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, 2015 to October 31, 2016
Annual Meeting Dates: October 24 – 25, 2016
Date of This Report: December 2016

Present participants

Name	Institution	E-mail	Notes
Rius, Agustin	University of Tennessee	arius@utk.edu	Outgoing Chair
Faciola, Antonio	University of Nevada	afaciola@cabnr.unr.edu	New member
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Benfield, David	The Ohio State University	benfield.2@osu.edu)	Administrative Assistant
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Ramirez, Hugo	Iowa State University	hramirez@iastate.edu	Incoming Chair, 2017
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Absent participants, submitted report

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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).

A. **Summary of Minutes of Annual Meeting**

Monday, October 24:

- Business meeting: Administrative Advisors

  - Dr. David Benfield, discussed the following items:
    Committee continues to demonstrate work
    Termination report is due in 2017
    Application for renewal is needed during 2017 for the official renewal date Sept. 2018
    Documentation of impact is very important for reports
    The committee should consider nomination for Experiment Station Section Award for Excellence in Multistate Research

  - Dr. Steve Smith, USDA-NIFA (via conference call), provided the following information:
    Budget:
    Hatch Capacity Funding maintained at $243.7 million for FY 2017
    Competitive programs:
    Anticipated release dates for RFA's are December 2016 through April 2017
    Consider these areas for funding opportunities for animal science research, extension and/or education
    Organic agriculture
    Beginning farmers and ranchers
    Engineering products and processing (AFRI)
    Small and medium sized farms (AFRI; Ag Economics and Rural Communities)
    Research in Biomedicine and Agriculture (dual purpose/dual benefit using ag domestic animal species as models).

- Business meeting: discussion for 2017 meeting
  The committee discussed inviting National Program Leader Steve Smith to attend our meeting. The committee can meet in Washington DC as first option if Steve Smith accepts invitation; otherwise the meeting is back in Chicago.

- Station reports presented

- Tour of Purina Animal Nutrition Center
  Drs. Jill Davidson and Katie Bradley gave a tour of the facilities including guided visits of:
  - Dairy farm
  - Heifer unit
  - Calf answer center (observation room)
  - Calf hutches
  - Large animal metabolism unit
  - Beef feedlot unit (observation/education room)

Tuesday, October 25

- Election of new officers for NC2040 2016-7
  Secretary: TBD
  Chair: Hugo Ramirez (Iowa State University)
  Next year's meeting
  Time: Oct 23 and 24
  Venue: TBD, Washington, DC or Chicago, IL

- Station reports presented

- Meeting adjourned at noon

B. Summary of Station Reports

Kansas State University

Station investigator: Barry Bradford

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

Project title: Effects of Cobalt Source on Rate and Extent of Dry Matter and Fiber Degradation In Vitro

Investigators: C. F. Vargas-Rodriguez*, A. J. Carpenter*, J. DeFrain†, and **B. J. Bradford***
*Department of Animal Sciences and Industry, Kansas State University, Manhattan
†ZINPRO Corporation, Eden Prairie, MN

Progress of work

Ruminal fluid was collected from heifers fed a high-forage diet with < 0.1 ppm supplemental Co and fermentation substrate contained no measurable Co. Different inclusion levels (0.0, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, and 15.0 ppm Co) of Co glucoheptonate (CoGH) and Co carbonate (CoCarb) were tested in vitro during Experiment 1. Gas production was recorded every 15 min and, after 24 h, pH was measured and contents of each flask were used to determine DMD and NDFD. Experiment 2 evaluated the effects of Co (CoGH and CoCarb at 0, 0.33, 1, 3, and 9 ppm Co) on gas production, VFA concentration and NH3 concentrations. In both studies, each treatment combination had 4 replicates and samples were incubated for 24 h. Asymptotic gas production curves were modeled with the NLIN procedure of SAS using the Gauss-Newton fit method. Gas production kinetic values and all other data were modeled to assess the effects of Co concentration, source, and their interaction. Regardless of source, Co significantly decreased ($P < 0.05$) the rate of gas production in Experiment 1. Gas production tended to decrease ($P < 0.10$) more rapidly with increasing CoGH relative to similar levels of CoCarb. Dry matter disappearance was greater for CoGH compared to CoCarb across levels of Co tested. Neutral detergent fiber disappearance was increased by 21% for CoGH at concentrations between 0.1 and 1.0 ppm but a pronounced drop in NDFD occurred at 15 ppm Co for this treatment. The effects of Co source on gas production kinetics and pH change were inconsistent between experiments 1 and 2, largely because of dramatic effects at 15 ppm. Relative to no Co supplementation, concentrations of branched chain VFA decreased ($P < 0.05$) with Co supplementation. CoCarb dose-dependently and linearly decreased ($P < 0.05$) the amount of unsaturated fatty acids and also linearly increased ($P < 0.05$) saturated fatty acids after incubation, presumably reflecting an increased extent of biohydrogenation. In the case of CoGH, the effect of concentration was minimal, resulting in substantial differences in FA profiles between the Co source at concentrations of 3 and 9 ppm. In summary, CoGH increased DMD and NDFD at 1 ppm Co or less relative to CoCarb but decreased NDFD at 15 ppm Co. Furthermore, CoGH had limited effects on the biohydrogenation of LCFA, whereas CoCarb $\geq$ 3.0 ppm appeared to stimulate this process.

