NC 2040 Annual Report - 2019

Project/Activity Number:
NC-2040

Project/Activity Title:
Metabolic relationships in supply of nutrients for lactating cows

Period Covered:
01 October 2018 – 30 September, 2019

Date of This Report:
30 December 2019

Annual Meeting Date(s):
21-22 October 2019

Annual meeting participants:

A. Faciola	University of Florida
R. Appuhamy Jayasooriya	Iowa State University
M. VandeHaar	Michigan State University
H. Paz	Mississippi State University
L. Dias de Moraes	Ohio State University
J. Firkins	Ohio State University
S. Donkin	Purdue University
J. Osorio	South Dakota State University
H. Rossow	UC Davis
T. Hackmann	UC Davis
A. Rius	University of Tennessee
S. Arriola Apelo	University of Wisconsin
M. Hanigan	Virginia Polytechnic Institute

Brief summary of minutes of annual meeting:

Monday, October 21 (8:00 am to 5:00 pm)

1. Ranga Appuhamy (Iowa State) and Sebastian Arriola Apelo (University of Washington, Madison) volunteered to serve as secretaries in 2019 and 2020, respectively
2. Membership updates
   - The importance of encouraging the attendance and station report submission was emphasized
   - The importance of formatting station report in compliance with guidelines posted in the website
3. David Benfield introduced the new administrator, George Smith from Michigan State University

4. Mike Vandehaar and Mark Hanigan presented some important updates of energy and protein systems in new dairy NRC.
5. Station Reports
    - Purdue University (Shawn S. Donkin):
    - University of California, Davis (Heidi A. Rossow)
    - University of Wisconsin, Madison (Sebastian Arriola-Apelo)
    - Mississippi State University (Henry Paz)
    - University of California, Davis (Tim Hackmann)
    - University of Tennessee (Augustin G. Rius)

Tuesday, October 22 (8:00 am to 1:00 pm)

1. 2020 annual meeting
    - Voted to be at Iowa State University
2. Molly cow model
    - Mark Hanigan emphasizes the significance of implementing new experimental approaches (possibly by the young scientists) in generating data to improve representativeness of the model
    - Luis Moraes pointed out the importance of representing cow to cow variability in the model
    - Heidi Rossow pointed out importance of including an epidemiologist to the team, if animal variability to be represented in the model.
3. Group discussed about a possibility of submitting a big grant targeting sustainability (e.g. AFRI Sustainable Agriculture Systems) as a collaborative effort of the group
    - A project primarily involving modeling (the Molly cow model)
    - Mark Hanigan suggested that individual collaborator would write a summary of his or her contribution and come up with a budget to it
    - Mike Vandehaar suggested collecting those ideas and writings in Google Docs.
    - Mike Vandehaar also stressed the importance of setting up a strict timeline to complete various tasks associated with compiling a large research grant proposal

4. Steve Smith - USDA grant opportunity updates via Zoom
    - RFA of competitive programs have been published for both 2019 and 2020 FYs
    - The application set for 2020 will be up in USDA website in November 2019
    - Program priorities did not change from 2019 to 2020
    - Some carryover funds from 2019 for interdisciplinary programs are available
    - Even though the deadline is passed, there will be some reservation for dual-purpose programs (program contact is not known at the moment)
    - Equipment essential to complete the project can be included in the budget
    - Be sure to include and adequately describe an education and extension component to any proposal targeting big sustainability grants
5. Station Reports
    - University of Florida (Antonio Faciola)

- Iowa State University, (Ranga Appuhamy)
- Ohio State University (Luis Moraes)
- Ohio State University (Jeff Firkins)

Accomplishments and impacts pertaining to each objective:

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

CA: The *in-vitro* gut system, previously established in our lab, was used to test various seaweeds for their effect on the production of greenhouse gases (GHG) and volatile fatty acids (VFA) during in-vitro enteric fermentation. Results from in-vitro trial were summarized and used to make an informed decision about inclusion rate of seaweed for in-vivo trial. We also conducted an in-vivo trial to test effect of seaweed on enteric GHG production, health, and productivity of dairy cows. We optimized an existing in-vitro rumen system to better represent the rumen microbiology and function over time and to decrease costs of existing of in-vitro systems. In a separate study, a goal-programming model was developed to serve as an optimization framework that could balance between minimization of dietary costs and dietary irrigation water usage under different environmental scenarios in dairy production and other livestock production systems. In this study, a set of unique solutions were generated with different water usage and provide different feeding options according to diet cost, water usage and available feeds. From the least cost diet to the least water diet, DMI increased, NDF proportion decreased, lignin proportion increased, energy concentration decreased, and ether extract decreased as the model, in general, tended to choose feedstuffs that have a lower energy concentration but are more water-saving such as wheat silage. When protein requirements were reduced, the results had a similar pattern as when they were hirer indicating the robustness of this modeling framework. Future research in this area will require a better understanding of plant nutrient changes under different environmental conditions. Three other studies were conducted to 1) examine the effect of a fibrolytic enzyme on milk yield and body weight changes during a lactation on a commercial dairy herd, 2) analyze three datasets on sub clinical and clinical ketosis and changes in milk yield in transition cows to determine interactions between management factors and milk yield, and 3) examine the impact of replacing canola meal with NovaMeal as the protein source for lactating dairy cattle.

DE: Our lab conduced two studies evaluating a new rumen protected methionine product. One study measured the plasma methionine response to feeding the product and the second measured the animal performance responses compared to a positive and negative control. The results of the first experiment were positive, showing high availability of the product. In the second experiment, there were no differences between the positive and negative control, so the experiment will be repeated.

