Figure 1). Pellet offered was greater for SBE60 cows compared to control cows during week 1-9 (*P* > 0.001) and tended to be increased across week 1-36 (*P* < 0.10). No overall treatment effect was seen from 64 – 252 DIM (*P* = 0.25); however, there was a treatment × week interaction (*P* < 0.001) with greater amounts of pellet offered to SBE60 than control in week 10-13, 15, 16, and a tendency for difference in week 17. Daily rumination time through 120 DIM was not different for control cows compared to either SBE5 or SBE60 from both week 1-9 and week 10-17 (all *P* > 0.55) and no treatment × week interaction observed (*P* = 0.39; Table 3).

*Milk production and composition*

Milk yield did not differ between SBE5 and control either during the treatment period (weeks 1-9; *P* = 0.35) or the carryover period (weeks 10-43; *P* = 0.73). Milk yield tended to increase for SBE60 compared to control during weeks 1-9 (*P* = 0.07) and was significantly increased during week 10-43 (*P* = 0.04). An overall treatment × week interaction was observed with tendencies for differences during weeks 4-6, 9-11, 15-16, 22, 24, and 28 and significant differences in weeks 17-21, 23, and 26-27 (Figure 2). Whole-lactation milk yield (305-day) was 11,245, 11,608, and 12,664 ± 465.3 kg for control, SBE5, and SBE60, with significant differences between SBE60 and control (*P* = 0.03), but not between SBE5 and control (*P* = 0.60). Milking frequency was not affected by either SBE5 (*P* = 0.60) or SBE60 (*P* = 0.19) during the first 63 DIM, but milking frequency was increased for SBE60 during the carryover period compared to control (*P* = 0.04) whereas no difference was detected between SBE5 and control (*P* = 0.48). As expected, milking frequency differed by week (*P* < 0.001), but no overall treatment × week interaction was observed (*P* = 0.11). Despite the difference in milking frequency, milk yield per milking did not differ by treatment during the treatment or carryover periods (all *P* > 0.65).

Milk composition data during the first 17 weeks of lactation are summarized in Table 4. There were no treatment effects on milk fat or protein concentration during the treatment or carryover periods (all *P* ≥ 0.15). Milk lactose concentration tended to be increased for SBE60 compared to control during the treatment period (*P* = 0.06), but not the carryover period (*P* = 0.25), and was not different for SBE5 compared to control during either weeks 1-9 or weeks 10-17 (*P* = 0.54 and 0.46, respectively). Milk fat yield was increased in SBE60 during both the treatment and carryover period compared to control (both *P* = 0.04), whereas SBE5 was not different from control in either period (both *P* ≥ 0.50). Milk protein yield tended to be increased for SBE60 compared to control in the treatment period (*P* = 0.09) and was statistically greater during the carryover period (*P* = 0.01), but again did not differ between SBE5 and control (*P* ≥ 0.13). Milk lactose yield was increased for SBE60 but not SBE5 compared to control during the treatment period (*P* = 0.03 and 0.26, respectively). During the carryover period, milk lactose yield continued to be greater for SBE60 compared to control (*P* = 0.02), and SBE5 tended to increase milk lactose yield compared to control (*P* = 0.07). There was a tendency for an overall treatment × week interaction for milk lactose yield (*P* = 0.08) with significantly greater values for SBE60 compared to control during weeks 5-6 and 8-11, and tendencies for increases during weeks 4, 14, and 15. Milk lactose yield was also greater for second lactation cows compared to cows in lactation 3+ (2.31 vs. 2.15 ± 0.06 kg/d; *P* = 0.03).

Somatic cell count was decreased by SBE60 compared to control during the treatment period (*P* = 0.02) with a tendency for a difference in week 3 and significant effects in weeks 4-6 and 8 (Figure 3). SBE5 did not affect SCC (*P* = 0.37) during weeks 1-9, and neither SBE5 or SBE60 affected SCC during the carryover period (*P* = 0.29 and 0.13, respectively).

Overall there was no treatment effect on BCS (*P* = 0.44) with means being 3.40, 3.30, and 3.31 ± 0.06 for control, SBE5, and SBE60. As anticipated, body condition score differed by week (*P* < 0.001), but there was no treatment effect on prepartum or postpartum BCS (treatment × week: *P* = 0.57). Treatment means for BCS from 3 weeks prior to calving and through 29 weeks of lactation are shown in Figure 3.5.

*Milk markers of inflammation and metabolism*

Neither milk haptoglobin nor milk BHBA showed significant treatment effects (*P* = 0.97 and 0.89, respectively; Table 5) or treatment × DIM effects (*P* = 0.45 and 0.47). Milk haptoglobin concentrations were greatest the day after calving (when inflammation is greatest) and subsequently declined for day 3 and day 5-12 milk samples (*P* < 0.001). BHBA concentration also had a DIM effect (*P* < 0.0001), increasing from day 1 to day 5-12 samples.

*Time to pregnancy, disease incidence, and herd retention*

Survival analyses through 305 DIM were completed for time to pregnancy (Figure 4) and removal from the herd (Figure 5). There was no treatment effect on time to pregnancy (*P* = 0.34). Incidence of several diseases are reported in Table 6. The only disease incidence affected by treatment was mastitis incidence (*P* = 0.07), being lesser for both SBE5 and SBE60 compared to control (*P* = 0.04 and 0.05, respectively). When accounting for multiple comparison tests, SBE60 tended to decrease the hazard of leaving the herd compared to control and SBE5 (*P* = 0.07). Proportional hazard ratios between treatments are as follows: SBE60 v. control = 0.36 (*P* < 0.05; 95% CI: 0.11, 0.99), SBE60 v. SBE5 (*P* = 0.03; 95% CI: 0.11,0.93), and SBE5 v. control = 1.03 (*P* = 0.94; 95% CI: 0.47, 2.29).

Michigan State University

Station Researcher: Mike VandeHaar

**Impact of Ammonia Fiber Expansion-treated wheat straw on the milk yield and body weight of lactating cattle and buffalo in India.**

Preeti Mor, Bryan Bals, Amrish Kumar Tyagi, Farzaneh Teymouri, Nitin Tyagi, Sachin Kumar, Bobby Bringi, and Michael VandeHaar.

Seasonal availability of lush green forages can force local dairy farmers in developing nations to rely on crop residues such as wheat and rice straw as the major feed source. We tested whether ammonia fiber expansion (AFEX) treatment of wheat residue would increase the energy available to Murrah buffalo and crossbred Karan-Fries cattle consuming 70% of their diet as wheat straw (WS) in India. Ten lactating animals of each species were blocked by parity and days in milk and randomly assigned to one of four treatment diets. Treatments were diets of 70% WS with no AFEX treatment (negative control), 25-30% AFEX pellets and 40-45% WS (low AFEX), 50% AFEX pellets and 20% WS (high AFEX), and a nutrient rich diet (positive control). Urea was added to the negative control and low AFEX diets to provide additional crude protein. Animals were individually fed a control diet for 14 d followed by 7 d of adaptation to treatments and then full treatments for 28-35 d and finally control diets for 14 d. In the buffalo experiment, AFEX treatment reversed body weight loss compared to the negative control (0.2 kg/day vs 1.4 kg/day), despite similar dry matter intake and milk yield. In the Karan-Fries cattle experiment, the high AFEX diet increased dry matter intake (11.4 kg/day vs 8.2 kg/day) and milk energy (7.4 vs 6.1 Mcal/day) relative to the negative control. Digestibilities of OM, DM, NDF, ADF, and CP were higher in AFEX treatments than in the negative controls for both experiments. The positive control diet outperformed all other diets in both experiments. In conclusion, AFEX-treatment increased the digestibility and energy avalability of wheat straw for lactating buffalo and cattle and has commercial potential to improve animal feeding and milk production during the dry season.

**Estimating urinary nitrogen using creatinine in cows fed adequate and protein deficient diets.** D. M. Andreen, E. Liu, and M. J. VandeHaar.

The objective of this study was to examine the accuracy of urinary creatinine excretion as a predictor for urinary nitrogen (UN) output in dairy cows fed adequate and protein de cient diets. To determine dietary protein requirements and measure ef ciency of N use in lactating dairy cows, N balance must be calculated. This requires measurement of N excreted in milk, feces and urine. Performing total urine collection via catheter to measure UN output is labor intensive and puts cows at risk for infection. As an alternative method, creatinine excretion is commonly used as a predictor of daily urine output. However, the accuracy of this method has been questioned, and previous research has not examined creatinine’s accuracy in cows fed adequate vs protein de cient diets. This study used 21 mid-lactation Holstein cows in 2 blocks. For 4 weeks, half the cows consumed protein-adequate diets and the other half consumed protein-de cient diets, then all cows were tted with urinary catheters connected to total collection cans containing 50% sulfuric acid for 72h. Every 9 h, urine in the can was removed and measured, acid was re- added, and urine samples were taken from the cows and collection cans. Cows were milked 2× daily, and BW was taken 3× in the week before total collection. Urine samples for each cow from the collection can and cow were composited, and creatinine and N content were measured. Daily total creatinine output was estimated as 29 mg/kg BW. Data were analyzed by *t*-test and correlation. In both experiments, creatinine con- centration in samples taken from cows accurately represented samples from cans. However, creatinine-estimated and actual daily urine output values were signi cantly different (*P* < 0.05), even though the correla- tions between them were moderate (0.51 in experiment 1 and 0.94 in experiment 2). When N output was calculated using creatinine-estimated vs actual urine output values, creatinine underestimated actual N output by 17% on average. Using creatinine to estimate urine volume can be used to see relative differences among cows but values are not always quantitative and should be used with caution.

**Key Words:** creatinine, urinary nitrogen

**Preliminary genomic predictions of feed saved for 1.4 million Holsteins.** P. M. VanRaden, J. R. Wright, E. E. Connor, M. J. VandeHaar, R. J. Tempelman, J. S. Liesman, L. E. Armentano, and K. A. Weigel.

Genomic predictions of transmitting ability (GPTAs) for residual feed intake (RFI) were computed using data from 4,621 42-d and 202 28-d feed intake trials of 3,947 US Holsteins born 1999–2013 in 9 research herds. The 28-d records had 8.5% larger error variance than 42-d records and received less weight (0.92 vs. 1.0) in the evaluation. The RFI averages were already adjusted to remove phenotypic correlations with milk energy output, metabolic body weight, and body weight change and for several environmental effects including other nutrition experiments during the feed intake trials. Traditional breeding values (BVs) for RFI of 74.3 million Holsteins were obtained by an animal model that also included effects for age-parity group, trial date, herd management group, permanent environment, herd-sire interaction, and regressions on inbreeding and on genomic evaluations for milk energy and body weight composite (BWC). The milk energy and BWC terms were specified with the intent to remove positive genetic correlations that remained after the phenotypic correlations were removed. Estimated heritability was 0.17 and repeatability across lactations was 0.42. Genomic BVs for RFI included 60,671 genetic markers for 1.4 million Holsteins and produced calculated genomic reliabilities for young animals averaging 19% compared with traditional reliabilities of 5%. A parallel test using somatic cell score (SCS) records for these same 3,947 cows indicated lower observed than expected genomic reliability (11% vs. 19%). The economic value of RFI is very large and could receive >20% of total emphasis in net merit, but the low reliability will limit the extra genetic progress to about 4% more than current progress. The RFI predictions were added to the extra feed associated with large BWC and then multiplied by −305 to convert from extra feed eaten per day to feed saved per lactation. Additional feed intake records could make feed saved a very important trait in future selection indexes for dairy cattle.

**Repeatability of residual feed intake across diets with two levels of dietary protein content.** E. Liu and M. J. VandeHaar.