University of Maryland

Station Investigator: Rich Erdman

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

Project title: Potential Interactions of Dietary Cation-Anion Difference and Monensin with Respect to Feed Efficiency in Lactating Dairy Cattle (Weidman MS Thesis)

Investigators: Rich Erdman, Tracy Catterton, Marie Iwaniuk, Sarah Potts, and Amy Weidman

Progress of work

Previous work at Maryland (Catterton and Erdman, 2016) demonstrated that rumen strong ion concentrations could be altered by dietary ion content. Additions of Na and K and to a lesser extent, Cl result in corresponding increases in their respective rumen ion concentrations. Rumen K and Na concentrations are inversely related and addition of dietary K results in reduced rumen Na concentration (Catterton and Erdman, 2016). Monensin preferentially binds sodium (Russell and Houlihan, 2003) and monensin sensitivity has been previously shown to be enhanced (Newbold et al., 2013) in the presence of higher Na concentrations in fermentation media. We reasoned that manipulation of the diet cation sources and DCAD could mediate production and feed efficiency responses to monensin supplementation. The objective of this experiment was to determine if there was an interaction between monensin supplementation, DCAD concentration and DCAD cation source on milk production, feed efficiency, and rumen fermentation in lactating dairy cows.

Thirty five early-to-mid lactation Holstein cows (12 primiparous and 33 multiparous) including 6 multiparous rumen fistulated cows) were used in the 11 wk study. Cows were individually fed a basal diet containing 66% forage and 34% concentrate (DM basis) in two replications over time. Treatments consisted of two concentrations of monensin (0 or ~300 mg/d) that were fed continuously for 9 wk after a 2 wk preliminary period that was used as a covariate in the analysis of covariance. Within each monensin treatment cows were fed 0, 200 mEq/kg added DCAD using potassium carbonate, or 200 mEq/kg added DCAD using sodium sesquicarbonate in a 3 x 3 Latin square design with 3 wk experimental periods. The 6 treatment combinations consisted of: 1) Control (**C0**), un-supplemented basal diet; 2) Control plus K (**200K**), + 200 mEq/kg DCAD using potassium carbonate sesquihydrate; 3) Control plus Na (**200Na**), + 200 mEq/kg DCAD using sodium sesquicarbonate; 4) Monensin (**M0**), 13.2 mg/kg added monensin; 5) Monensin plus K (**M200K);** 1.32 mg/kg added monensin + 200 mEq/kg DCAD using potassium carbonate sesquihydrate; and 6) Monensin plus Na (**M200Na**); 1.32 mg/kg added monensin + 200 mEq/kg DCAD using sodium sesquicarbonate. Measured diet Na, K, Cl, and S composition corresponded closely with the expected changes due to treatment. Monensin and DCAD treatments had no effect on feed intake, milk production and composition, and feed efficiency (Table 3). The lack of production and intake responses may have been due to the relatively short (3 wk) experimental periods used in the experiment. In the rumen fistulated cows, rumen pH was increased by DCAD concentration but was not affected by strong ion source (Table 4). There was a monensin by DCAD interaction where monensin treated cows showed higher rumen pH at 3 to 9 hours post-feeding. Rumen concentrations of K and Na were respectively increased with K and Na supplementation. Monensin, DCAD concentration, and strong ion source had no effect of rumen VFA and strong ion concentrations. The results of this study did not demonstrate any significant interactions between DCAD concentration and cation source and monensin that would suggest that production and rumen function responses to monensin were altered by DCAD concentration or source.