FL: We evaluated a range of feed additives on ruminal fermentation. The goal is to understanding ruminal fermentation and nutrient outflow as it is critical to improve nutrient utilization efficiency in lactating dairy cows. We successfully use continuous culture system in these experiments in assessing ruminal fermentation

IA: Growth of pre-weaned calves and calves undergoing weaning are positively related to future milk production and nutrient utilization efficiency. Under the light of this idea, we demonstrated that beginning to offer drinking water to newborn dairy calves right at birth improved growth during pre-weaning period and fiber digestibility at three weeks post weaning. An accompanying fecal microbiome analysis indicated that drinking water offered to new born calves increased microbiota richness and the abundance of beneficial bacterial species in the gut partly explaining those improved growth performance. In another calf trial, the group showed that supplementation of glutamine (a dispensable amino acid) increased feed intake, water intake, and body weight in calves undergoing weaning. Improved gut health, particularly the integrity of gut epithelium appear to be partly responsible for improved feed intake and growth during weaning.

IN: At Purdue University, three lactating multiparous Holstein dairy cows weighing 724 kg (± 28 kg) and producing 31.4 kg of milk/d (± 5.1 kg) were used in a 3x3 Latin square design. Each square consisted of 3 periods of 7 d, including 6 d of wash out and one day of infusion and sampling. Cows were housed in tie-stalls, milked twice daily, and were fed individually at 0600 h throughout the experiment. Milk production was recorded daily. Cows were fed a basal TMR to meet or exceed protein, lysine and methionine requirements NRC (2001). Dry matter intake was assessed daily and the TMR was fed to provide 10% refusals. The intestinal bioavailability of the RP-Lys product was assessed using changes in plasma Lys concentrations after an abomasal bolus dose of lysine-HCl (59g in 300 ml) following the procedure of Gressley et al. (2006). The rise in blood concentrations of Lys was determined prior to and following abomasally infused Lysine-HCl (positive control) and water bolus (Negative control) and a rumen protected source of lysine (test material). After infusion, lines were flushed with an additional 1,500 mL of water to ensure complete delivery of the dose. Blood samples were collected 10 and -5 minutes prior to and at 30, 60, 90, 120, 240, 360 and 480 min following of each treatment. Plasma was analyzed for amino acid concentration. Plasma amino acid appearance from the treatments was estimated from the area under curves (AUC) with respect to time. There was no effect of treatments on milk production or DMI ($P > 0.05$). The effects of the bolus dose of the rumen-protected RP Lys, HCl-Lys or water on plasma amino acid concentration are presented on Table 1. Free Lys increased lysine ($P < 0.01$), tended to increase Arginine ($P \leq 0.09$) plasma concentration, and as a result, tended to increase TAA ($P \leq 0.09$) and EAA ($P < 0.05$) plasma concentration. The infusion decreased plasma Citruline ($P < 0.05$) concentration. There was a time x treatment interaction for aminoadipic acid, alanine, hydroxyproline, ornithine, lysine, 1- methyl histidine, arginine, EAA, TAA and also for Lys, His and Met when evaluated as % of EAA. The data indicate an inability of the form of rumen protected lysine to deliver the amino acid for absorption. Blood profiles of lysine indicate an inverse relationship with methionine and an effect of lysine infusion on lysine catabolism and circulating concentrations of key essential amino acids.

KS: Twenty-four multiparous Holstein cows were fed 4 diets with a different protein concentrate source including soybean meal (SBM), high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP) and canola meal with bypass protein (CANBP) in a replicated 4 × 4 Latin Square with 28 d periods. Feed and water intake, and milk yield and composition were measured daily. Fecal grab samples were collected for determination of total tract digestibility and Urine samples were collected via vulva stimulation every 9 h during the 3-d collection period. The CANBP increased dry matter intake compared with SBM and HPCP ($P < 0.01$). The HPCP decreased milk yield compared with all other treatments, but SBMBP and CANBP increased milk yield compared with other treatments ($P < 0.001$). HPCP decreased milk protein concentration, milk urea nitrogen concentration ($P < 0.01$), and yields of fat, protein and energy-corrected milk ($P < 0.001$). HPCP decreased urine nitrogen excretion compared with SMB and CANBP ($P < 0.02$), and it increased fecal nitrogen excretion compared with all other treatments ($P < 0.001$).

MI: In two studies, we showed that 1) the ammonia-fiber-expansion process increased the available energy content of wheat straw fed to lactating cattle and buffalo in India and 2) increases in acetamide found in milk could be attenuated by feeding management. We published two studies including new equations for predicting intake of lactating dairy cows and showed that changes in feed intake explain much of the change in milk production to many dietary factors. We conducted meta-regression analysis aiming at determining the effect of dietary fatty acid (FA) composition on digestibility of dry matter (DM), neutral detergent fiber (NDF), and FA and digestible energy intake (DEI). Our database comprised 423 individual observations from 124 lactating Holstein cows receiving diets that varied in FA composition from five studies. The models included fixed effects of dietary FA contents and their quadratic terms, 2-way interactions between FA and starch contents, forage NDF content, and DMI. Random effects were study-specific intercepts and slopes on DMI, and cow, period and block factor nested within study. Best fitting models indicated that dietary C16:0 and C18:0 had significant impact on digestibility and DEI. C16:0 increased NDFD and DEI when increased from 0.33 to 1.27% of DM, while C18:0 decreased DEI when increased from 0.35 to 0.88% of DM. Results could aid the development of models to predict DEI from dietary ingredients including dietary FA. Finally, we examined effects of bioactive fatty acids on health of calves. In one study, a colostrum supplement of n-3 FA administered in volumes of 30, 60, and 120 mL linearly increased plasma concentrations of n-3 FA and n-3 FA metabolites, but did not alter overall oxidant status. In a second study, we found that a colostrum supplement of 60 ml of n-3 FA and α-tocopherol decreased oxidant status and increased plasma n-3 FA concentrations in the first week of life. This improved oxidation status likely was not due to the vitamin E alone but a synergistic action of the vitamin E and n-FA, and might translate into improved health. In a third study, feeding transition milk or colostrum replacer for 3 d after first colostrum increased growth rate of calves throughout the pre-weaning period.