Our objective was to examine the repeatability of residual feed intake (RFI) across diets with different levels of dietary protein. Mid lactation Holstein cows with initial MY 42 ± 10 kg/d (n = 88, in 3 blocks) were fed either a high protein diet (18% CP; 18P) or a low protein diet (14% CP; 14P) in a crossover design with 2 28-d treatment periods. Diets were similar and both contained at least 10% RDP (DM basis). The 18P diet contained expeller soybean meal, which was replaced by soybean hulls and ground corn in the 14P diet; 18P diet provided 4 percentage units more CP, 2 units less starch and 2 units less NDF than 14P. Cows were milked 2×/d; DMI and MY were recorded daily. Milk composition was measured during 4 consecutive milkings each week and BW was measured 3× weekly. Fixed effects of experiment, parity, diet and period nested within experiment and random effect of cow were included in the model to compare intake and production performance of treatments. RFI value was calculated for each cow on each treatment based on her actual intake, milk energy output, metabolic BW, and body energy change. Cows were ranked as high (>0.5 SD), medium (±0.5 SD) or low (<-0.5 SD) RFI. Compared with the 14P diet, the 18P diet increased DMI by 1.3 kg/d (27.1 vs. 25.8 kg/d; *P* < 0.01), milk yield by 5.1 kg/d (43.8 vs. 38.7 kg/d; *P* < 0.01), and BW gain by 0.22 kg/d (0.49 vs. 0.27 kg/d; *P* < 0.01). Ranking of cows for RFI was relatively repeatable (r = 0.64; *P* < 0.01). Of all cows, 64% maintained their group ranking across treatments whereas 33% changed ranking by 1 group. Only 2% moved in the ranking from the high to the low RFI group or vice versa. Compared with the previous studies where RFI repeatability was 0.73 across starch levels and 0.44 across forage levels, we presume that nutrient digestibility and protein efficiency are the 2 main sources for RFI variation. In conclusion, RFI was relatively repeatable across 2 diets varying in protein content enough to cause marked changes in production. We suggest this supports the use of RFI as part of a genomic breeding index to enhance feed ef ciency.

**Key Words:** deficient dietary protein, residual feed intake, breeding index

**Relationship of mid-lactation feed efficiency with early and late lactation body condition score in Holstein dairy cows.** L. Hardie, K. Maxwell, M. VandeHaar, and D. Spurlock .

The objective of this study was to investigate the relationship between feed efficiency in mid-lactation primiparous cows with change in body condition score (BCS) measured in late first parity and early second parity. Individual daily feed intakes, daily milk production, weekly body weight (BW), weekly BCS, and weekly samples for milk component analysis were collected over 8 weeks on 173 primiparous Holstein cows between 50 and 215 d in milk (DIM). For each cow, 3 measures of feed ef ciency were calculated: the ratio of milk to feed (MtoF), calculated as her average milk energy (MilkE) output divided by her average dry matter intake (DMI); gross ef ciency (GE), calculated as the ratio of the sum of MilkE and energy in body weight change (BWCE) divided by gross energy consumed; and residual feed intake (RFI), calculated as the regression of DMI on MilkE, metabolic body weight, and BWCE. Measures were adjusted for replicate and DIM. Weekly BCS were observed during late first parity and the first 45 DIM in second parity and used to estimate BCS at the start and end of each time period along with the change in BCS. For each feed efficiency measure, BCS traits were compared between the 18 most feed efficient and inefficient cows. Between feed efficiency group, mean RFI differed by 3.62 kg, GE by 0.10, and MtoF by 0.30 Mcal/kg per day. At dry off, low RFI (feed efficient) cows carried significantly more body condition than high RFI cows (3.5 ± 0.08 vs 3.25 ± 0.08). Furthermore, they tended to carry more condition throughout the first 45 d of second parity. When measured as MtoF, feed ef cient cows tended to carry less condition 35 d before dry off (3.21 ± 0.08 vs 3.39 ± 0.08), though at the initiation of second parity, there was no difference in BCS. However, these cows tended to lose more condition during the first 45 DIM (−0.013 ± 0.002 vs −0.008 ± 0.02 points/d). There was no difference in BCS or change in BCS at any time period for GE. In conclusion, defining feed efficiency as RFI or GE will likely identify cows that maintain body condition throughout lactation, whereas defining feed efficiency as MtoF may favor cows prone to greater body condition loss during early lactation.

**Feed efficiency and reproductive performance are genomically independent in lactating Holstein cows.** E. M. Bart, M. D. Hanigan, D. M. Spurlock, M. J. VandeHaar, and R. R. Cockrum.

For residual feed intake (RFI) or dry matter intake (DMI) to be used as indirect measures of feed ef ciency for selection, they must not be unfavorably associated with reproduction. Previous research in other livestock species suggests there may be a phenotypic relationship between feed ef ciency and reproduction; however, the underlying genomic relationship is unknown. Therefore, we hypothesized that associated variants will be shared between feed ef ciency and repro- ductive traits. The objectives of this study were to (1) identify single nucleotide polymorphisms (SNP) associated with DMI, RFI, and reproductive traits, (2) determine concordant variants among these SNP, and (3) identify the underlying genes of any shared variants. Feed, milk, and reproductive data were collected on lactating Holstein cows (n = 1,513) from Virginia Tech (VT), Iowa State University (ISU), and Michigan State University (MSU). Measurements of feed intake, milk yield, milk composition, and BW were used to calculate RFI. Repro- ductive performance was measured using number of services (NS), days open (DO), and days to first calving (DFC). Genotypic data were available on 677 cows using the Illumina Bovine SNP50 Beadchip. Markers were ltered by call rate (<0.9), allele number (>2), and minor allele frequency (<0.05), which resulted in 54,734 SNP. Genome-wide association analyses were performed using a multi locus mixed model. Signi cant variants for RFI (n = 55), DMI (n = 54), DO (n = 65), NS (n = 59), and DFC (n = 54) were identi ed. There were 3 common variants between RFI and DMI on BTA2 (rs109734679 and rs29010223) and BTA17 (rs41845355). A protein coding gene within the intronic region of *PDE1A* underlied rs109734679. Previous association studies in beef cattle have also identi ed *PDE1A* as associated with RFI. There were no concordant SNP between RFI or DMI with any of the reproductive traits. Though weak, there was a genomic relationship between RFI and DMI. However, both RFI and DMI were genomically independent of reproduction. Overall, genomic selection for RFI or DMI would not unfavorably impact reproduction.

**Development of equations to predict dry matter intake of lactating cows using animal factors.** R. Souza, R. Tempelman, D. Spurlock, E. Connor, L. Armentano, M. Allen, and M. VandeHaar.

Our objective was to model dry matter intake (DMI) in mid-lactation Holstein dairy cows based on milk energy (MilkE), energy required for maintenance, change in body weight (ΔBW), body condition score (BCS, scale 1 to 5), height (Ht), days in milk (DIM), and parity. The database contained weekly DMI of 4,031 lactations from 3,393 Holstein cows from research stations across the US. The average and standard deviation of all variables were 25 ± 4 kg DMI, 30 ± 6 Mcal/d MilkE, 125 ± 12 kg0.75 BW0.75, 630 ± 80 kg BW, 124 ± 12 kg0.75 BWBCS30.75, 620 ± 80 kg BWBCS3, 0.36 ± 1.29 kg/d ΔBW, 3.0 ± 0.4 BCS, 114 ±

37 DIM, and 149 ± 6 cm Ht, where BWBCS3 is the BW adjusted to a BCS of 3. Four full models were generated to model DMI wherein each model contained 1 of the 4 ways of expressing BW shown above. The full models contained xed effects of the covariates described previously, parity, and all possible 2-way interactions between parity and the other covariates. Cow, diet, experiment, and location were included as random effects. The full models were first subjected to forward selection. The resulting models were then analyzed using HPMIXED from SAS 9.4, where the non-signi cant covariates (*P* > 0.05) were removed. In this process the effects of parity, height, and change in BW were removed in all models. The nal models were compared based on the root mean square error of prediction (RMSEP), decomposition MSEP, mean bias, and concordance correlation coef cient (CCC). The suggested model is: DMI (kg/d) = 2.62 + 0.326 × MilkE (Mcal/d) + 0.0243 × BW (kg) − 0.89 × BCS (RMSEP = 2.59 kg, Mean bias, %MSEP = 0.001, Slope bias, %MSEP = 0.001, Mean bias = −0.001 kg, and CCC = 0.78). The selected model has smaller mean bias and higher CCC than the equation suggested by NRC (2001, Mean bias = −2.33, CCC = 0.57) to predict DMI and has potential to bene t nutritionists during diet formulation. To apply this equation for early-lactation cows it is necessary to use an adjustment factor for DIM.

**Development of equations to predict dry matter intake of lactating cows using factors related to the filling effect of rations.** D. O. Sousa, M. J. VandeHaar, and M. S. Allen.

Our objective was to predict dry matter intake (DMI) by lactating cows using factors related to the lling effect of rations. A database of 156 treatment means from 44 experiments reported in the literature was developed. The database included data for cows ranging from 60 to 309 d postpartum and included diet forage NDF (FNDF) content (23.6 ± 5.8, mean ± SD), diet ADF/NDF (0.61 ± 0.08, mean ± SD), and a laboratory measure (in vitro or in situ) of NDF digestibility (LNDFD) of the sole forage or major forage (51.2 ± 12.1, mean ± SD). Models included the random effect of study as well as its interaction with LNDFD to account for differences in methods used to determine LNDFD. The full model also included linear and quadratic effects of FNDF, ADF/NDF, and LNDFD, as well as their linear and quadratic interactions, mean milk yield (MY) for each study and its interaction with the diet factors. Equations were developed by stepwise regression with backward elimination and treatment means were weighted by the inverse of their variance. The prediction equation for DMI (r2 = 0.80, RMSE = 2.76 kg/d) is: DMI (kg/d) = 13.4 - 0.078 x FNDF + 8.264 x ADF/NDF + 0.0126 x LNDFD + 8.453 x (ADF/NDF-0.602)2 + 0.178 x MY – 0.172 x (ADF/ NDF-0.602) x (MY-33.1) + 0.0060 x (LNDFD-48.2) x (MY-33.1) + 8.183 x (ADF/NDF-0.602)2 x (MY-33.1). DMI was positively related to MY and ADF/NDF and negatively related to FNDF, while LNDFD was negatively related to DMI for cows with low MY but positively for cows with high MY. Response to higher ADF/NDF was greater as MY increased. ADF/NDF was included to represent differences in forage fragility between grasses and legumes but it is also affected by the fraction of cereal grain in the diet. The following equation was developed for when LNDFD data is not available (r2 = 0.77, RMSE = 3.01 kg/d) using the same database: DMI = 11.6 – 0.097 x FNDF + 8.31 x ADF/ NDF + 0.268 x MY. DMI was related positively to MY and ADF/NDF and negatively to FNDF with no interactions detected with MY. These equations might be useful to evaluate DMI response to factors related to the filling effects of rations.

University of Minnesota

Station Researcher: Brian Crooker

**Objective 1: To quantify supply, availability, and interaction of nutrients and bioactive compounds utilized for efficient milk production while reducing environmental impact.**

**Evaluation of a product to produce a negative dietary cation-anion difference (DCAD)**

**Overview**

About 10% of dairy cows experience clinical hypocalcemia (milk fever) and some 50% experience subclinical reductions in blood calcium concentrations with increased age and parity associated with more severe cases. Cows that consume a prepartum diet with a negative dietary cation-anion difference (DCAD) have improved postpartum calcium status and less incidence of hypocalcemia (milk fever). This study compares the impact of a new DCAD product with a traditional mix of anionic salts on performance of multiparous cows.

**Outcomes:**

The study is underway and the animal portion expected to be completed in spring of 2018.

**Outputs:**

Training for 1 graduate student

Training for 4 undergraduate students

**Objective 2: To identify and quantify molecular, cellular, and organismal signals that regulate partitioning and efficient conversion of nutrients to milk**

**A. Regulation and integration of hepatic function with mammary and adipose metabolism in Holstein cows during the periparturient period**

**Overview**

Coordinated regulation of nutrient use among tissues is critical for successful transitions to new a physiological condition such as the onset of lactation. This is the most stressful

period of a cow’s life and continued increases in milk yield per cow present a constant challenge for producers to effectively manage this transition. Our experimental model utilizes unique Holsteins from the University of Minnesota that have not been selected for milk yield since 1964 (unselected UMN Holsteins) and contemporary Holsteins that produce more milk (> 4,000 kg/305 d) than the unselected UMN Holsteins. Our hypothesis is transcript and targeted metabolite profiling of unselected UMN and contemporary Holsteins represents a unique and powerful opportunity to gain a greater understanding of key regulatory genes and networks known to be involved and to identify novel components that regulate metabolism and nutrient partitioning in Holsteins. An improved understanding of these factors is consistent with the priority area emphasis on cellular, molecular, genomic/genetic or whole-animal aspects of nutrition, growth and lactation and will positively impact the dairy industry.