Michigan State University

Station Investigator: Mike VandeHaar

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

Project title: Metabolic Relationships in Supply of Nutrients for Lactating Dairy Cows

Investigators: **Mike VandeHaar**, Jim Liesman, Rob Tempelman, Mike Allen, Adam Lock, Rodrigo Souza, Enhong Liu, Martin Mangual, Yongfang Lu

Progress of work

Project 1: Estimating intake and diet effects on digestibility of nutrients. Data reported on this last year. The abstract was presented at the 2016 American Dairy Science Association annual meeting and is currently in final preparation. Digestibility of fiber was decreased by adding starch and increasing intake. The formulas are similar to that shown in last year's report.

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 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

All projects listed are part of USDA AFRI-NIFA Competitive Grant no. 2011-68004-30340 (Genomic and farm decision tools to enhance feed efficiency). Coinvestigators include R. Tempelman, D.K. Beede (Michigan State); L. E. Armentano, K. Weigel, V. Cabrera (Wisconsin); D. M. Spurlock (Iowa State), Mark Hanigan (Virginia Tech); C. Staples (Florida); and R. Veerkamp (WUR, Netherlands)..

Project 2: Determine the genetic architecture of feed efficiency in lactating dairy cattle and build a foundation for genomic selection of more efficient animals.
During the past year, analysis on the genetic architecture of feed efficiency continued. A genomic analysis of the first 4000 cows in our database estimated the variation in residual feed intake due to genotype to be 0.15, slightly below the pedigree-based heritability of 0.17. Candidate genes for the RFI trait that are independent of the related traits of milk production, body weight, and feed intake were identified. However, none of these were of remarkable effect, indicating that feed efficiency is under the control of many genes in coordination.

Project 3: Facilitate genomic selection tools. In our original timeline, activity for Aim 3 was scheduled for years 4 and 5. We used RFI along with feed saved for maintenance from smaller BW to develop a Feed Saved index. Genomic Breeding Values for Feed Saved on 16,000 Holstein AI bulls in North America were predicted from a reference population of 3,500 cows using 57,000 SNP markers per animal. The range in Feed_Saved from top to bottom bulls was 2000 kg per lactation (10% above and below the mean for DMI). The reliability of the EBV for RFI was 0.3. The data looks promising but more data are needed to improve the reliability. In the past year, meetings were held with members of the USDA Animal Genomics and Improvement Laboratory and the Council for Dairy Cattle Breeding on how to build upon our foundational database into the future.

Project 4: Development of decision-support tools. Three model evaluations were either published or are in review for publication, and further evaluations are on-going using data from published literature sources and from this project.

Utah State University

Station Investigator: Jong-Su Eun

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

Project Title: Effects of supplementing rumen-protected methionine on lactational performance of Holstein dairy cows during early and mid-lactation.

Investigators: M. A. Fagundes, S. A. Blaser, S. Y. Yang, **J.-S. Eun**, and J. O. Moon.

Progress of work

Supplementing rumen-protected methionine (RPMet) has been shown to maintain milk and milk protein yields when dietary metabolizable protein is decreased by 5% due to its direct impacts on milk protein synthesis in the mammary gland. The present study investigated production responses of lactating dairy cows to RPMet supplementation in sub-optimal protein [SOPD, 15.5% crude protein (CP)] and normal protein diet (NPD, 16.5% CP). Eight lactating dairy cows (53 days-in-milk on average) were blocked by parity and days-in-milk, and the experiment was performed in a duplicate 4 × 4 Latin square design. Within each square, cows were randomly assigned to a sequence of 4 diets during each of the four 21-d periods (14 d of treatment adaptation and 7 d of data collection and sampling). A 2 × 2 factorial arrangement was used; SOPD or NPD was combined without or with RPMet: SOPD without RPMet, SOPD with RPMet (S+Met), NPD without RPMet, and NPD with RPMet (N+Met). An experimental RPMet product from CJ CheilJedang (Suwon, South Korea) were supplemented in the S+Met and the N+Met at 30 g/cow/d. Supplementation of RPMet did not affect dry matter intake (DMI; 25.4 kg/d) and milk yield (40.6 kg/d). Supplementing RPMet resulted in a similar milk true protein concentration (2.80%) with a numerical increase in milk protein yield at 3.6%. In contrast, supplementing RPMet increased milk fat concentration ($P = 0.02$) and yield ($P = 0.03$), 3.5% fat-corrected milk (FCM) yield ($P = 0.05$), and tended to increase energy-corrected milk (ECM) yield ($P = 0.06$) regardless of CP level. In addition, trends were observed for increased 3.5% FCM yield/DMI ($P = 0.09$) and ECM yield/DMI ($P = 0.10$), and the positive effects were greater under NPD than SOPD, resulting in trends toward interaction between CP and RPMet ($P = 0.06$). Overall results in the current study suggest that supplementing RPMet in SOPD and NPD improved milk fat concentration possible due to increases in apolipoprotein and phospholipid syntheses in the liver, leading to an increase in fatty acid supply to the mammary gland via very low density lipoproteins.