OH: We conducted three studies aiming at 1) assessing recovery of ^{13}C-enriched branched-chain VFA (BCVFA) and AA (BCAA) into rumen bacterial fatty acids (FA), 2) determining the effects of BCVFA supplementation with solids passage rate and pH on NDF degradation and microbial function in continuous culture, and 3) quantifying the relationship between intake of BCAA and production of lactating dairy cows. First two objectives were accomplished with continuous cultures. We found that adding BCVFA or BCAA increased ($P < 0.05$) 13:0, *iso* 14:0, and total odd chain FA. Total dose recovered in FA was not affected ($P > 0.15$) by BCAA (0.169%), or BCVFA (0.206%). There was a linear increase ($P < 0.05$) in enrichment for BCVFA substitution over BCAA (Q was $P > 0.15$). There was a BCAA/BCVFA × feed interaction ($P < 0.02$) for *anteiso* 14:0, 15:0, *anteiso* 17:0, and total odd chain FA (OCFA) enrichment. The bacterial FA profile was similar among treatments, but the enrichment of the OCFA and several BCFA was greatly increased by dosing BCVFA over dosing BCAA, supporting a potential benefit from higher concentration of elongation primers regardless of substrate. Results of the second objective showed that flows of bacterial N, nonammonia-nonbacterial N, NDF degraded, and total daily net production of VFA were not affected by BCVFA ($P > 0.15$). The net production of BCVFA was numerically lower when BCVFA was dosed. High pH increased ($P < 0.05$) isobutyrate and isovalerate production but decreased ($P < 0.05$) 2-methylbutyrate and valerate net production suggesting increased use by microbes. 2-methylbutyrate appears to play a more critical role among the BCVFA. Pertaining to the last objective we found that Milk yield had a positive response to isovalerate (ival, % of VFA) and isobutyrate (ibut, % of VFA) in the rumen but negative to pH and NH_3. Rumen variables including ival and ibut did not significantly explain variability in milk protein and milk fat. Dietary Ile content had a positive relationship at a decreasing rate, suggesting an important role of Ile in milk production**.**

PA: The Penn State group continued to investigate into dietary strategies that would mitigate enteric methane (CH_4) emissions from dairy cows. They examine the effects of feeding 3-nitrooxypropanol (3NOP), and macroalgae *Asparagopsis taxiformis* (AT) on enteric CH_4 emissions, dry matter intake, and lactational performance of dairy cows. They also examined the effects of enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* (ENZ) on milk production, blood metabolites, and in situ neutral-detergent fiber degradability in dairy cows. 3NOP decreased enteric CH_4 daily emission, yield, and intensity without affecting DMI and milk yield, but increased milk fat. The methane mitigation effect of 3-NOP is highest immediately after feeding and lowest before feeding. In the short-term *in vivo* experiment, inclusion of AT at 0.50% of DMI decreased CH4 emission in lactating dairy cows by 80% and had no effect on DMI or MY. Dietary supplementation of ENZ increased DMI and milk and milk protein yields in dairy cows, and ruminal rate and effective degradability NFD in an *in situ* experiment. Moreover, they studies effects of rumen-protected capsicum (RPC) and an artificial sweetener on productivity and fat mobilization in early lactation, and effects of graded levels of histidine on lactational performance and plasma amino acid concentrations, and compared two sampling techniques (rumen-cannulated cows vs. oral stomach tube) for evaluating ruminal fermentation in dairy cows. Dietary supplementation of RPC, but not artificial sweetener, appeared to increase milk production and feed efficiency in dairy cows following feed restriction to induce sub-clinical ketosis and had no effect on fat mobilization. Supplementation of digestible histidine at 80 g/d or 2.95% of MP optimally increased milk yield. Plasma concentration of His increased quadratically ($P < 0.001$) by His supplementation, as did Lys ($P < 0.01$) and the branched-chain AA ($P < 0.02$). In terms of rumen sampling techniques, rumen fluid samples collected through an oral stomach tube were found to be not representative of protozoa counts and VFA concentrations or molar proportions as measured in samples of ruminal fluid collected through the rumen cannula.

VA: We have completed trials to assess the potential of adding ruminal infusions of ^{15}N-ammonium sulfate to label microbes to our protocol for deriving amino acid bio-availability values from individual ingredients in a mixed diet so that a proportion of the total entry flux can be assigned to microbial protein thus yielding microbial and feed protein entry rates. To assess this concept, we first verified the length of infusion required to achieve steady state enrichment of ^{15}N arriving in blood. Having established the time required achieving steady state labeling, we conducted a 2nd trial using a 2 x 2 factorial design. The factors were sufficient and deficient ruminally degraded protein (RDP), and high and low ruminally degraded starch. Both factors elicited a change in milk protein output, however, urinary purine derivative output was only reduced in response to deficient RDP ($P<.07$). Absorption of Lys, Phe, and Val from RUP were significantly greater for the low RDP (high RUP) diet, and absorption of Lys and Phe from microbial protein was significantly reduced for the low RDP diet with a trend for reductions in Ile. Leu, and Val. The SEM for the assessments of absorption from microbial protein were similar to those for assessments from RUP ranging from 8 (Met) to 20 (Leu) g/d with mean absorption estimates ranging from 24 (Met) to 129 (Lys) g/d.