The animal portion of this study has been completed. Next Generation Sequencing technology (RNA-Seq) was used to determine transcript expression in liver, adipose, and mammary biopsies (80/tissue) collected at -14, 3, 14, and 42 days postpartum. Bioinformatic analysis of effects of day postpartum, genotype and their interaction are underway to identify networks, pathways, and specific mechanisms associated with regulating and coordinating nutrient use among tissues. Gene expression data generated from these hepatic samples by digital multiplexed analysis (nanoString nCounter) and liquid chromatography-mass spectrometry (LCMS) analyses of metabolite concentrations in milk collected weekly have been completed and reported. Serum LCMS analysis has been completed and the results are being analyzed. Hepatic tissue LCMS analysis is underway.

**Outcomes**

Expression results indicate postpartum reduction in hepatic sensitivity to somatotropin which lasted longer in CH cows. During the postpartum interval, expression of INSR and genes for enzymes related to gluconeogenesis were greater in CH than UH which is consistent with their greater need for lactose synthesis in the contemporary cow. Milk from CH cows contained more triacylglycerides (TAG) species with at least two preformed fatty acid moieties while TAG species with at least two de novo synthesized fatty acid moieties were more prevalent in milk from UH cows. Quantitative fatty acid analysis further confirmed that the increase in preformed fatty acids in CH cows was mainly responsible for different TAG profiles between the two genotypes.

**Output**

Training for 4 graduate students

Training for 6 undergraduate students (one undergrad research project)

3 abstracts presented in 2016 and manuscripts being developed

**B. Effect of bovine genotype on immune-endocrine-metabolite interactions in dairy cows**

**Overview**

Selection practices over the last 50+ years have increased milk yield of contemporary Holsteins and have altered other important phenotypic traits, including immune response, that impact productive performance. Selection strategies and management practices differ between beef and dairy breeds and likely result in genetic and epigenetic factors that contribute to breed differences in immune responses. We have evaluated immune and stress parameters in periparturient UH and CH cows and have used immunological (lipopolysaccharide, LPS) challenges of live animals and dermal fibroblasts to investigate potential genetic influences on the innate immune response of growing Angus and UH and CH heifers and of UH and CH cows.

**Impact of milk yield genotype and stress on accumulative cortisol concentrations in hair from Holstein cows.** Hair accumulates cortisol over time and concentrations during intervals (IT) are used to assess chronic exposure of animals to situations that increase cortisol secretion. Our objectives were to assess impact of milk yield genotype on hair cortisol when unique unselected (stable milk yield since 1964; n = 12) and contemporary (n = 12) Holsteins that differed in milk yield by more than 4,500 kg milk/305 d were subjected to stressors during IT of the periparturient period and early lactation. Cows were blocked by expected calving date and hair removed from both shoulders and left side 4 wk before expected calving. Shoulder hair was collected at 2 d (IT1), 7 wk (IT2) and 14 wk (IT3) and left side hair at 14 wk (IT4) of lactation. Liver, mammary and tailhead adipose samples were collected at -12 ± 1 DIM (during IT1) and at 4, 14, and 42 ± 1 DIM (during IT2). During IT3, saline or LPS (0.25 μg/kg BW, Escherichia coli 055:B5) was administered twice by iv injections 4 d apart and liver sampled at 0, 4, and 24 h after each injection. Left side samples represented the entire 18 wk study (IT4). Cortisol in washed, ground hair was extracted with methanol and measured by a validated ELISA. Right and left shoulder values were averaged. The IT1 and IT2 samples were assessed for effect of genotype, interval, and interaction by repeated measures using PROC MIXED (SAS) with IT as the repeated effect. The IT3 and IT4 samples were analyzed separately to assess effect of genotype, treatment (saline, LPS) and interaction. Means differed when P < 0.05. There was no interaction in either model. Results indicate hair cortisol accumulation did not differ between genotypes (2.90 ± 0.24 pg/mg) but was increased when cows were in early lactation and subjected to 3 multiple biopsies (IT1 vs. IT2; 2.41 vs. 3.39 ± 0.26 pg/mg; P = 0.002). During IT3 and IT4, there was no effect of genotype and the short duration of the LPS-induced response was insufficient to elicit a change in hair cortisol. Although there is concern that increased milk yield increases stress in cows, it had no effect on hair cortisol concentrations in this study.

**Outcomes**

Expression of genes associated with immunity was determined by digital multiplexed analysis (nanoString nCounter). Results indicate a less robust hepatic TLR-4 response in the contemporary cow. This has prompted total transcriptome analysis, including micro-RNA expression, via RNA-Seq and proteome analysis. These chemical analyses have been completed and bioinformatic analyses of the results are underway.

Hair cortisol concentrations increased in early lactation but did not differ between UH and CH cows during the transition period. Although there is concern that increased milk yield increases stress, hair cortisol accumulation during periparturient period (lactogenesis and early galactopoiesis, 10 weeks of lactation) did not differ between UH and CH cows.

Pennsylvania State University

Station Researcher: Kevin Harvatine and Alex Hristov

**Study 1. Production effects of phytonutrients alone or in combination with yeast culture in lactating dairy cows.** J. Oh, M. Harper, A. Melgar, E. H. Wall, and A. N. Hristov.

As rumen modifiers with distinct modes of action, yeast and phytonutrients may have additive effects on ruminal fermentation and animal productivity. To test this concept, an 8-wk, randomized complete block design study with 36 Holstein cows (average days in milk, 117 ± 37.5 d; average body weight, 651 ± 83.3 kg) was conducted to investigate the effects of a blend of phytonutrients alone or in combination with yeast culture on feed intake and milk production and composition. Cows were housed in a free-stall barn equipped with Calan Feeding System for monitoring individual feed intake. Following a 2-wk covariate period, cows were blocked in blocks of three based on days in milk, milk yield, and parity. Cows within a block were randomly assigned to one of the following treatments (12 cows/treatment): 14 g/cow/d yeast culture (XPC), 1.0 g phytonutrients (XT), and a combination of XPC and XT (XPCXT). Cows were fed ad libitum once daily. Treatments were top-dressed at the time of feeding. The basal diet consisted of (dry matter basis): 42.5% corn silage, 12.5% alfalfa haylage, 5.0% grass hay, and 40% concentrates and contained 16.3% crude protein and 32.4% neutral detergent fiber. Dry matter intake was not affected (P = 0.35) by treatment (29.1, 28.2, and 28.6 kg/d for XPC, XT, and XPCXT, respectively). Milk yield was also similar (P = 0.82) among treatments (42.3, 42.7, and 42.2 kg/d, respectively). Treatment did not affect feed efficiency (average 1.50 kg/kg; SEM = 0.030, P = 0.24) and concentrations and yields of milk fat, true protein, and lactose (average 3.5, 3.0, and 4.8%; SEM = 0.10, 0.02, and 0.01, respectively; P ≥ 0.20). Concentration of milk urea nitrogen was also similar among treatments. In this experiment, there was no difference in lactational performance between cows supplemented with yeast culture or a blend of phytonutrients. In addition, additive effects of the two dietary supplements were not observed.

**Study 2. Effects of feeding brown midrib dwarf pearl millet silage on lactational performance and enteric methane emission in dairy cows.** Harper, M. T., A. Melgar, G. Roth, and A. N. Hristov.

The objective of this experiment was to evaluate the production effects of replacing corn silage (CS; serving as the control) with brown midrib dwarf pearl millet silage (PM) in the total mixed ration of lactating dairy cows. Sixteen Holstein cows (65 ± 21 DIM; BW 630 ± 71 kg) were used in a replicated 2 × 2 Latin square design experiment with 2, 28 d periods. Feeding was ad libitum for 5 to 10% refusals. The control diet consisted of (DM basis): 50% CS, 6% alfalfa haylage, 4% hay/straw mixture, and 40% concentrate feeds. For the PM diet, 20% of CS was replaced with PM (on DM basis). Control and PM diets were 16.7 and 17.2% CP, 30.3 and 32.4% NDF, and 28.0 and 24.1% starch, respectively. Metabolizable protein balance of control and PM diets was 27 and 208 g/d, respectively; NEL balance was -0.7 and -0.5 Mcal/d. Enteric methane emission was measured using the GreenFeed system. The PM diet resulted in equal DMI as the control (29.0 vs 29.1 kg/d; SEM = 0.65, P = 0.78, respectively) but lower milk yield (49.6 vs 51.3 kg/d; SEM = 2.02, P < 0.001) and lower feed efficiency (1.72 vs 1.77 kg/ kg milk; SEM = 0.05, P = 0.01). Energy corrected milk (ECM) yield (46.7 kg/d; SEM = 1.92, P = 0.86), and ECM feed efficiency were not different between diets. The PM diet tended to increase milk fat content compared with the control diet (3.71 vs 3.47%; SEM = 0.118, P = 0.06, respectively) but true protein and lactose content were not affected. Yields of the individual milk components were not affected (P ≥ 0.23) by diet and averaged 1.81 kg/d fat, 1.45 kg/d true protein and 2.51 kg/d lactose. Enteric methane emission was increased by the PM diet over the control (454 vs 396 g/d; SEM = 18.4, P < 0.001) as was methane yield (15.7 vs 13.8 g/DMI; SEM = 0.54, P = 0.001) and methane intensity (9.6 vs 8.3 g/kg ECM; SEM = 0.39, P = 0.001). Brown midrib dwarf pearl millet silage has potential to partially replace CS in the diet of dairy cattle without affecting ECM yield and milk components. This replacement, however, is likely to increase enteric methane emission.

**Study 3. Effects of phytonutrients or ionophore on productivity, blood cells, and fat mobilization in lactating dairy cows.** J. Oh, M. Harper, E. H. Wall, and A. N. Hristov.

Phytonutrients exhibit both ruminal and post-ruminal effects in ruminants and there may be additive effects on performance when phytonutrients are combined with other rumen modifiers such as ionophores. The objectives of this experiment were to investigate the effects of phytonutrients alone or in combination with monensin on productivity, blood cells, and fat mobilization in lactating dairy cows. Thirty six Holstein cows (average days in milk, 120 ± 23.1 d; average body weight, 676 ± 75.8 kg) were used in a 9-wk randomized complete block design study. Cows were blocked in blocks of 3 based on days in milk, milk yield, and parity following a 2-wk covariate period. Cows within a block were randomly assigned to one of the following treatments (12 cows/treatment): 450 mg/cow/d monensin (MO), 1,000 mg/cow/d of a mixture of cinnamaldehyde, eugenol, and capsicum (XT), and 250 mg/cow/d of a product containing capsicum oleoresin in addition to MO (MOCAP). Cows were housed in a free stall barn equipped with Calan Feeding System for monitoring individual feed intake and fed ad libitum once daily. Treatments were top-dressed at the time of feeding. Dry matter intake and milk yield were not affected (P ≥ 0.18) by treatments (average 31.0 and 47.4 kg/d; SEM = 0.52 and 0.81, respectively). Compared with MO, XT increased (P = 0.04) feed efficiency (1.48 and 1.58 kg/kg, respectively). Concentrations of milk fat, true protein, and lactose were similar (P ≥ 0.46) among treatments. The expression of hormone-sensitive lipase in adipose tissues tended to increase (P = 0.09) for MOCAP compared with MO. However, blood non-esterified fatty acids were not affected (P = 0.26) by MOCAP. Treatments had no effect (P ≥ 0.15) on blood urea nitrogen, red blood cells, and white blood cells except a slight decrease (P = 0.10) in monocyte counts for XT. Relative to monensin, phytonutrients had no or subtle effect on feed intake, milk yield and composition, blood cells, and fat mobilization in dairy cows. However, a mixture of cinnamaldehyde, eugenol, and capsicum increased feed efficiency compared with monensin.

**Study 4. Effect of a *Saccharomyces cerevisiae*-based direct-fed microbial product and an enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* on productivity and enteric gas emission in lactating dairy cows.** J. Oh, M. Harper, A. Melgar, D. M. Paulus Compart, and A. N. Hristov.