Project Title: Effects of supplementing rumen-protected methionine on lactational performance of Holstein dairy cows during early and mid-lactation.

Investigators: T. G. Grisenti, S. Y. Yang, **J.-S. Eun**, S. Sharp, C. L. McCary, J. O. Hall.

Progress of work

Effects of supplementing methionine derivative at varying doses on lactational performance of Holstein dairy cows during mid to late lactation.

The current experiment was conducted to test a hypothesis that lactating dairy cows fed with N-acetyl-L-methionine (NALM), a rumen-protected methionine derivative, would increase milk production by increasing N and energy utilization efficiencies. Eight multiparous Holstein cows that were mid lactation (124 ± 13 days-in-milk), with similar milk production were used in a 4 × 4 Latin square design for 84 d. Four dietary treatments included 0 g (control), 15 g, 30 g, and 45 g/d/cow of NALM supplementation.

Supplementing NALM sizably increased dry matter intake (linear effect; $P < 0.01$), while milk yield tended to increase quadratically ($P = 0.07$). A linear decrease in milk fat concentration was seen due to NALM treatments relative to the control ($P = 0.02$). However, milk fat yield was similar across treatments. A trend toward an increase in milk protein yield was observed between the control and the 45 g NALM (1.18 vs. 1.21 kg/d; $P = 0.10$). There were no differences in energy-corrected or 3.5% fat-corrected milk yields in response to treatments. It is likely that the supplementation of NALM to mid to late lactating dairy cows may have shifted nutrient and energy utilization toward tissue gain rather than lactation, which resulted in a decrease in feed efficiency for lactation ($P = 0.02$). Overall results from the present study suggest that supplementing NALM to lactating dairy cows in mid to late lactation would have minor impact on lactational performance, but it would be beneficial to rapidly growing beef cattle.

University of California Davis
Station Investigators: Heidi A. Rossow, Ermias Kebreab, Matthias Hess, and James Fadel

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

Project Title: Glucose precusor supplementation in Holstein and Jersey cows as a preventative treatment for ketosis in the transition period