OBJECTIVE 2: *To identify and quantify molecular, cellular, and organismal signals that regulate intake, partitioning and efficient utilization of nutrients.*

CA: Acetate is an important VFA formed during fermentation in the rumen. The biochemical pathways for forming it have been studied for over 80 years. Here we report a pathway that was previously unknown in bacteria. We performed experiments with propionibacteria from cheese and skin, as many of these bacteria are missing known pathways. With enzymatic assays, we found many propionibacteria formed acetate using a pathway involving two enzymes. The first enzyme, succinyl-CoA:acetate CoA-transferase (SCACT), forms acetate from acetyl-CoA. The second enzyme, succinyl-CoA synthetase (SCS), synthesizes ATP. This pathway is common in eukaryotes, but it has not been found in bacteria or other organisms. The next step is to investigate if this pathway is used by rumen bacteria. Genome sequences show that several rumen bacteria encode the pathway. This includes rumen propionibacteria that are close relatives of the bacteria studied here. In a separate experiment, mitochondrial enzyme activities were quantified periodically to find markers that identify future high producing cows with longevity in the herd. Additionally, effects of antibiotics at dry-off on mitchondrial function of high- and low-SC cows at dry off, 7 d after and 60 days into next lactation were examined.

IA: At Iowa State, we conducted two in-vitro experiments using primary bovine mammary epithelial cells to examine some potential effects of branched chain amino acid (BCAA) availability on some rate limiting steps of milk fat and lactose synthesis. In one experiment, they examined the impact of extracellular BCAA deficiency on the abundance glucose transporter 1 (GLUT1). The results showed that BCAA deficiency (0.40 vs. 0.04 mM) was associated with increased abundance of GLUT1 in the cell membrane. These findings are in line with some literature data demonstrating a negative relationship between lactose yield and BCAA deficiency. In another experiment, they conducted a proteomics analysis to examine the impact of BCAA deficiency on the abundance of proteins regulating milk component synthesis in bovine mammary epithelial cells. They detected 35000 proteins, only 21 of which responded significantly to BCAA deficiency. Of those 21 proteins, nine proteins were found to be involved in synthesis of fatty acids and milk fat synthesis. The abundance of those proteins decreased more than two folds for BCAA deficiency. Further, they verified those proteomics data using Western immunoblotting analysis and immuno-cytochemistry analysis.

IN: At Purdue university, Madin-Darby bovine kidney epithelial cells (MDBK) were cultured as described previously (White et al., 2012) and selected to contain doxycycline (DOX) inducible shRNA for pyruvate carboxylase (PC) or a non-targeting DNA sequence (control, CON). Following induction of shRNA, PC diminished (PCd) cells and control cells (PCn) were incubated with 1.0 mM U-[^{14}C] lactate or 2-[^{14}C] propionate for 3 h. Total ^{14}CO2 was collected by addition of 0.2 mL of phenethylamine to the hanging center well trap (Donkin and Armentano, 1994). The rate of radiolabeled precursor to product was determined for PC diminished and Pc normal cells. Parallel incubations were conducted with 1.0 mM [^{13}C3]pyruvate to the media and cell homogenates removed after 3 h to determine TCA cycle fluxes through PC and pyruvate dehydrogenase (PDH), as well as flux to gluconeogenesis.

In another experiment, RNA was prepared from milk samples collected from six early lactation dairy cows and used for gene expression profiling analysis on the Illumina platform. Gene signatures were derived and then subjected to Ingenuity Pathway Analysis (IPA) to identify conserved pathways. One hundred thousand reads were extracted from each sample. Magic-Blast was used to align the reads to a nucleotide database and those that had 500 reads or greater were aligned to a bacterial database to remove reads from bacterial contamination. Noncoding regions within the database were identified by downloading bovine noncoding RNA regions from ENSEMBL and NCBI. Noncoding regions that did not match noncoding regions from ENSEMBL and NCBI were used in further analysis. FAST-X Toolkit was used to ensure each gene consisted of at least 50 base pair read length and 30 base quality score. STAR aligned the reads to the bovine reference genome. Raw read counts were generated by HT-seq. Gene transcript reads for 12,730 gene transcripts with a treatment mean of a least 5 reads were used for analysis. The Database for Annotation, Visualization, and Integrated Discovery (DAVID; version 6.8) can only have an input file with 3,000 genes at one time, therefore, the data had to be organized into categories with different read cut-offs in order to be uploaded into DAVID. These cut-offs were selected by the range of the reads and by the potential for more than 3,000 genes to be in a category. The data were organized into 8 different categories with the frequency of genes per category of >50,000, 50,000 to 1,000, 1,000 to 500, 500 to 200, 200 to 100, 100 to 50, 50 to 10, and 10 to 5. Genes in each category were uploaded into DAVID. Then, functional annotation clustering was selected to organize each uploaded category. The output of the functional annotation clustering sorted each category by a generalized "keyword" category, gene ontology (GO) terms (biochemical processes, cellular components, molecular function), or an Interpro output. An enrichment score was also a part of the output. A cluster with an enrichment score of 2.0 or higher was used. A higher enrichment score indicates greater similarity of the genes to the denoted GO term, keyword, or Interpro classification. Ingenuity Pathway Analysis (IPA) (Qiagen, Hilden, Germany) was to cluster the expressed genes into canonical pathways for protein synthesis, lactose synthesis, fat synthesis and lactogenic cellular signaling. The data indicate the most highly expressed genes for each pathway. Identification of the most highly expressed genes for each pathway will be useful in the identification of impact of changes in nutrition and management to alter milk composition at the level of mammary gene expression.