Dietary supplementation of live yeast and fungal enzymes may have beneficial effects on productivity and rumen fermentation in ruminant animals. The objective of this experiment was to investigate the effects of a Saccharomyces cerevisiae-based direct-fed microbial product (DFM) and an enzyme extract from Aspergillus oryzae and Aspergillus niger (ENZ) on feed intake, milk production and composition, and enteric gas emission in lactating dairy cows. Eighteen Holstein cows (115 ± 42.0 days in milk; 609 ± 77.9 kg body weight) were used in a 3 × 3 Latin square design experiment with 3, 28-d periods. Treatments were: (1) control (no additive), (2) 28 g/cow/d DFM, and (3) 10 g/cow/d ENZ. Cows were housed in a tie-stall barn and fed ad libitum once daily. Treatments were top-dressed at the time of feeding in the morning. The basal diet consisted of (DM basis): 44.5% corn silage, 10.5% alfalfa haylage, 5.0% grass hay, and 40% concentrates and contained 16.5% CP and 32.0% NDF. Feed intake and milk production were monitored daily and gas emission was measured during the last week of each experimental period using the GreenFeed System. Dry matter intake was not affected (P = 0.53) by treatments (average 25.3 kg/d; SEM = 0.97). Compared with control, DFM increased (P = 0.03) milk yield (39.7 vs. 41.9 kg/d, respectively). Feed efficiency was similar among treatments (average 1.61 kg/kg; SEM = 0.04). Concentrations of milk fat, true protein, and lactose (average 3.50, 3.03, and 4.84%, respectively) and energy-corrected milk (ECM) yield (average 38.1 kg/d) were similar (P ≥ 0.38) among treatments. Milk urea nitrogen was also not affected (P = 0.39) by treatment. Treatments had no effect (P ≥ 0.17) on enteric methane (average 344 g/d, SEM = 16.2) or carbon dioxide emission and methane yield (average 13.8 g/kg DMI; SEM = 0.56) or intensity (9.5 g/kg ECM; SEM = 0.49). In this experiment, a Saccharomyces cerevisiae-based microbial product increased milk production without affecting enteric methane emission in dairy cows.

**Study 5. County-level gridded livestock methane emissions for the contiguous United States.** A. N. Hristov, A. N., M. Harper, R. Meinen, R. Day, J. Lopes, T. Ott, A. Venkatesh, and C. A. Randles

Livestock is considered to be the second largest source of anthropogenic methane emissions in the United States. Top-down approaches for methane source attribution have questioned existing bottom-up estimates of methane emissions, such as the U.E. Environment Protection Agency’s (USEPA) Inventory of Greenhouse Gas Emissions and Sinks, suggesting that livestock emissions are underestimated. This analysis used a spatially-explicit, bottom-up approach, based on animal inventories, feed dry matter intake, and dry matter intake-based emission factors to estimate county-level enteric (cattle) and manure (cattle, swine, and poultry) methane emissions for the contiguous United States. Counties with the largest combined livestock methane emissions included: Tulare, Merced, Stanislaus, and Kings, CA (217, 123, 80, and 78 Gg methane/year, respectively); Gooding, ID (75 Gg/year); Weld, CO (63 Gg/year); Kern, Fresno, and San Joaquin, CA (62, 59, and 49 Gg/year), Maricopa, AZ (47 Gg/year), and Sampson, NC, Yakima, WA, and Sioux, IA (43 to 44 Gg/year). Overall, the bottom-up approach used in this analysis yielded total livestock methane emissions (8,888 Gg/yr) that are comparable to current USEPA estimates (9,117 Gg/yr) and to estimates from the global gridded Emission Database for Global Atmospheric Research (EDGAR) inventory (8,657 Gg/yr), used previously in a number of top-down studies. However, the spatial distribution of emissions developed in this analysis differed significantly from that of EDGAR. As an example, methane emissions from livestock in Texas and California (highest contributors to the national total) in this study were 36% lower and 100% higher, respectively, than estimates by EDGAR. The difference for these two states between the current analysis and the latest USEPA gridded inventory was 15 and 4%, respectively. The spatial distribution of emissions in gridded inventories (e.g., EDGAR) likely strongly impacts the conclusions of top-down approaches that use them, especially in the source attribution of resulting (posterior) emissions, and hence conclusions from such studies should be interpreted with caution.

**Study 6. Enteric methane emissions: Prediction and mitigation, the GLOBAL NETWORK project.** A. N. Hristov, A. N., E. Kebreab, M. Niu, J. Oh, C. Arndt, A. Bannink, A. R. Bayat, A. F. Brito, D. Casper, L. A. Crompton, J. Dijkstra, P. C. Garnsworthy, N. Haque, A. L. F. Hellwing, P. Huhtanen, M. Kreuzer, B. Kuhla, P. Lund, J. Madsen, S. C. McClelland, P. Moate, C. Muñoz, N. Peiren, J. M. Powell, C. K. Reynolds, A. Schwarm, K. J. Shingfield, T. M. Storlien, and M. R. Weisbjerg.

Ruminant production systems are important contributors to anthropogenic methane (CH4) emissions. There is a need for more accurate prediction of enteric CH4 emission to help address discrepancies in global and regional CH4 inventories and emission source attribution. Although different types of models have been developed to predict enteric CH4 emissions, rather simple empirical (statistical) models have been in common use due to the ease of use compared with more detailed empirical and process based mechanistic models. However, extant empirical models suffer from narrow spatial focus, limited observations and limitations of the statistical technique employed. The GLOBAL NETWORK project was established to collate and analyze available CH4 emission and mitigation data from live ruminant animals in relation to nutrition. Three databases have been developed: a treatment means database (TMD), an individual animal database (IAD), and a rumen microbial database (MICD). The objective of the TMD is to summarize and recommend science-based enteric CH4 mitigation options to stakeholders. This database consists of 1,796 experimental treatment means from 410 studies. Over 30 dietary treatments or treatment groups (along with their control measurements) were identified and allocated to 2 general categories, “Feed supplements” (i.e., lipid/individual fatty acids, yeast products, ionophores, CH4 inhibitors, nitrates, etc.; a total of 879 observations) and “Nutritional treatments” (i.e., forage quality, type and proportion of concentrate feeds, processing, etc.; a total of 917 observations). Preliminary analysis confirmed that some mitigation strategies (inhibitors, nitrates, lipids) do have a potential for a sizable reduction in enteric CH4 emissions from cattle but also revealed large variability in the response. The purpose for the collation of the IAD, was to develop robust enteric CH4 emission prediction models for the predominant farmed ruminant species (dairy and beef cattle, sheep) and nutritional, animal, and farm management scenarios. The dairy cattle database within IAD currently contains 5,233 individual animal observations from 154 studies from North and South America, Europe, and Oceania. In total, 11 models each were developed from global, European and North American databases. As expected, a global CH4 emission (g/d) model with a greater number of independent variables fitted the data best. Less complex models requiring only dry matter intake, or dry matter intake plus neutral-detergent fiber concentration had a predictive ability similar to more complex models. These prediction models, along with recommendations from the TMR analysis, provide robust prediction equations for enteric CH4 inventories and indicate reliable dietary mitigation options for ruminant farming systems.

**Study 7. The effects of feeding rations that differ in neutral detergent fiber and starch within a day on the daily pattern of rumen microbial populations.** I.J. Salfer, C.E. Crawford, L.W. Rottman, Y.Ying, K.J. Harvatine

The cow’s daily pattern of feed intake creates in differences in nutrient consumption across the day. Feeding a high-fiber diet during the high-intake period of the day and a low-fiber diet during the low-intake period of the day is a potential strategy to stabilize rumen fermentation and nutrient absorption. A study was conducted to examine how the daily pattern of rumen microbial populations is modified in response to feeding multiple rations that provide different amounts of neutral detergent fiber and starch across the day. Diets included a control diet (33.3% NDF), a low fiber diet (LF; 29.6% NDF), and a high-fiber diet (HF; 34.8% NDF). Nine cannulated Holstein cows were fed one of three dietary combinations in a 3x3 Latin square design: (1) 100% daily offering of the control diet at 0900 h (**CON)**; (2) 70% daily offering of HF at 0900 h and 30% daily offering of LF at 2200 h (**HL**); or (3) 30% daily offering of LF at 0900 h and 70% daily offering of HF at 1300 h (**LH**). Rumen digesta representing every 3 h across the day was collected, and microbial DNA was extracted. Quantitative PCR (qPCR) was used to determine the relative abundances of Total bacteria, total fungi, total protozoa, *F. succinogenes*, *R. albus*, *B. fibrisolvens*, *S. ruminantium*, *B. hungatii*, *P. bryantii*, *M. elsdenii*, and *S. bovis*. The relative abundance of all microbial groups exhibited a daily pattern across the day and nearly all were affected by feeding strategy. Notably, both the HL and LH treatments caused a dramatic spike in the relative abundances of *S. bovis*, *S. ruminantium*, and *B. hungatii* at 0900 h, that was not observed in the control (P < 0.05). A similar peak in *B fibrisolvens* abundance occurred at 0900 h in the HL treatment, but not CON or LH (P < 0.05). Total bacteria abundance did vary by treatment, but the lowest daily variation was detected within HL. Alternatively, the abundances of ciliated protozoa and total fungi were the most stable in the CON treatment.

**Study 8. Physical characterization of fat supplements highly enriched in palmitic and stearic acid.** R.P. Shepardson, E. Bazilevskaya, K. J. Harvatine

Fatty acid (FA) supplements are widely used in lactating cow diets to increase energy intake. Previous published research has reported that supplements with moderate enrichment (~85%) of palmitic acid have expected digestibility, while very high enrichments (~98%) have lower digestibility. Saturated FA have the potential to form organized secondary structures at high purity. Differential Scanning Calorimetry (DSC) is a thermal technique commonly used in material science to measure the change in heat flow as energy is absorbed or released from a sample during heating. Our hypothesis was that a supplement with a very high enrichment would differ in physical characteristics due to the formation of a secondary structure, which may contribute to a decreased digestibility. A 98% stearic acid (SA; 98.5% C18:0, 0.4% C16:0), 98% palmitic acid (PA; 98.5% C16:0, 0.7% C18:0), and a mixture of palmitic and stearic (PA/SA; 54.5% C16:0, 44.5% C18:0) sample were used for this characterization. Sample FA profile was determined by gas chromatography with a flame ionization detector. Data from DSC was gathered on a Q600 SDT (TA Instruments). Briefly, ~40 mg of sample was heated at 5°C/min from 25°C to 110°C. TA Universal Analysis software (TA Instruments) was used to determine melting temperature, and total enthalpy of melting (area under the curve). Triplicate runs were highly reproducible. Melting temperature of the PA/SA mixture was 61.2°C, similar to the reported melting temperature of PA (62.9°C). However, the melting temperatures of the high purity PA and SA were increased to 67.8°C and 73.7°C, respectively. The enthalpy of melting was increased 12.5% in the high purity PA compared to the PA/SA blend. Melting enthalpy for the high purity SA supplement was increased 3.5% from the high purity PA and 16.4% from PA/SA mixture. Although melting temperatures are well above body temperature, increasing the energy required to melt the sample is indicative of physical characteristics of the supplement expected to decrease digestibility.

**Study 9. The effects of U.S. region on the annual rhythms of milk yield and fat and protein concentration and yield of dairy cattle at the herd level.** I.J. Salfer, C.D. Dechow and K.J. Harvatine

The annual or seasonal rhythm of milk yield and composition is important for dairy producers and it may represent an underlying adaptation of the cow to yearly changes. It is well appreciated that milk fat and protein concentration peak during the winter and reach a nadir in the summer. Summarized monthly production data from individual Federal Milk Marketing orders has suggested that the region of the U.S. may impact the difference between mean and peak (amplitude) fat and protein concentration and the timing of peak production (acrophase). Less data is available on yields of milk, fat and protein. Our objective was to determine the seasonal rhythm of milk production and the effect of U.S. region at the herd level. Monthly DHIA records of all herds in Pennsylvania, Minnesota, Texas and Florida from the years 2003 to 2016 were obtained from Dairy Records Managements Systems. Milk yield, fat and protein yield, and fat and protein concentration were fit to the linear form of the cosine function with a 12-month period using a linear mixed effects model in ASreml. Model parameters included the fixed effects of state, cosine parameters, the interaction of state and cosine parameters, and breed and the random effects of herd and year. A zero-amplitude test was performed to determine the fit of the linear form of the cosine function. Milk yield and fat and protein yield and concentration fit a cosine function in all four states, indicating an annual rhythm (P<0.001). The amplitude of the rhythm of milk yield varied by state, and was lower in PA (2.8 kg) and MN(2.4 kg) compared to TX (6.9 kg) and FL (8.1 kg; P<0.05). Fat and protein yield similarly showed a greater amplitude in the southern versus northern states (P<0.05). The concentrations of fat and protein was opposite, with greater amplitudes occurring in MN and PA than in TX and FL (P<0.05). The acrophase of milk yield, fat and protein yield, and concentration also varied by state, but all peaked between October and March (P<0.05). Results suggest that region of the U.S. impacts annual production rhythms, with a greater yearly variation in milk, fat and protein yield occurring in the south.