Investigators: K. A. Mitchell, **H. A. Rossow**

Progress of work

Glucogenic substances can treat subclinical or clinical ketosis by lowering β-hydroxybutrate (BHBA) levels and raising glucose (Glu) levels. Subclinical ketosis is defined as BHBA ≥1.0 mmol/L and Glu <60 mg/dl and clinical ketosis is defined as BHBA >1.2mmol/L and Glu <60 mg/dl. The objectives of this study are to determine if supplementation with a glucose precursor powdered product (GP; Glucose Booster, Stuhr Enterprises, LLC.; GP) during transition would decrease subclinical or clinical ketosis and have an effect on health and milk production of multiparous Jersey and Holstein dairy cows. Holstein (n=106) and Jersey (n=105) cows at a commercial dairy were systematically enrolled into either a control (C; odd numbered ear tags) or GP (even number ear tags) treatments. Glucose precursor was top dressed on the prepartum pen(PreP) TMR and postpartum pen (PPost) TMR at a rate of 300g/cow/d and mixed in using a pushup tractor. Cows were then allowed access to the TMR. Daily feed samples were pooled weekly and sent to Analab (Agriking, Fulton, IL) for nutrient analyses. Weekly blood samples were analyzed for Glu(mg/dl) and BHBA (mmol/L) using NovaMax® (Nova Diabetes Care, Inc., Billerica, MA). Weekly milk samples were taken to approximately 21 DIM followed by monthly tests. Holstein (n_{GP}=52, n_C=54) and Jersey (n_{GP} =53, n_C=52) data were analyzed using the MIXED procedure of SAS (v. 9.4, SAS Institute 2015) with repeated measures by cow, parity as a random effect and fixed effects treatment, previous lactation fat milk or protein yield, period of lactation and DIM . Jersey cows did not show a response to treatment. Holstein cows supplemented with GP increased production by 4.05 kg/d milk yield (P=0.0011), 0.22 kg/d fat yield (P=0.0002), and 0.12 kg/d protein yield (P=0.0042) in the first 21 d DIM while on treatment. After treatment, GP Holsteins production was still greater than C Holsteins by2.45 kg/d milk (P=0.0487), 0.08kg/d fat (P=0.17), and 0.08kg/d protein (P=0.055) until 120 DIM. Total number of health events in the first 60 DIM for GP Holstein cows decreased (N_{GP}=32, N_C=44) and incidence of clinical and subclinical ketosis decreased by 15%. Holsteins and Jerseys responded differently to treatment; therefore, different breeds face different issues during early lactation. Holsteins tend to have a difficult transition period and are more likely to benefit from GP. For Holsteins, supplementation with GP prevented ketosis, decreased health events, and increased milk yield and milk component production.

The objectives of this study were also to identify a monitoring program for ketosis detection and determine when cows are most at risk. For detection of ketotic events, both Glu and BHBA measurements are needed. Using Glu alone identified 285 ketotic events, but 85% were false positives. Using BHBA alone identified 61 ketotic events, but 28% were false positives. At least 77% of the cows that experienced ketosis were Holsteins. Monitoring Holstein cows would be beneficial since the breed is much more likely to have ketosis in early lactation. For both breeds,58% of the cows that experienced ketosis were identified in week 1. Of those cows, 67% were chronic and had ketosis in later weeks. Therefore week 1 the best week to monitor for ketosis. Monitoring cows in week 2, however, would identify 50% of chronic cows from week 1. Using both Glu and BHBA decreases false positives and aids in identification of ketosis.

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

Project Title: The effects of age on PBMC mitochondrial enzyme activity in Holstein cattle.

Investigators: A. M. Niesen, and **H. A. Rossow**

Progress of work

There is a substantial cost to the dairy when heifers are culled in their first or second lactation before they have produced enough milk to become profitable. If heifers that were more prone to these problems could be identified before the costs of feeding them to a productive age were incurred, they could be culled early saving the dairy industry in feed, labor and environmental costs (manure and methane production). Mitochondrial variation and function has been shown to be important in milk production and reproduction in cows as well as disease states in humans. Mitochondria are central to metabolism and are sensitive to changes in inflammation, disease, and energy supply. Therefore assays of mitochondrial function could serve as a marker of future milk production, reproduction rates and survivability of a heifer. The purpose of this study is to compare the activity rates of various mitochondrial enzymes isolated from peripheral blood mononuclear cells (PBMCs) in 4 Holstein heifers (3 mo) and 4 adult Holsteins. Crude mitochondrial extracts were isolated from the PMBCs using a mitochondria isolation kit from Abcam (Cambridge, MA). Aliquots from each sample were assayed to determine the enzyme activity of citrate synthase, complex I, complex IV, complex V and the concentration of mitochondrial protein. Plasma total protein ($P<0.02$) was higher in calves while complex I ($P<0.05$) activity rates were higher in adult animals. Citrate synthase ($P=0.1$) and Complex IV ($P=0.1$) activity rates had a tendency to be higher in adults. No difference in packed cell volume was observed between the groups, indicating that both groups had similar hydration status. These findings suggest that variation in mitochondrial enzyme activity occurs in dairy cattle as they age and that this data could be used as an indicator in calves of future performance on a dairy.