MN: The Minnesota group studied calcium metabolism in lactating dairy cows by examining the effects of oral calcium supplementation bolus on calcium dynamics (e.g., blood calcium concentration) and evaluating a new product compared to a successful commercial product in producing a negative dietary cation-anion difference (DCAD). Further, they continued in studying hyperketonemia (HYK) in early lactation by evaluating associations between HYK occurring in early lactation with milk yield and reproductive performance in Holstein dairy cows throughout an entire lactation. The findings indicate that HYK does not affect all cows in a similar fashion and that elevated BHB in the first 10 d postpartum is not necessarily detrimental to all cows. In another study, they investigated into regulation and integration of hepatic function with mammary and adipose metabolism during the periparturient period using genome-wide single nucleotide polymorphism marker analysis of contemporary Holstein cows (CH) and Holstein cows not selected for milk yield since 1964 (UH). UH cows had a consistently greater abundance of phospholipids containing polyunsaturated fatty acid than CH cows during parturition and lactation. In contrast, CH cows had a greater abundance of hepatic triglycerides containing saturated fatty acids and monounsaturated fatty acids, especially in the early phase of lactation. In two other studies, the group examined genome changes due to artificial selection and effects of bovine genotype on immune-endocrine-metabolite interactions in dairy cows. The results support the hypotheses that selection for increased milk yield has also increased the presence of genes associated with negative effects on fertility and immunity. Finally, they worked on developing a software resource for quantitative proteo-transcriptomic data analysis that would help in understanding potential posttranscriptional regulation and provide researchers with important new insights into underlying biological and pathological disease mechanisms.

MS: We evaluated diurnal and longitudinal variations of the rumen and fecal bacterial community composition in Holstein and Jersey cows fed the same diet. The results showed that bacterial community composition in both rumen and fecal samples differed between Holstein and Jersey cows. Collection day (0, 14, and 28) influenced overall bacterial community composition in both rumen and fecal samples, whereas collection time (0, 6, 12, 18, and 24 h) did not

PA: Penn State group studied the effects of protein and fatty acid absorption on the daily rhythms of milk synthesis and plasma hormones and metabolites, effects of oleic soybean and sodium acetate and bicarbonate on milk fat yield in dairy cows. The time of protein infusion influenced daily rhythms of milk and milk protein synthesis. Night infusion abolished rhythms of protein concentration and induced rhythms of milk yield. Day infusion increased the amplitude of protein concentration. Fatty acid infusion during the daytime modified the daily rhythms of milk synthesis by increasing the amplitude of milk yield and decreasing the amplitude of fat and protein concentration, whereas infusion at night had little effect. High oleic soybeans increased milk fat through decreased biohydrogenation-induced milk fat depression. Additionally, increasing soybeans increased milk fat through providing preformed fatty acids. Sodium acetate and sodium bicarbonate both increased milk fat production, but sodium acetate achieved this through increased mammary gland *de novo* lipogenesis. Furthermore, the relationships between rumination time, milk fat production, and milk fatty acid profile were determined using real-time rumination data. As per the results, rumination time was not related directly to milk fat but was associated with differences in *trans* and odd and branched-chain FA that also change during SARA or milk fat depression, which may impact milk fat and other production variables.

TN: Two projects were conducted to assess the effect of an additive from Aspergillus oryzae (AO) on milk production, body temperature, and inflammation responses in dairy cows exposed to heat stress, and to compare the effect of two levels of rumen-protected Met (RPM) prototype with a commercial product in dairy cows. During heats stress, AO increased ($P < 0.01$) yields of ECM, milk protein, and fat by 3.8, 0.08, and 0.16 kg/d (CTL = 36.4, 0.98, and 1.37 kg/d). Furthermore, AO linearly decreased SCC ($P = 0.02$) and morning vaginal temperature ($P < 0.01$) but did not affect DMI. Moreover, AO decreased ($P = 0.01$) serum amyloid A concentrations in plasma by 65.6% and tended to decrease ($P \leq 0.10$) haptoglobin and LPS binding protein concentrations by 35.4 and 23.3%, respectively. From the ex vivo LPS challenge, AO linearly decreased ($P = 0.02$) the IL-6 expression ratio (LPS to no LPS stimulation) by 65.6%. In summary, AO postbiotic increased milk yield parameters and reduced vaginal temperature and decreased markers of inflammation and cytokine production in cows exposed to heat stress.

In the second experiment, Cows were randomly assigned to the following treatments: 1) control (CTL; 0 g of RPM), 2) 6.0 g of MP-Met from Smartamine M (SM6), 3) 12.0 g of MP-Met from Smartamine M (SM12), 4) 6.0 g of MP-Met from RPM prototype (PT6), and 5) 12.0 g of MP-Met from RPM prototype (PT12). The basal diet was formulated for -9.5 g/d in MP-Met balance (2.02% of MP-Met supply), 16.0% CP, and 52% forage (corn and ryegrass silages) to 48% concentrate (corn grain and soybean meal). Treatments were top-dressed twice daily. Milk and blood samples were collected and analyzed. The RPM treatments increased ($P = 0.01$) milk protein percent compared with the CTL treatment. Relative to SM12, the SM6, PT6, and PT12 treatments did not differ in milk protein percent (3.19, 3.17, and 3.17 vs. 3.27%). Relative to CTL, the SM6, PT6, and PT12 treatments numerically improved milk protein percent. The SM6, SM12, and PT6 treatments showed no differences in milk fat percent. Relative to PT12, the SM12 treatment increased (P = 0.03) milk fat percent (4.70 vs. 4.42 ± 0.10%). Relative to SM12, the PT12 treatment tended to increase (P = 0.08) milk lactose yield (1.83 vs. 1.78 ± 0.03 kg/d). Relative to CTL, the RPM treatments increased (P = 0.01) Met concentration in coccygeal vessels and the abdominal mammary vein. Relative to RPM treatments, the CTL treatment increased (P < 0.01) mammary clearance rate of Met. In summary, the RPM treatments improved milk protein percent. The prototype at both levels sustained milk protein percent at the same level as SM6 and SM12. The RPM treatments increased plasma concentration and mammary capture of Met, which may have contributed to greater milk protein percent.