**Study 10: Kinetics of *trans*-10*, cis*-12 and *cis*-9*, trans-*11 conjugated linoleic acid (CLA) transfer to plasma and milk following an abomasal bolus in lactating dairy cows.** Natalie L Urrutia, Rebecca Bomberger, Michel Baldin, Kevin J Harvatine

Dietary fatty acids (FA) are directly transferred directly to milk through chylomicrons and indirectly through tissue recycling. The objective of this study was to characterize the direct and indirect transfer rates of the *cis*-9, *trans*-11 (c9t11) and *trans*-10, *cis*-12 (t10c12) CLA through plasma to milk following a single abomasally infused bolus. Five ruminally cannulated multiparous mid-lactation cows (148 ± 86 DIM; Milk 44.1 ± 11.2 kg/d) received a single abomasal bolus infusion of an enriched CLA mixture providing 15 g of each CLA isomer (c9t11, t10c12) over a 30 min period. Total transfer of CLA was analyzed in a model that included cow as a random effect and CLA isomer as a fixed effect (JMP Pro). Time course data was analyzed as repeated measures in SAS and least-square means were fit to a double exponential decay function by non-linear curve fitting (JMP Pro) to characterize direct (fast pool) and indirect (slow pool) transfer of CLA isomers to milk. Plasma CLA concentration peaked at 2 h, reaching 0.32 and 0.31% of plasma FA for c9t11 and t10c12, respectively, and returned to baseline at 72 h. Milk t10c12 concentration peaked at 14 h (0.5% of FA) and returned to baseline at 86 h post infusion. Milk c9t11 concentration initially peaked at 14 h (0.98 % of FA), returned to baseline at 86 h post infusion, and then had a second peak between 146 to 158 h (0.56% of FA) post infusion. Total transfer of CLA to milk differed between isomers and was 79.3 and 40.8% of the bolus for c9t11 and t10c12, respectively (*P* < 0.001). Time course of CLA isomers transferred to milk fit a biexponential model (R2=0.99). The area (% of total) under the first exponential representing direct transfer was 17 and 73% and the second exponential representing indirect transfer was 83 and 27% of the total CLA isomers transferred for c9t11 and t10c12, respectively. In conclusion, although plasma kinetics of c9t11 and t10c12 were similar, transfer of CLA isomers to milk differed greatly in their transfer efficiency and major pool of transfer.

The Ohio State University

Station Researcher: Jeffrey Firkins

PROGRESS ON RESEARCH RELATED TO: Objective 1. To quantitatively evaluate chemical and physical properties of protein and energy sources which determine the availability of nutrients critical to milk protein secretion in lactating dairy cows.

# Inhibition of the Rumen Ciliate Entodinium caudatum by Antibiotics (with Z. Yu).

Axenic cultures of free-living aerobic ciliates, such as *Tetrahymena thermophila* and *Paramecium aurelia*, have been established and routinely used in laboratory research, greatly facilitating, or enabling characterization of their metabolism, physiology, and ecology. Ruminal protozoa are anaerobic ciliates, and they play important roles in feed degradation and fermentation. Although, repeatedly attempted, no laboratory-maintainable axenic culture of ruminal ciliates has been established. When axenic ciliate cultures are developed, antibiotics are required to eliminate the accompanying bacteria. Ruminal ciliates gradually lose viability upon antibiotic treatments, and the resultant axenic cultures can only last for short periods of time. The objective of this study was to evaluate eight antibiotics that have been evaluated in developing axenic cultures of ruminal ciliates, for their toxicity to *Entodinium caudatum*, which is the most predominant ruminal ciliate species. Scanning and transmission electron microscopy (TEM) showed that the antibiotics damaged both the cell surface and nuclei of *E. caudatum* and increased accumulation of intracellular glycogen. Combinations of the three least toxic antibiotics failed to eliminate the bacteria that are present in the *E. caudatum* culture. The combination of ampicillin, carbenicillin, streptomycin, and oxytetracycline was able to eliminate all the bacteria, but the resultant axenic *E. caudatum* culture gradually lost viability. Adding the bacterial fraction (live) separated from an untreated *E. caudatum* culture reversed the viability decline and recovered the growth of the treated *E. caudatum* culture, whereas feeding nine strains of live bacteria isolated from *E. caudatum* cells, either individually or in combination, could not. Nutritional and metabolic dependence on its associated bacteria, accompanied with direct and indirect inhibition by antibiotics, makes it difficult to establish an axenic culture of *E. caudatum*. Monoxenic or polyxenic cultures of *E. caudatum* could be developed if the essential symbiotic partner(s) can be identified.

# Amino acid composition of rumen bacteria and protozoa in cattle (with Helene Lapierre).

Because microbial crude protein (MCP) constitutes more than 50% of the protein digested in cattle, its AA composition is needed to adequately estimate AA supply. Our objective was to update the AA contributions of the rumen microbial AA flowing to the duodenum using only studies from cattle, differentiating between fluid-associated bacteria (FAB), particle-associated bacteria (PAB), and protozoa, based on published literature (53, 16, and 18 treatment means were used for each type of microorganism, respectively). In addition, Cys and Met reported concentrations were retained only when an adequate protection of the sulfur groups was performed before the acid hydrolysis. The total AA (or true protein) fraction represented 82.4% of CP in bacteria. For 10 AA, including 4 essential AA, the AA composition differed between protozoa and bacteria. The most noticeable differences were a 45% lower Lys concentration and 40% higher Ala concentration in bacteria than in protozoa. Differences between FAB and PAB were less pronounced than differences between bacteria and protozoa. Assuming 33% FAB, 50% PAB, and 17% of protozoa in MCP duodenal flow, the updated concentrations of AA would decrease supply estimates of Met, Thr, and Val originating from MCP and increase those of Lys and Phe by 5 to 10% compared with those calculated using the FAB composition reported previously. Therefore, inclusion of the contribution of PAB and protozoa to the duodenal MCP flow is needed to adequately estimate AA supply from microbial origin when a factorial method is used to estimate duodenal AA flow. Furthermore, acknowledging that hydrolysis of 1 kg of true microbial protein yields 1.16 kg of free AA substantially increases the estimates of AA supply from MCP.

# Technical note: Methodological and feed factors affecting prediction of ruminal degradability and intestinal digestibility of essential amino acids (with P. Kononoff, R. White).

We hypothesized that ruminal degradability of essential AA (EAA) and the intestinal digestibility of the ruminally undegraded EAA residue in feeds could be evaluated in a meta-analysis. The objective was to characterize methodological factors for ruminal incubation (time of incubation of feed in situ) and method of simulating digestion of the ruminally undegraded AA (incubation of residue in digestive enzymes in vitro or in mobile bags inserted into the duodenum). To increase numbers of observations, feeds were categorized before ANOVA. An approach is described to predict differential ruminal degradability (or undegradability) of individual EAA by normalizing them as a proportion of total AA (TAA) degradability (undegradability) and similarly to normalize the intestinal digestibility of EAA using TAA. Interaction of feed category with individual EAA justifies future studies with a broader range of feeds and more replication within feed to bolster this approach. With broader data, the approach to normalize EAA as a proportion of TAA should allow a better defined EAA library to be integrated with more robust CP databases (that can be updated with specific feed information from more routine laboratory analyses) in dairy supply-requirement models.

University of Tennessee

Station Researcher: Agustin Rius

Objectives 1:

1) Qualitative analysis of nine forage mixtures designed for Southeastern U.S. organic dairy production.

The warm climate in the Southeastern U.S. allows for extended grazing seasons; however, there is limited information on the nutritional quality of forage mixtures to sustain pasture-based organic dairy production. A study was conducted to evaluate the nutritional quality of nine forage mixtures designed for this region. Mixtures of cool-season perennial and annual legumes (red clover, alfalfa, crimson clover), cool-season perennial and annual grasses (tall fescue, orchard-grass, annual rye-grass), warm-season annual grasses (sorghum-X sudan-grass hybrids, crabgrass), and warm-season annual legumes (annual lespedeza, cowpea) were established in a randomized complete block design at the East Tennessee Research and Education Center-Organic Crops Unit. Plots (2 x 10m, four replicas per plot) were harvested twice at the appropriate growth stages. Grab samples were analyzed using near-infrared spectroscopy (Foss-DS2500, Foss America, Eden Prairie, MN) to determine crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, and in vitro dry matter digestibility for 48 hours (IVTDMD48h). Data were analyzed in SAS 9.4 using the GLIMMIX procedure and the model included the fixed effects of forage mixture, replica, time, and their interactions. Data from the first set of harvests indicate that average CP ranged from 8.2-15.0%, with greatest concentrations in mixtures that contained legume species (p<0.01). Concentrations of ADF ranged from 40.2-45.2%, with the lowest concentration in monoculture orchard-grass stands. Concentrations of NDF ranged from 54.1-67.6%, with the lowest concentrations in mixtures containing red clover (p<0.01). Lignin content ranged from 4.2-8.5%, with the lowest content in red clover stands (p<0.01). Prediction means of IVTDMD48h ranged from 63.5-68.8%, with the greatest digestibility in orchard-grass-red clover mixtures (p<0.01). Results indicate that mixed grass-legume stands should provide nutrients to enhance organic dairy production in the Southeast. Additional information is needed over multiple seasons in combination with dairy cow responses to mixed stands.

Objective 2:

2) Circulating insulin resistance biomarker lignoceroyl sphingosine is not elevated in Holstein dairy cows in response to heat stress.

The sphingolipid ceramide (Cer) mediates the development of insulin resistance. Lipidomics has revealed that lignoceroyl sphingosine (C24:0-Cer) is a circulating biomarker for insulin resistance in dairy cattle. Environmental heat stress conditions compromise milk production, a response that may involve enhanced insulin action. Our objective was to investigate the effects of heat stress on circulating ceramide concentrations. Twelve multiparous, lactating Holstein dairy cows were assigned to 2 environmental conditions [thermoneutral (TN) or heat stress (HS)] for 7 d in a crossover design. Temperature-humidity index was maintained below 66 for TN treatment, and above 68 (peaking at 76) for HS treatment. Blood was collected at 0800 (AM) and 1900 h (PM) on d 6 and 7 of conditioning, and plasma samples pooled to reflect AM and PM metabolic status. Plasma concentrations of Cer, monohexosylceramide (GlcCer), and lactosylceramide were determined using mass spectrometry. Data were analyzed using a mixed model with fixed effects of treatment (HS and TN) and time (AM and PM). As previously established, heat stress increased rectal temperature and respiration rate, and reduced DM intake and milk production (P < 0.05). Circulating free fatty acids were elevated during AM, relative to PM (P< 0.05). Circulating β-hydroxybutyrate was increased by HS, relative to TN (P < 0.05). Relative to TN, HS did not increase C24:0-Cer or C24:0-dihydroceramide. Mild reductions in GlcCer levels were observed in response to HS treatment (e.g., 20% C20:0-GlcCer, P < 0.05), while lactosylceramide levels were unchanged. In contrast, C16:0-Cer and C16:0-dihydroceramide levels increased 14 and 19%, respectively. Plasma fatty acid levels were moderately associated with the majority of Cer quantified (r = 0.3 - 0.4; P < 0.05). For instance, C24:0-Cer was positively associated with circulating fatty acids (r = 0.38; P < 0.05). We conclude that short-term heat stress conditioning did not increase the insulin resistance biomarker C24:0-Cer. Our results suggest insulin resistance likely did not develop in heat-stressed cows.

Objective 3:

3) Evaluation of low concentrations of rumen degradable protein in the diet of lactating dairy cows: A meta-analysis.