Objective 3: To use 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

Project Title: Models to predict enteric methane emissions from lactating dairy cows

Investigators: **E. Kebreab, M. Hess, and J. G. Fadel**

Progress of work

The objectives of this study were to (i) evaluate extant models for predicting enteric CH4 emissions from lactating dairy cows in North America (NA), Europe (EU), and Australia and New Zealand (AUNZ)

using literature data and (ii) examine performances of the best-performing models using DMI estimated from routinely available information. Models were often developed on region or country-specific data and may not be able to predict the emissions successfully in every region. The majority of extant models require dry matter intake (DMI) of individual animals, which is not routinely measured. The objectives of this study were to (i) evaluate performance of extant models in predicting enteric CH4 emissions from dairy cows in North America (NA), Europe (EU), and Australia and New Zealand (AUNZ) and (ii) explore the performance using estimated DMI. Forty extant models were challenged on 55, 105, and 52 enteric CH4 measurements (g per lactating cow per day) from NA, EU, and AUNZ, respectively. The models were ranked using root mean square prediction error as a percentage of the average observed value (RMSPE) and concordance correlation coefficient (CCC). A modified model of Nielsen et al. (Acta Agriculturae Scand Section A, 63, 2013 and 126) using DMI, and dietary digestible neutral detergent fiber and fatty acid contents as predictor variables, were ranked highest in NA (RMSPE = 13.1% and CCC = 0.78). The gross energy intake-based model of Yan et al. (Livestock Production Science, 64, 2000 and 253) and the updated IPCC Tier 2 model were ranked highest in EU (RMSPE = 11.0% and CCC = 0.66) and AUNZ (RMSPE = 15.6% and CCC = 0.75), respectively. DMI of cows in NA and EU was estimated satisfactorily with body weight and fat-corrected milk yield data (RMSPE < 12.0% and CCC > 0.60). Using estimated DMI, the Nielsen et al. (2013) (RMSPE = 12.7 and CCC = 0.79) and Yan et al. (2000) (RMSPE = 13.7 and CCC = 0.50) models still predicted emissions in respective regions well. Enteric CH4 emissions from dairy cows can be predicted successfully (i.e., RMSPE < 15%), if DMI can be estimated with reasonable accuracy (i.e., RMSPE < 10%).

Project Title: Predicting water intake in cattle

Investigators: **E. Kebreab, M. Hess, and J. G. Fadel**

Progress of work

The objectives of the study were to (1) develop a set of new empirical models for predicting fresh water intake (FWI) of lactating and dry cows with and without DMI using literature data, and (2) evaluate the new and the extant models using an independent set of FWI measurements made on modern cows. Random effect meta-regression analyses were conducted using 72 and 188 FWI treatment means with and without dietary electrolyte and daily mean ambient temperature (TMP) records, respectively, for lactating cows, and 19 FWI treatment means for dry cows. Milk yield, DMI, body weight, days in milk, dietary macro-nutrient contents, an aggregate milliequivalent concentration of dietary sodium and potassium (NaK), and TMP were used as potential covariates to the models. A model having positive relationships of DMI, dietary dry matter (DM%), and CP (CP%) contents, NaK, and TMP explained 76% of variability in FWI treatment means of lactating cows. When challenged on an independent data set (n = 261), the model more accurately predicted FWI [root mean square prediction error as a percentage of average observed value (RMSPE%) = 14.4%] compared with a model developed without NaK and TMP (RMSPE% = 17.3%), and all extant models (RMSPE% ≥ 15.7%). A model without DMI included positive relationships of milk yield, DM%, NaK, TMP, and days in milk, and explained 63% of variability in the FWI treatment means and performed well (RMSPE% = 17.9%), when challenged on the independent data. New models for dry cows included positive relationships of DM% and TMP along with DMI or body weight. The new models with and without DMI explained 75 and 54% of the variability in FWI treatment means of dry cows and had RMSPE% of 12.8 and 15.2%, respectively, when evaluated with the literature data. The study offers a set of empirical models that can assist in determining drinking water needs of dairy farms.

Mechanistic models of ruminant digestion and metabolism have advanced our understanding of the processes underlying ruminant animal physiology. Deterministic modeling practices ignore the inherent variation within and among individual animals and thus have no way to assess how sources of error

influence model outputs. We introduce Bayesian calibration of mathematical models to address the need for robust mechanistic modeling tools that can accommodate error analysis by remaining within the bounds of data-based parameter estimation. For the purpose of prediction, the Bayesian approach generates a posterior predictive distribution that represents the current estimate of the value of the response variable, taking into account both the uncertainty about the parameters and model residual variability. Predictions are expressed as probability distributions, thereby conveying significantly more information than point estimates in regard to uncertainty. Our study illustrated some of the technical advantages of Bayesian calibration and discussed the future perspectives in the context of animal nutrition modeling.