VA: A portion of the postabsorptive efficiency of use of amino acids is determined by the affinity of mammary tissue for amino acids. Greater affinity results in greater proportional extraction of amino acids from blood and reduced amino acid catabolism. Some of the essential amino acids are transported into the cells in exchange for a non-essential amino acid whose uptake was driven by ATP use. It was hypothesized that varying the non-essential amino acid supply and composition may result in variable essential amino acid affinity and transport. Alterations in the rate of transport of essential amino acids will often result in altered rates of milk protein synthesis. Depending on the sensitivity of essential amino acid transport, it is possible that a shortage of non-essential amino acids could negatively impact essential amino acid transport and milk protein synthesis. A trial was conducted to assess the impact of varying concentrations of non-essential amino acids on the rate of transport of the essential amino acids. Sample analyses is complete and modeling work to interpret the isotope data is ongoing.

WI: Roles of hepatic lipases during the onset and recovery of fatty liver during the transition to lactation period in dairy cows were studied. We previously completed a pilot study where we induced fatty liver by a combination of overfeeding energy prepartum and feed restricting postpartum (fatty liver induced, FLI, n=8) and compared liver lipases with cows that were not under an induction protocol (control, n=3). The study yielded compelling results but needed to be repeated in a larger block of cows. During 2018, the study was repeated (n= 12 control, 12 FLI). Production and health responses as well as blood and liver samples are currently being analyzed. In another study, SNP that are associated with hyperketonemia, aka. Sub-clinical ketosis, in postpartum Holstein cows were determined. Four blood samples were collected between 3 and 18 DIM postpartum to have a good indication of if a cow had development sub clinical ketosis or not.

The role of mTORC1 on AA regulation of milk production in C57BL/6J mice was determined. Lactating mice were fed a breeder diet (HP, 18% CP from casein, n=4), or a protein reduced diet (LP, 9% CP from casein, n=5) from parturition until lactation day (LD) 13. From LD 3, a group of HP fed mice were intraperitoneally injected with the mTORC1 inhibitor rapamycin (4 mg/kg every other day), while all other mice were injected with vehicle on the same schedule. Dietary protein restriction reduced final pup weight by 30% and milk production by 63% (P< 0.05). Additionally, protein restriction reduced mammary mTORC1 substrates (S6K1, T389 and 4E-BP1 S65) phosphorylation (*P*< 0.05). Treatment with rapamycin decreased pup weight to similar degree than LP (27%, *P*< 0.05) and milk production by 37% (*P*< 0.05). Our findings suggest that mammary mTORC1 plays a major role on AA regulation of milk production.

Moreover, the role of lactogenic hormones on mTORC1 signaling in mammary epithelial cells was analyzed. Primary bovine mammary epithelial cells were treated with prolactin, hydrocortisone, insulin, or the triple lactogenic hormone. Then, we tested the role of insulin and lactogenic hormones on mTORC1 stimulation by AA. Intracellular proteins were isolated and analyzed by Western blotting for total and phosphorylated forms of the mTORC1 substrate and protein synthesis regulator S6 kinase 1 (Thr 389), its downstream substrate ribosomal protein S6 (Ser 240/244), and the mTORC1 substrate and autophagy initiator factor ULK1 (Ser 757). Amino acid dose response parameters (linear and quadratic) were estimated for the phosphorylated to total ratio of each of the above-mentioned proteins. Only the essential AA Leu, Ile, Met, Arg and Thr significantly stimulated mTORC1 activity as determined by phosphorylation of its substrates S6K1 and 4E-BP1. Importantly, total essential AA and TOR AA (i.e. those AA that stimulate mTORC1), individually or as a group, require the presence of insulin to maximize their effect on mTORC1 activity. In the absence of insulin, linear and quadratic parameter estimates were no different than zero (P > 0.05) for any of the groups of AA tested. Our results indicate that EAA have insulin dependent saturable effects on mTORC1 activity within physiological levels, suggesting that disruption of insulin signaling would negatively affect milk protein synthesis.

IMPACTS/ OUTCOMES

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

CA: *Asparagopsis taxiformis* harvested in Australia was confirmed as having methane mitigating impact on in-vitro rumen fermentation. Local seaweeds, *Asparagopsis taxiformis*, harvested offshore Southern California have been identified as potentially potent feed additive for methane mitigation from enteric fermentation. This opens up new opportunities for local seaweed-based aquaculture. *Asparagopisis armata* reduced enteric methane emissions by up to 67%.
A modeling framework is now available to integrate irrigated water used for growing crops and feeding these crops for dairy and other livestock feeding systems. Feeding recommendations were developed for how to feed new feed products and supplements.

DE: A new rumen protected methionine product having high availability was identified as evidenced by increased plasma methionine concentrations.

FL: Canola meal was shown an important protein supplement in the US and abroad. Several important ruminal fermentation parameters were explained.

IA: A number of scientific evidences supporting the importance of offering drinking water to newborn calves was published (an average dairy farmer in US still wait for more than two weeks to first offer drinking water to newborn calves).

IN: The postruminal infusion model was shown to be successful as an evaluation tool to evaluate postruminal delivery of rumen protected amino acid supplements.

KS: Results of the studies provide a cautionary tale to feed merchandisers developing new feed products and relying primarily on nutrient profiling to assess product value. This novel high-protein corn product substantially decreased productivity and would have resulted in dramatic losses for both farms and, eventually, to the source company if it had been taken to the market in this form.