The objective of the present study was to conduct a meta-analysis that summarizes the effect of reducing dietary rumen degradable protein (RDP) proportions on dry matter intake (DMI) and milk production in dairy cows. The data set was identified using 2 search engines comprising of 41 studies with 109 observations. Means were weighted by the inverse of their variance. Treatments of RDP (% of DM) were organized as very low (<8% RDP), low (8% ≤ RDP <9%), medium (9% ≤ RDP <10%), and high (>11% RDP) and compared with a control (10% ≤RDP ≤11%). Comprehensive Meta-Analysis v3 (Biostat, Englewood, NJ) was used to evaluate the raw mean difference (RDP treatment – RDP control) in a random-effects model. Variables evaluated were DMI and yields of milk and milk protein. Moderators included were experimental design of the studies (change-over or continuous), concentration of dietary energy [medium (<1.6 Mcal of net energy of lactation per kg ofDM] or high (≥1.6 Mcal of net energy of lactation per kg of DM)], and concentration of dietary rumen undegradable protein (RUP; < 6 or ≥6% of DM). Statistical significance was identified at P ≤ 0.05. Heterogeneity was present, and publication bias was absent. High RDP did not affect DMI and production (n = 47). Compared with control RDP, low RDP sustained DMI and milk production (n = 12), and medium RDP sustained yields of milk and milk protein (n = 40). However, medium RDP reduced DMI in diets with medium and high energy content (0.45 kg/d; P < 0.02). Low and high RDP reduced DMI and milk production in continuous design trials (n = 56; P < 0.05); whereas, RDP did not affect the evaluated variables in change-over trials. In trials with RUP <6% (n = 26), medium RDP decreased DMI (0.50 kg/d; P < 0.03), but at RUP ≥6%, lowering RDP did not affect production. In summary, lowering RDP proportions may not always reduce DMI and milk production in dairy cows. However, experimental design of the study, dietary energy, and dietary RUP may influence production responses to lowering RDP.

4) Evaluation of the NRC predictions in response to changes in dietary rumen degraded and undegraded protein on dairy cows exposed to warm climates.

A study was conducted to evaluate the prediction accuracy of the National Research Council (2001) model for metabolizable protein (MP) allowable for milk production. Thirty multiparous Holstein cows were used in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Dietary treatments comprised 2 levels of rumen degradable protein (RDP; 10 and 8%) and 2 levels of rumen undegradable protein (RUP; 8 and 6%) as follows: 10RDP:8RUP, RDP:8RUP, 10RDP:6RUP, and 8RDP:6RUP. The 10RDP:8RUP diet was fed from d 1 to 21 followed by respective treatments from d 22 to 42. Cows were exposed to the warm climates of July and August in Tennessee without supplemental cooling. Least squares means of dry matter intake, milk, and body weight from each treatment were input into the model, and tabular crude protein and fiber contents were adjusted to reflect chemically derived values. Treatments did not affect feed intake. The RDP treatment did not affect milk yield but, at 6% RUP, milk yield declined compared with the 10RDP:8RUP diet (RDP x RUP interaction; P < 0.01). The NRC model overpredicted a decline in milk yield (1.9 kg) in response to lowering RDP at 8% RUP (10RDP:8RUP vs.RDP:8RUP). At 6% RUP, the model predicted a decline in milk yield (2.0 kg) in response to lowering RDP (10RDP:6RUP vs. 8RDP:6RUP); however, milk yield increased by 2.3 kg. At 10% RDP, the model overpredicted a decline in milk yield (5.1 kg) in response to lowering RUP (10RDP:8RUP vs. 10RDP:6RUP). At 8% RDP, the model overpredicted a decline in milk yield (7.5 kg) in response to lowering RUP (8RDP:8RUP vs. 8RDP:6RUP). Reduction of dietary RDP decreased predicted RDP supply and increased RUP requirements. Reduction of dietary RUP decreased predicted RUP supply but did not affect RUP requirements. In summary, the NRC model underestimated RDP supply and overestimated RUP requirements in response to low dietary RDP. The model underestimated the RUP supply in response to low dietary RUP. An improvement in predictions of MP allowable for milk production should increase the accuracy of the NRC model.

Key Words: National Research Council, rumen degradable protein, rumen undegradable protein

Virginia Polytechnic Institute and State University

Station Researcher: Mark Hanigan

**OBJECTIVE 1:** To quantify supply, availability, and interaction of nutrients and bioactive compounds utilized for efficient milk production while reducing environmental impact.

**Absorbed Supply of Amino Acids:** We have continued development work on an isotope based method of assessing amino acid bioavailability from individual ingredients within a mixed diet . We conducted a trial last year that demonstrated the validity of the method and derived bioavailabilties for blood mean and feather meal. We conducted a 2nd trial this year to assess assess amino acid bioavailabilities for corn silage, alfalfa hay, grass hay, distillers grains, brewers grains, corn, and soyhulls. The animal and sample analyses work have been completed, and the modeling work is in progress. We have completed the animal work for a 3rd trial to assess the potential of adding ruminal infusions of 15N-ammonium sulfate to label microbes so that a proportion of the total entry flux can be assigned to microbial protein thus yielding microbial and feed protein entry rates. Sample analyses is almost complete.

**Effects of hydroxyl methylthio butanoic acid on microbial protein synthesis:**

Methionine is an important essential AA for milk protein synthesis in dairy cows. Supplementation of unprotected, free Met is nearly 100% degraded by ruminal microorganisms which complicates supplementation. 2-hydroxy-4-methylthio-butanoic acid (HMTBa) can be converted to Met in the body, and is used as a Met source in dairy production. However, results of published studies assessing the effects of supplementing Met sources, including HMTBa, on performance variables are inconsistent. A meta-analysis was performed to quantitatively summarize the accumulated results of HMTBa supplementation on animal performance and nutrient digestibility. Data pertaining to HMTBa dose, dietary composition, and major performance variables (rumen VFA composition, milk performance, nutrient digestibility) were collected from 48 articles containing 204 treatment means. Publications were from scientific journals and internal Novus International, Inc. (St. Charles, MO) reports published from 1970 to 2016. The HMTBa effects on response variables were analyzed using linear mixed models that included random study effects when significant. To fully investigate the effects of HMTBa supplementation, it was tested both as a continuous (Met equivalent dosage) and a categorical (fed/not fed) predictor. Other explanatory variables tested included study type (in vitro or in vivo), forage percent, NDF percent, CP percent and days in milk (DIM). Results showed that HMTBa supplementation increased microbial N output, blood Met concentration, milk fat yield, and DM, OM and CP digestibility. Although HMTBa supplementation increased milk fat yield, further studies are needed to elucidate the mechanisms by which HMTBa generates this response. As a categorical variable, supplementation of HMTBa increased digestibility of OM and CP. To further evaluate how HMTBa supplementation should be accounted for in nutrient requirement models like the NRC (2001), standard residual errors for digested NDF and CP, and microbial N (observed minus predicted as a percentage of predicted) were regressed against Met equivalent of HMTBa to identify adjustments that can be used to better model the effects of HMTBa supplementation on nutrient supply. The predicted amount of NDF and CP digested was found to increase by 1.08% and 1.36% of predicted digested NDF and CP, respectively for each g of HMTBa added to the diet.

**OBJECTIVE 2:** To quantify metabolic and molecular interactions that alter synthesis of milk components.

**Extracellular amino acids and lysine to methionine ratio affect cell signaling in mammary epithelial cells.**  
Extracellular amino acid (AA) profile may affect intracellular AA concentrations and profile as well as signaling proteins that regulate translation rate. The objective of this work was to assess the effects of various extracellular AA profiles and Lys to Met ratio to determine signaling protein sensitivity. Six AA profiles of DMEM, blood meal (BM), corn gluten meal (CM), casein (CS), blood plasma of cows milking 45 kg/d (CW), and a negative control (NC) represented the profile factor (AAPROF) and Lys/Met ratio unchanged or set to 3:1 was the Lys/Met factor (ML) for a total of 12 treatments with 4 replications. The concentrations of total AA for all treatments except NC (0 mg/L) were set to 659 mg/L (63% of DMEM) which previously was shown to result in maximal stimulation of casein synthesis. Confluent mammary epithelial cells were exposed to treatments for 75 min. Intracellular concentrations of Met, Lys, Leu, Ile, and Thr were affected by AAPROF (*P*< 0.02) whereas only Met and Lys were affected by ML, increasing by 13.6 μmol/L and 11.5 μmol/L (*P* < 0.01). Intracellular Met and Lys concentrations were 145 and 274% (*P* < 0.01) greater for the NC and ML at 3:1 ratio treatment versus other ML 3:1 ratio treatments despite similar extracellular concentrations indicating greater uptake. Within mTOR pathway, mTOR, ribosomal protein S6 kinase 1 (S6K1), and eukaryotic initiation factor 4 E binding protein 1 were induced by AAPROF (*P* < 0.01) while only S6K1 was affected by ML (*P* = 0.11). mTOR pathway proteins had greater phosphorylation for DMEM, BM, CM, CS, and CW versus NC (*P* < 0.01). Blood meal (*P* = 0.15) and CM (*P* = 0.06) had higher phosphorylation than CS and CM tended to be higher than CW (*P* = 0.14) for S6K1 respectively. For mTOR, BM, CS, and CM tended to have higher phosphorylation than CW (*P* = 0.07). Eukaryotic initiation factor 2 α subunit was unaffected by PROF and ML factors (*P* = 0.23). Setting Lys/Met at a 3:1 ratio had a small positive effect on S6K1 regardless of AA profile. Changes in extracellular AA profiles largely translated to intracellular AA and these varying profiles in general stimulated mTOR pathway related proteins.

**Lactational performance responses to ruminally protected methionine and lysine prototypes.**  
Methionine (Met) and lysine (Lys) are often limiting amino acids in lactating cow diets. The objective of this work was to assess a lipid encapsulated Lys (RP-Lys) and 3 lipid encapsulated Met (RP-Met) prototypes (P1, P2, and P3) to determine animal performance responses. Twenty Holstein cows were randomly assigned to 2 trials (n = 10 each) in a replicated Latin square design with 14 d periods. Both trials were analyzed using a linear mixed effect model, however, the Lys trial was analyzed using a dose response technique. The base diet was predicted to be deficient in metabolizable Met (−14.8 g/d) and Lys (−16.1 g/d). In the Met trial, the base diet was supplemented with RP-Lys to meet the lysine requirement. The treatments included no added RP-Met (NC), Smartamine (SM), and P1, P2, or P3 at 148% of the Met content of SM. In the Lys trial, the base diet was supplemented with RP-Met to meet the methionine requirement. Treatments included no added RP-Lys (NC), AjiProL (AL), or the RP-Lys prototype at 55%, 78%, or 102% of the Lys in AL. Performance results are listed in Table 1. Milk protein percent significantly increased when diets were supplemented with P2 or P3 compared with NC, but none were different from SM. Overall, P2 had the greatest numerical production response among the 3 Met prototypes suggesting it had the greatest efficacy when supplemented into these rations. There was a significant linear increase for milk protein percent for the RP-Lys prototype compared with AL when fed at a range of 55–102% of lysine content, indicating that it could support comparable performance. Table 1. Performance results when supplementing RP-Met or RP-Lys Prototypes

**Milk protein and intake responses to isoleucine, leucine, methionine, and threonine.**

In vitro experiments have demonstrated independent, additive casein synthesis responses to supplies of Ile, Leu, Met, and Thr. We hypothesized that lactating cattle would respond in a similar manner. Forty-eight Holstein cows were fed a diet containing 75% of NRC (2001) predicted, metabolizable protein (MP) requirements (LoMP, 13.5% CP) in a randomized block design with replicated 4 × 4 Latin squares within each block. Each of the 4 ruminally protected (RP) amino acids (AA) represented a block. Period length was 12 d. Treatments within each block were LoMP and LoMP plus RPIle, RPLeu, RPMet, or RPThr at doses of 0, 50, 100, and 150% of the difference between absorbed AA supplied by the LoMP and MP sufficient diets. Intestinal availability of each RPAA was assessed by abomasal dosing of the RPAA after 8 h of ruminal incubation. The RPAA doses were 0, 8.5, 17, and 25.5 g of absorbed Ile/cow/d; 0, 14, 28, and 42 g of absorbed Leu/cow/d; 0, 3, 6, and 9 g of absorbed Met/cow/d; and 0, 8, 16, and 24 g of absorbed Thr/cow/d. DMI increased linearly with increasing dose of Ile (*P* = 0.02), and tended to increase quadratically with respect to Met and Thr. Leu had no effect on DMI. Milk yield (kg/d) increased quadratically (*P* < 0.05) in response to Ile, Met, and Thr, and decreased quadratically in response to Leu. Milk protein yield (kg/d) tended to increase quadratically (*P* = 0.11) in response to Met and linearly (*P* = 0.12) in response to Thr, and decreased quadratically in response to Leu. Ile had no effect. Body weight (kg/d) decreased quadratically (*P* < 0.0001) with Met dose, and tended to increase linearly (*P* = 0.11) with Leu dose, suggesting that changes in milk protein yield for animals supplemented with Leu may be driven by non-mammary tissue use. Conversely, DMI and BW responses for animals supplemented with Met and Thr do not explain the trend for increased milk protein yield, suggesting that Met and Thr stimulated milk protein synthesis. Revising dairy requirement models to include animal responses to individual AA may improve milk production predictions leading to increased N efficiency, and reduced N excretion from lactating dairy animals.