MI: New dry matter intake equations were provided to the NRC committee for updating nutrient requirements for dairy cattle

OH: We showed 2-methylbutyrate appears to support a more critical role among the BCVFA to improve NDF digestibility with higher K_p and higher pH.

PA: The impact of novel feed additives that significantly reduce enteric methane production without affecting feed intake or milk production of dairy cows were determined.
Important aspects of histidine requirements in lactating dairy cows were also discovered.

VA: The addition of a ruminal microbe labelling by ^{15}N to the existing protocol for assessing absorbed AA from RUP provides an estimate of microbial AA absorption with similar errors of measurements as for the RUP estimates.

OBJECTIVE 2*: To identify and quantify molecular, cellular, and organismal signals that regulate intake, partitioning and efficient utilization of nutrients.*

CA: We identified a new biochemical pathway by which bacteria form acetate during fermentation. This work can guide genetic engineering or other efforts to manipulate acetate production in the rumen. We measured mitochondrial enzyme activities in calves and cows to determine metabolic efficiency in response to age, environment, and management (feeding and milking) and trained graduate students on implementing research methods in commercial farm setting.

IA: More evidence supporting the idea that amino acids are not only a nutrient but also powerful cellular signal regulating nutrient metabolism in mammary epithelial cells.

IN: Transcript profiling of mammary gene expression from milkfat RNA provides information on the most highly expressed genes in mammary epithelial cells and identifies target genes to evaluate in response to changes in nutrient supply. Experiments using the shRNA provided information that would improve our understanding about the basic biology of control of gluconeogenesis and TCA cycle activity. This information will help us to determine the signals that enhance metabolic activity and those that impair metabolism in ruminants.

MN: The results of genome-wide SNP marker analysis indicated selection has increased the extent and duration of hepatic lipidomic alterations consistent with the greater milk yield of the CH cow. They developed the QuanTP program to provide an accessible and effective software resource, which can be used to confirm existing and identify new multiomic relationships among quantitative proteo-transcriptomic data sets.

MS: Our findings suggest that a single sample is adequate to characterize the overall composition of the bacterial community in rumen and fecal samples

PA: Roles of amino acids and fatty acids in entraining the molecular clock of the mammary gland were demonstrated.

TN: Graduate students and undergraduate students were involved in these projects and trained in methodology, sample analysis, statistical analysis and presenting their research at ADSA national meeting. We determine the effect of a feed additive heat stress on nutrient-use efficiency and performance in lactating dairy cows. We improved our understanding of the efficacy and the relative effectiveness of rumen-protected amino acids in diets of lactating dairy cows.

VA: Maximal feed efficiency of production animals is generally achieved when animal productivity is maximized thereby diluting maintenance and reproductive costs and dietary nutrients are precisely matched with animal requirements for those nutrients. This also minimizes nutrient excretion thus reducing environmental impact. Additional gains in animal productivity and efficiency are possible if one takes advantage of potential interactions among nutrients and animal management to target nutrients to the desired food product. This strategy relies upon the ability to accurately assess nutrient supplies to the animal.

WI: Understanding the role of energy source on essential (TOR and non-TOR AA) AA regulation of milk components synthesis is critical to increase digested AA efficiency in lactating dairy cows, to reduce production cost and the environmental impact of dairy production systems.

GRANTS

CA

- Hackmann TJ (PI), Daley VE. The NANP Nutrition Models Workshop: Training a new generation of scientists in mathematical modeling. USDA-NIFA Foundational Program Grant 2019-67015-29841. ($40,878)
- Hackmann TJ (PI). Tackling the low efficiency of protein production by rumen microbes: a cellular and whole-animal approach. USDA-NIFA Foundational Program Grant 2018-67015-27495. ($500,000)
- Hackmann TJ (PI). Using fluorescent compounds to unveil substrates used by uncultured gut bacteria USDA-NIFA Foundational Program Grant 2019-67015-29841. ($99,961)
- Title: Investigating the effect of plant halogenates on microbial methanogenesis; Funder: Envision Low Methane
- Title: PAPM EAGER: Engineering a modular artificial gut system for high-throughput analysis of gut microbiome function; Funder: U.S. Department of Agriculture/NSF
- Ruminant digestion in the environment; Funder: Envision Low Methane
- Effect of *A. armata* on dairy cattle; Funder: Elm Innovations and FFAR

FL

- CBET - Track 2 INFEWS – National Science Foundation $2,263,330 (2019-2023). Project: NEWIR Manure: Nutrients, Energy, and Water Innovations for Resource Recovery. Role:

Co-Principal Investigator. Other investigators: Charles Coronella, PI (UNR); Pablo Cornejo, Co-PI (CSU); Sage Hiibel, Co-PI (UNR); and Kimberly Rollins, Co-PI (UNR).

- USDA-NIFA-AFRI: Foundational. - $500,000 (2018 – 2022). Project: Tackling the low efficiency of protein production by rumen microbes: a cellular and whole-animal approach. Role: Co-Principal Investigator. Other investigators: Timothy Hackmann, PI (UF/UC, Davis) and Nicolas DiLorenzo, Co-PI (UF).
- Multiple industry contracts that cannot be disclosed due to signed confidentiality agreements.

KS

- Bradford, B. J. The effect of gluconic acid (calcium gluconate) supplementation in diets for mid-lactation Holstein cows on lactation performance and feed efficiency. Nutreco. $135,774. 3/1/19 – 12/31/19.
- Bradford, B. J. Productivity and nitrogen balance of lactating cows fed a high-protein corn feed product. Ace Ethanol. $104,000; 9/25/18 – 8/31/19.
- Bradford, B. J. Effect of calcium carbonate supplementation on metabolic acid-base status and feed intake of cows with compensated metabolic acidosis. Landus Cooperative. $70,000; 7/1/18 – 12/31/19.
- Bradford, B. J., M. Garcia, J. Daniel, and B. Whitlock. Is orosomucoid a mechanistic link between inflammation and impaired intake during the transition period? USDA-NIFA Agriculture and Food Research Initiative. $483,500; 5/15/17 – 5/14/20.
- Vipham, J. L., D. L. Pendell, B. J. Bradford, T. O'Quinn, D. Min, Z. P. Stewart, A. Tolera, B. Sinote, S. Yigrem, K. Abegaz, Y. Mummed, and A. Mekasha. Linking cattle nutrition to human nutrition: A value chain approach to improving the production, handling, and consumption of animal source foods in Ethiopia. USAID Feed the Future Innovation Lab for Livestock Systems. $1,040,000; 10/1/16 – 9/30/20.
- Bradford, B. J., L. Mendonça, L. Hulbert, and J. McGill. Evaluating impacts of OmniGen-AF on immune/reproductive interactions during the transition to lactation. Phibro Animal Health. $178,978; 7/1/17 – 6/30/19.

IA

- Appuhmy, JADRN (PI). Effects of Glutamine Supplementation in Early-weaned Dairy Heifer Calves. Ajinomoto Animal Nutrition North America Inc. 2019 ($30,429)
- Appuhmy, JADRN (PI). Effects of Sangrovit supplementation on growth, feed conversion efficiency, gut morphology, and residue concentrations in tissues in young calves. Phytobiotics. 2019 ($130,721)

MI

- VandeHaar, M, R Tempelman, K Weigel, H White, J Koltes, H Ramirez-Ramirez, F eñagaricano, C Staples, E Connor, and P Van Raden, Improving dairy feed efficiency, sustainability, and profitability by impacting farmer's breeding and culling decisions. US Foundation for Food and Agriculture Research and Council for Dairy Cattle Breeding. $2,000,000 (Feb, 2019-Feb, 2024).
- VandeHaar, M, L Sordillo, M Weber-Nielsen, F Cullens, M Mangual, and P Sanguesa. Enhancing calf health by boosting the omega-3 fatty acids in colostrum. MSU-Michigan Alliance for Animal Agriculture. Total Funding: $91,726 (Feb 2018-Feb 2020).
- Weber-Nielsen, M, M VandeHaar, M F Cullens, and P Bacigalupo. Effect of feeding

transition milk on growth and health of dairy calves. MSU-Michigan Alliance for Animal Agriculture. Total Funding: $61,967 (Feb 2018-Feb 2020).

- VandeHaar, M. (100%). Testing of floury gene mutation of BMR corn silage. Mycogen Seeds. $117,820. Aug, 2017 - Aug, 2020).
- Vandehaar, M. (100%), "Increasing the profitability and efficiency of protein use of lactating dairy cows," MSU-Michigan Alliance for Animal Agriculture. Total Funding Requested: $124,069 (Feb 2017 - Feb 2019).
- Vandehaar, M. (100%), "Feed intake prediction system to identify inefficient cows on commercial farms," MSU-Michigan Alliance for Animal Agriculture. Total Funding Requested: $136,984 (Feb 2017 - Feb 2019).

PA

- Effect of High Protein Distillers Grains on Intake and Milk Production in Dairy Cows. 6/20/19 to 6/30/2020. Ace Ethanol. P.I. K.J. Harvatine
- Effect of acetate supply on milk fat synthesis.
  2019-2023. USDA AFRI Lactation and Nutrient Utilization. $500,000 total. P.I. K.J. Harvatine
- Ability of whole cottonseed to increase milk fat yield. 2019-2020. Cotton Inc. P.I. K.J. Harvatine
- Ability of high oleic soybeans to increase milk fat yield. 2019-2020. PA Soybean Board. P.I. K.J. Harvatine

TN

- Effect of a prebiotic additive on heat-stressed lactating dairy cows. Funded by BioZyme Inc.
- Impact of rumen protected methionine on methionine availably and dairy cow performance. Funded by Balchem Corporation.

VA

- Assessing intestinal absorption of amino acids from individual feed ingredients and microbes.
  M. D. Hanigan. 2017-2020. Balchem Corp. $221,524.
- A role for valerate, isovalerate, and isobutryate in rumen fermentation kinetics.
  R. R. White, M. D. Hanigan, and J. L. Firkins. 2017-2019. Pratt Foundation. $144,688.
- Integration of livestock feeding strategies into a nutrient loading, watershed model. M. D. Hanigan, R. R. White, Z. Easton, and D. Bosch. 2017-2019. Pratt Foundation. $198,670.
- A field application model for lactation responses to amino acids. M. D. Hanigan, R. R. White, and G. Ferreira. 2017-2019. USDA AFRI. $480,000.

PUBLICATIONS (peer-reviewed articles only)

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2. Wickramasinghe, J, A. J. Kramer, and J. A. D. R. N. Appuhamy. 2019. Drinking water intake of newborn dairy calves and its effects on feed intake, growth performance, health status, and nutrient digestibility. J. Dairy Sci. 102: 377-387.
3. Ríus, A.G.. 2019. Invited Review: Adaptations of protein and amino acid metabolism to heat stress on dairy cows and other livestock species. Applied Animal Science, 35(1), pp.154-161.
4. Bailey, H.R., Kaufman, J.D., Estes, K.A., Zimmerman, C.A., Barton, B.A. and Ríus, A.G., 2019. Rumen-protected lysine supplementation increased milk production in dairy cows fed a lysine-deficient diet. Applied Animal Science, 35(5), pp.482-490.
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