**Objective 3:** To use this knowledge of feed properties and metabolic and molecular quantitative relationships to challenge and refine nutrient requirement models leading to more precise feeding systems for dairy cattle.

**Development of a revised representation of postabsorptive amino acid metabolism and milk protein synthesis.** We have made additional progress on our postabsorptive model which contains representations of amino acid removal by portal-drained viscera, liver, mammary, and the remaining tissues in aggregate, and the production of milk protein from mammary amino acid use. Modeling work has been completed on the representation of amino acid uptake by each tissue and an analytical solution for predicting arterial concentrations of each essential AA has been derived and validated. Amino acid uptake predictions have been evaluated for each of the tissues and found to be of high quality. Remaining work includes evaluation of arterial concentration predictions and derivation of an equation for milk protein production.

**Revised representation of urea recycling and ruminal nitrogen metabolism for the Molly cow model.** Accurately predicting nitrogen (N) digestion and utilization will allow diet optimization to achieve improved N efficiency. The objectives of this study were to revise the representation of urea recycling in Molly cow model and evaluate the revisions. The work included 1) modification of the existing urinary urea excretion equation to include BW as a scalar; 2) supplement of urea gut entry rate to derive parameters related to urea return to rumen; and 3) reparameterization of equations related to urea N recycling and ruminal N metabolism. Model parameters were changed from initial values to optimized values. Model predictions were compared with a data set from 12 published studies with 54 treatment means before and after the revisions. Mean squared errors were assessed to characterize model performance. Residual analyses demonstrated that the modifications improved the accuracy of predictions of ruminal N digestion, absorption and recycling. After the modifications, prediction errors for duodenal flows of total N, microbial N, non-ammonia N, and non-ammonia non-microbial N were 14.8, 22.4, 17.8 and 28.2%, respectively, which were all approximately 2% units less than observed with the initial model due primarily to the decreased mean bias. Compared with the initial model, predictions of ruminal ammonia and blood urea concentrations were greatly improved with substantial decreases in mean and slope bias. Prediction errors for gut entry rate were 19.2% with 0.93% mean bias and 1.73% slope bias, which indicated that urea N recycling mechanisms were properly represented in the model. Although the accuracy of urinary urea flow was improved, it still had 81.7% prediction error, which implies high variation of urinary urea N secretion may exist in collected studies, and therefore the reparameterization is not necessarily more accurate even though it fits the observed data. In summary, the model modifications led to a robust representation of urea N recycling and ruminal N metabolism which enabled more accurate and precise predictions of the effects of feeding and management decisions on N efficiency, thus contributing to sustainability of the dairy industry.

**Comparison of Molly and Karoline model to predict methane emissions in cattle.** *With Estonian University of Life Science and the Swedish University of Agricultural Sciences, Umeå, Sweden.*  
Models originally compiled to predict nutrient absorption from the digestive tract and metabolized in various tissues could be adapted for CH4 predictions. Numerous empirical equations and mechanistic models to predict CH4 emission are available. The Molly cow model is a mechanistic, dynamic model describing digestion and metabolism of dairy cattle with the ability to predict the animal-related factors that affect the environment, including CH4 emission (Hanigan et al., 2013). The Nordic cow model Karoline is a dynamic, mechanistic model describing digestion and metabolism in dairy cows (Danfær et al., 2006), and it was confirmed by Ramin and Huhtanen (2015) to be a useful tool in predicting CH4emissions in cattle. The aim was to evaluate these models for predicting CH4 emissions in cattle using a data set consisting of 267 treatment means from 55 respiration chambers studies. The data set contained DMI, (14.2 ± 5.82 kg/d); ingredient proportions; dietary contents of CP (156 ± 30.8 g/kg) and NDF (356 ± 105.9 g/kg); BW (531 ± 131.1 kg); and CH4 (303 ± 118.7 g/d) which covers the range of typical cattle diets. The simulations were conducted using observed DMI, BW and dietary nutrient concentrations and digestion rates. Each treatment mean was simulated and predictions of nutrient digestibility and CH4 output were collected in a database. The relationships between observed and predicted CH4 (pCH4) were assessed by regression analysis. Root mean square error (RMSpE) was calculated as: RMSpE = √ [∑ (Obs – Pred)2/n]. Molly predictions were: CH4 (g/d) = 0.81 ± 0.018 × pCH4 (g/d) + 38 ± 6.4 (RMSpE = 54.9 (18.1% of observed mean) CCC = 0.910). The corresponding equation for Karoline was: CH4 (g/d) = 1.00 ± 0.019 × pCH4 (g/d) + 5 ± 6.0 (RMSpE = 34.6 (11.4%) CCC = 0.955). Both mean (−27 g/d) and linear bias (−0.19) were significant (*P* < 0.001) with Molly, but only mean bias (4 g/d) was significant (*P* = 0.04) with Karoline. Proportions of MSE attributable to mean and linear bias and random error were 23, 24 and 53% for Molly, and 2, 0 and 98% for Karoline, respectively. Based on predictions it can be concluded that both models predicted CH4 emissions reasonably well in terms of high CCC, but Karoline was more accurate based on smaller RMSE, mean and slope bias.

University of Wisconsin-Madison

Station Researcher: Heather White

**Objective 1. To quantify properties of feeds that determine availability of nutrients critical to milk production.**

Project 1. The effect of fermented ammoniated condensed whey supplementation on hyperketonemia incidence in transition dairy cows

INVESTIGATORS: Heather White, Rafael Oliveira

The objective of this trial was to examine the effect of fermented ammoniated condensed whey supplementation on hyperketonemia incidence in transition dairy cows. Holstein cows were individually housed in a tie-stall facility from 28 days prepartum to 45 days postpartum. Cows were blocked by expected calving date and randomly assigned to control (ctl; n=20) or GlucoBoost® (GB; Fermented Nutrition; n=19). All cows received the same high energy prepartum ration precalving in order to achieve postpartum hyperketonemia incidence consistent with industry averages. Cows began treatment rations (GB 0.9 kg AF/day replacing DM equivalent in ctl) on the day of calving. Hyperketonemia incidence was calculated from blood samples collected on days 1, 3, 5, 7, 9, 11, 14 and 17 after calving. Categorical data was analyzed by logistic regression (GLIMMIX, SAS) fitting a binary distribution response. Continuous variables were analyzed with the MIXED procedure of SAS. Models containing the fixed effect of treatment (GLIMMIX) or treatment, time, and the interaction of treatment x time (MIXED) and random effects of block and cow(block x treatment).

Milk volume was not altered by treatment (41.1 vs. 42.2 ± 1.53 kg, ctl vs. GB), but there was a tendency (P = 0.1) for a treatment by time interaction. Supplementation did not alter fat, protein, or lactose yield. Dry matter intake was reduced from three to seven weeks (treatment x time P < 0.01) in cows supplemented with GB and feed efficiency was increased (treatment x time P < 0.01) from four to seven weeks. Cows supplemented with GB had a numerically reduced hyperketonemia incidence (60.0 vs. 36.8 ± 11.28%, P=0.17). Body weight change (-69.7 vs. -60.4 ± 8.78 kg, ctl vs. GB) and body condition score change (-0.39 vs. -0.30 ± 0.052 units, ctl vs. GB) from calving to 45 d did not differ (P>0.25) by treatment. Concentration of NEFA was lower at 5 and 7 DIM (treatment x time P = 0.02) differed over time but was not affected (P=0.70) by treatment.

Overall, during a metabolic challenge, supplementation of an ammoniated lactate product numerically reduced HYK while improving milk efficiency. This may be a valid nutritional strategy for herds with high HYK. Gene expression work on liver tissue samples is yet to be completed. Further work needs done to understand the potential benefit of the supplement when cows are not metabolically challenged (ie, no induced HYK).

**Objective 2. To identify and quantify molecular, cellular, and organismal signals that regulate partitioning and efficient conversion of nutrients to milk.**

*Project 1. Hepatic and adipose patatin-like phospholipase domain-containing protein 3 as a part of coordinated lipase activity.*

*Experiment 1:* *Response of patatin-like phospholipase domain-containing protein 3 abundance to fatty acid treatment in bovine primary hepatocytes*

Hepatic PNPLA3 abundance is increased postcalving during remobilization of liver lipids, and is correlated with nonesterified fatty acids (NEFA) concentrations. Fatty acids are known regulators of hepatic gene and protein abundance. Therefore, the objective of this study was to determine if hepatic PNPLA3 abundance is responsive to incremental changes in concentration of fatty acids in primary hepatocytes. Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 hours before treatment. Treatments consisted of a NEFA free control, palmitic acid (C16:0), oleic acid (C18:1n6), α-linolenic acid (C18:3n3), and a fatty acid cocktail with a profile of fatty acids reflective of NEFA at parturition. The fatty acid cocktail consisted of: 3% myristic acid (C14:0), 27% palmitic acid (C16:0), 23% stearic acid (C18:0), 31% oleic acid (C18:1n6), 8% linoleic acid (C18:2 n6), and 8% α-linolenic acid (C18:3n3). Abundance of PNPLA3 was determined by Western blot analysis and normalized to total lane protein. For analysis, PNPLA3 was expressed relative to a fatty acid free control, and transformed as log(relative abundance +1) because data were not normally distributed. Data were analyzed for main effects of treatment and concentration, and linear and quadratic contrasts of concentration using PROC MIXED in SAS 9.4. Fatty acid treatment had an effect (P=0.04) on PNPLA3 abundance with the fatty acid cocktail tending to result in greater PNPLA3 abundance than palmitic acid. Increasing fatty acid concentrations decreased (P=0.05) PNPLA3 abundance. These data indicate that PNPLA3 abundance may be regulated by fatty acids. This could suggest that decreasing NEFA concentrations observed around two weeks postpartum could play a role in coordinating increased PNPLA3 abundance at this time, to facilitate remobilization of liver lipids.

*Experiment 2: Coordinated response of hepatic lipolysis during the transition to lactation in dairy cows*

The objective of this study was to examine the coordinated response of hepatic lipolysis-associated proteins during the transition to lactation. Liver biopsies were collected at -14, +1, and +14 days relative to calving (DRTC) from multiparous cows. Liver TG were quantified and used to retrospectively assign cows to either a high (>20% liver lipids, dry matter; n=5) or low (<20% liver lipids, dry matter; n=3) treatment based on the maximal liver TG concentration. Protein abundance of hepatic abhydrolase domain containing 5 (ABHD5), hormone sensitive lipase (HSL), lipase A and C (LIPA, LIPC), lipoprotein lipase (LPL), perilipin 1 (PLIN), patatin-like phospholipase domain containing 2 and 3 (PNPLA2, PNPLA3) were determined through Western blot analysis and normalized to total lane protein. For analysis, each lipase was expressed relative to -14 DRTC and transformed as log(relative abundance +1) because data were not normally distributed. Data were analyzed for main effects of treatment, DRTC, and treatment x DRTC using PROC MIXED (SAS 9.4). Differences were declared at P<0.1 and tendencies at P<0.15. Cows with low liver TG had greater ABHD5 (P=0.05) and HSL (P=0.049), and tended to have greater LIPA (P=0.146), LPL (P=0.11), and PHSL (P=0.12) abundance compared to cows with high liver lipids. Abundance of ABHD5 tended to be greater (P=0.13) and PLIN was greater (P=0.06) at +14 compared with -14 and +1 DRTC. Abundances of LIPA (P=0.0006) and PNPLA3 (P=0.04) were decreased at +1 and then increased by 3.3 and 1.8 times by +14 DRTC, respectively. These data indicate that cows with lower liver TG postpartum had a greater abundance of some hepatic lipases while other hepatic lipases are increased postpartum regardless of liver TG concentration. This suggests that there may be a coordinated response of several hepatic lipases to mediate both liver TG accumulation and subsequent remobilization during fatty liver recovery.

*Experiment 3:*

Lipolysis is mediated by lipases; however, known regulators of lipolysis in nonruminants are not upregulated during the transition period in dairy cows. The objective of this study was to determine the coordinated response of bovine adipose lipases to mobilize TAG during the transition period. Multiparous pregnant dairy cows were blocked by anticipated calving date and randomly assigned to either the control group (n=3), fed an ad libitum diet, or fatty liver induction (FLI) group (n=8; overfed prepartum and feed restricted postpartum) until clinical ketosis onset. Adipose tissue and blood samples were collected at -14, +1, and +14 days relative to calving (DRTC) for NEFA and lipase quantification. Additional samples (n=3) were taken once during a period of positive energy balance (PEB) to utilize as PEB controls. Protein abundance of abhydrolase domain containing 5 (ABHD5), lipoprotein lipase (LPL), perilipin 1 (PLIN), and patatin-like phospholipase domain containing 3 (PNPLA3) were determined through Western blot analysis, normalized to total lane protein, and expressed relative to -14 DRTC samples. Data were non-normal; therefore, the log(relative abundance +1) was analyzed (PROC MIXED, SAS 9.4) for effect of DRTC, treatment, and DRTC x treatment. Correlations were explored using PROC CORR. During the transition period, ABHD5 (P=0.04) was increased at +14 DRTC compared with +1 DRTC. Abundance of LPL was greater (P=0.05) in FLI cows, and +1 LPL was correlated to NEFA (r=0.62) at +14 DRTC across treatments. At +14 DRTC, abundance of ABHD5 (r=0.64) and PLIN (r=0.60) were correlated with NEFA concentration at +1 DRTC. Interestingly, PNPLA3 abundance (P=0.04) was increased 3.8-fold from PEB to -14 DRTC, and was highly correlated to abundance of both PLIN (r=0.71) and phosphorylated PLIN (r=0.74) at +1 DRTC. Previously thought to not be expressed in bovine adipose tissue, PNPLA3 may be a key lipase during the transition period; however, the mechanistic relationship between these lipases needs to be further explored.

*Experiment 3: Screening for siRNA that knockdown bovine hepatic PNPLA3.*

Initial attempts to knockdown PNPLA3 resulted in a 33% knockdown. We designed several new siRNAs and exposed cells to 20, 60, or 80 pmol. Quantification of relative protein abundance indicated that one siRNA was able to knockdown PNPLA3 97% in primary hepatocytes and another was able to upregulate PNPLA3 by 220%. Also of interest is the 120 kDa “unknown” band. Several antibodies for PNPLA3 all result in the 55 and 120 kDa band and the 120kDa band is labeled as “unknown” or “nonspecific binding” by the antibody companies. We suspect that it is a dimer, complex, or phosphorylated PNPLA3 because it is present with any PNPLA3 antibody used, dynamic over the transition period, and responds to siRNA. We are in the process of pursuing this further.

Figure 1. Relative abundance of 55kDa PNPLA3 band (open bars) and 120kDa unknown band (gray bars) during siRNA effectiveness screen.

*Project 2: Influence of methyl-donors choline and methionine on hepatic triglyceride metabolism.*

*Experiment 1:*

Choline and methionine can both serve as methyl-donors; however, previous works suggests they may serve unique functions to regulate carbon metabolism within bovine hepatocytes. The objective of this experiment was to trace the flux of carbon through pathways of fatty acid (FA) metabolism in bovine hepatocytes exposed to choline and methionine. Primary hepatocytes isolated from 3 neonatal Holstein calves were maintained as monolayer cultures for 24 h. At 24 h media was refreshed and treatments of choline chloride (CC; 0, 0.01, 0.1, or 1.0 mM) and D,L-Met (DLM; 0, 0.1, or 0.3 mM) were applied in a factorial design along with 1.0 mM FA that reflected the blood FA profile at calving. After 21 h of treatment, [1-14C]palmitate was added to the media and both CO2 and acid soluble products (ASP) were collected after a 3h incubation. Parallel treatments were incubated without radiolabeled substrate for 24h to quantify media reactive oxygen species (ROS) and cellular triglyceride (TG). Cellular oxidation and TG were normalized to DNA. Data were expressed relative to a no FA/CC/DLM control within each cell prep, and analyzed by PROC MIXED (SAS 9.4) in a model accounting for fixed effects of CC, DLM, interaction of CC and DLM, and random effect of calf. Contrasts evaluated for CC were: 0 mM vs. (0.01, 0.1, and 1.0 mM) and linear contrast 0.01 vs. 0.1 vs. 1.0 mM. Contrasts evaluated for DLM were: 0 mM vs. (0.1 and 0.3 mM) and 0.1 vs. 0.3 mM. Data are reported as least squares means ± SE and differences declared at P≤0.10 and tendencies at P<0.15. No interactions of CC and DLM were detected. DLM did not alter (P>0.15) cellular TG, ROS, or recovery of substrate label as CO2 or ASP. Recovery of palmitate label as CO2 or ASP was not altered (P>0.15) by CC treatment, indicating no change in complete or incomplete oxidation of FA with CC treatment. Presence of CC decreased (P=0.03) cellular TG accumulation (3.3 vs. 2.9 ± 0.03 arbitrary units) and concomitant secretion of ROS into media (P=0.04). Treatment with CC did not affect fatty acid oxidation but exhibited a hepatoprotective effect by decreasing cellular TG accumulation and ROS secretion, despite methionine treatment.

*Experiment 2:*

Adipose tissue mobilization increases circulating fatty acid (FA) concentration, hepatic FA uptake, and influences hepatic metabolism. The objective of this experiment was to examine the effect of FA challenge on complete and incomplete oxidation, glucose output, and oxidative stress in bovine primary hepatocytes. Primary hepatocytes isolated from 3 neonatal Holstein calves were maintained as monolayer cultures for 24h. At 24h, media was refreshed with a glucose-free media containing only pyruvate as a gluconeogenic precursor, and cells were exposed to 0 or a 1 m*M* FA cocktail that reflected the circulating FA profile at calving. After 21h of treatment, 14C-labeled palmitate or pyruvate was added to the media and both CO2 and acid soluble products (ASP) were collected after a 3h incubation. Media was harvested to quantify glucose and reactive oxygen species (ROS). Cell lysates were collected and DNA quantified to normalize all data. Data were analyzed by PROC MIXED (SAS 9.4) in a model accounting for fixed effect of FA treatment and random effect of calf. Data are reported as least squares means ± SE and differences declared at *P*≤0.10 and tendencies at *P*<0.15. Fatty acid treatment decreased (*P*=0.01) the relative recovery of pyruvate label as CO2 (3.8 vs. 1.94 ± 0.31 arbitrary units) and increased (*P*=0.02) label recovery as ASP (20.0 vs. 27.3 ± 1.4 arbitrary units). Recovery of palmitate label as CO2 tended to be greater (*P*=0.14) in FA treated cells (0.87 vs. 1.05 ± 0.15 arbitrary units) but ASP from palmitate was not affected (*P*>0.18) by FA treatment. Glucose output from cells exposed to FA was increased (*P*<0.007) by 35%. Treatment with FA increased (*P*=0.07) ROS in cell culture media. Fatty acid challenge appears to shift oxidative and gluconeogenic capacity in a substrate-specific manner. Decreased complete, and increased incomplete oxidation of pyruvate indicates a shift of pyruvate conversion towards ASP or glucose production. Conversely, fatty acid challenge increased complete oxidation of palmitate, consistent with previous indications that FA can upregulate key oxidative enzymes at the time of calving.

**C. Publications**

Peer-reviewed articles

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| 1. Acetoze, G., J. Champagne, J.J. Ramsey, H.A. Rossow. 2017. Liver mitochondrial oxygen consumption and efficiency of milk production in lactating Holstein cows supplemented with Copper, Manganese and Zinc. J Anim Physiol Anim Nutr (Berl), DOI: 10.1111/jpn.12836 |
| 1. Baldin, M., D.E. Rico, M.H. Green, and K.J. Harvatine. 2017. Technical note: An in vivo method to determine kinetics of unsaturated fatty acid biohydrogenation in the rumen. J. Dairy Sci. Submitted. |
| 1. Baldin, M., G.I. Zanton, and K.J. Harvatine. 2017. Effect of 2-hydroxy-4-(methylthio)butanoate (HMTBa) on risk of biohydrogenation-induced milk fat depression. J. Dairy Sci. Submitted. |
| 1. Bradford, B. J., C. A. Cooper, M. L. Tizard, T. J. Doran, and T. M. Hinton. 2017. RNA interference-based technology: what role in animal agriculture? Anim Prod Sci. 57:1–15. |
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**D. Impacts**

Many members of the group currently have competitive research funding and are involved in collaboration with other research within and outside of the NC2040 group. These funded projects and collaborations are advancing our understanding of nutrients, fed technologies and supplements, ruminant nutritional physiology and metabolism, and animal impact on the environment. Members of this group are nationally and internationally recognized for their research.

In addition to research findings, an additional outcome of this group is the training of future researchers and leaders. Across all three objectives, researchers have trained many masters and doctoral students, as well as undergraduate students. The following are specific outcomes and impacts from this committee:

1. We have previously described the dynamic regulation of adiponectin and FGF21 in transition dairy cows but primary factors driving this regulation were unknown. Identification of some of these factors contribute to a better understanding of the functional impact of adiponectin and FGF21 in transition dairy cows.
2. Previous findings around the impact of non-steroidal anti-inflammatory drugs on sustained milk yield responses have attracted a lot of interest, but currently there is no legal means to use such approaches in the United States. These findings suggest that early lactation supplementation of a polyphenol source may be able to induce similar responses without the use of pharmaceutical agents.
3. Most rumen bacteria are uncultured and thus have unknown niches in rumen fermentation. Along with other fluorescent compounds, 2-NBDG has potential to identify uncultured, glucose-consuming species and thus better define microbial niches in the rumen.
4. Fermentation pathways of rumen bacteria were thought to have been well-established decades ago. However, our results show nearly half of bacteria have atypical pathways.
5. We have demonstrated that canola meal can be a suitable protein supplement to dairy cows, capable of replacing soybean meal and sustain or improve milk production.
6. We evaluated the nutritional value of western plants that may be suitable for ruminant consumption, these include cheatgrass and forage kochia.
7. We demonstrated that cell culture media has an impact on inflammation response in MAC-T cells.
8. Results are consistent with the premise that our animal model provides a unique and powerful opportunity to gain a greater within and among tissue understanding of key genes and gene networks involved in regulating metabolism of the cow and how these components have been altered by selection for increased milk yield per cow. Our ongoing genomic investigations are therefore expected to provide additional insight to specific factors that regulate nutrient metabolism and health of the cow.
9. Established 2 commercial dairy herds where both daily milk yield and daily bodyweight changes can be quantified to examine the whole herd response to changes in feed management and supplementation.
10. Examined animal responses to feeding fibrolytic enzymes, glucose precursors and a calf starter supplement.
11. Developed a method to measure and quantify mitochondrial enzyme activities in calves and cows to examine metabolic efficiency in response to age, environment, and management (feeding) in dairy cattle.
12. We further characterized bioavailability of methionine, lysine, and histidine products in development, moving them closer to commercial use.
13. We found that feeding of calcium carbonate and magnesium oxide may help to buffer the intestines, potentially improving health of the intestines.
14. We have improved methods of assssing amino acid supply to the animal, and have gained an increased understanding of the effects of HMTBa on microbial growth.
15. Profiles of extracellular amino acids were shown to largely be reflected in intracellular concentrations, and those changes affected mTOR signaling. We have collected additional data on responses ot Lys and Met that can be used to help define requirements for those amino acids. Milk protein output was shown to respond independently to 2 different amino acids within the same diet demonstrating that the single, limiting amino acid concept is not correct. Threonine responses were observed in a moderately MP deficient diet suggesting this amino needs to be further evaluated for consideration in feeding programs.
16. A model of postabsorptive amino acid use has been developed and evaluated. Preliminary milk protein predictions indicate a substantial improvement over the 2001 NRC model. The Molly cow model was revised with an improved representation of N cycling between the gut and blood which improved predictions of ruminal ammonia concentrations. This will help resolve issues with biased microbial growth rates and ruminal pH as ammonia concentrations affect pH. Predictions of methane by the model are biased, but track well with observed changes in output. Additional model parameterization is required to more accurately predict methane output.