Project Title: Urea hydrolysis in dairy manure

Investigators: **E. Kebreab, M. Hess, and J. G. Fadel**

Progress of work
The examination of urea hydrolysis rates with cattle manure under two temperature (15°C, 55°C), three urea concentration (500, 1000, and 1500 mg urea-N/dL), and three pH (6, 7, 8) conditions showed that urea hydrolysis rates are higher at 35°C than at 15°C, are higher at medium urea concentration compared to high, are higher at pH 6 compared to pH 8 at medium urea concentration. The rapid loss of urea in the first few hours indicates that careful experiments need to be conducted in the future to account N through mass balance experiments. The rapid early loss of urea should be considered in management practices aimed at mitigating ammonia emissions from dairy cattle manure.

The Ohio State University

Station Investigator: Jeffrey Firkins

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

Project Title: Nitrate or live yeast culture on methane mitigation in Jersey dairy cattle.

Investigators: **J.L. Firkins**, M.L. Eastridge, Y. Roman-Garcia, B. Wenner, and Z. Yu. *Note*: integrated work with T.J. Hackmann (Univ. of FL), M. Hanigan, and R. White (VT)

Progress of work
Concern over the environmental impact of dairy production has stimulated research to decrease enteric CH4 production. One approach is feeding the electron acceptor, NO3, to be reduced by bacteria such as the selenomonads, thus outcompeting methanogens for aqueous H2. We hypothesized that a live yeast culture, [Yea-Sacc® (YS); Saccharomyces cerevisiae; Alltech, Inc.] would stimulate the reduction of NO3 completely to NH3 and thereby improve the ratio of CH4 emission:energy-corrected milk production while decreasing blood methemoglobin concentration. Twelve lactating Jersey cows (8 multiparous and non-cannulated; 4 primiparous and ruminally cannulated) were used in a replicated 4 x 4 Latin square design with a 2 x 2 factorial arrangement of treatments. Cattle were fed diets either containing 1.5% NO3 (from calcium nitrate) after an adjustment period or a control diet (containing urea isonitrogenous to NO3) and were given a top-dress of ground corn without or with YS. All non-cannulated cows were spot-measured for CH4 emission by mouth using GreenFeed (C-Lock Inc., Rapid City, SD). The main effect of NO3 decreased ($P < 0.01$) methane 17% but decreased ($P < 0.01$) DMI by 10% (from 19.8 to 17.8 kg/d) such that CH4:DMI tended ($P = 0.14$) to decrease by 8%. Milk and milkfat production were not affected, but NO3 decreased ($P < 0.01$) milk protein from 758 to 689 g/d. Ruminal

pH was decreased more after feeding diets without NO3, and acetate:propionate was greater for cows fed NO3, especially when combined with YS (interaction, P = 0.01). Others who have noted lower palatability and lower consumption per meal, which is consistent with our observations. Methemoglobin was higher (P = 0.01) for cattle fed NO3 than those fed urea but were still low (1.5 vs 0.5% and only once exceeded 5%), documenting minimal risk for NO2 accumulation at our feeding levels of NO3. Although neither apparent OM nor NDF digestibilities were affected (P > 0.15), apparent N digestibility had an interaction (P = 0.06) such that, compared with those fed either diet without NO3, N absorption was slightly higher for those fed NO3 without YS but slightly lower for those fed NO3 with YS. Under the conditions of this study, NO3 did mitigate ruminal methanogenesis but was not particularly effective after considering that it depressed DMI and milk protein. Based on few interactions detected, YS had a minimal role in attenuating either of these responses.

Project Title: Potential for nitrate in combination with defaunation to suppress methanogenesis in continuous culture

Progress of work

Research is needed to suppress enteric methane (CH4) in ruminant production systems. Nitrate serves as an alternative sink for aqueous hydrogen [H2(aq)] accumulating in the rumen, producing ammonium via nitrate reduction pathways, thereby decreasing CH4 production. However, there is evidence that nitrate or nitrite inhibits methanogens or perhaps other H2-producing microbes directly. Defaunation has also been correlated with decreased methanogenesis because rumen protozoa produce acetate or butyrate through enzymatic pathways that either produce H2 or formate. In the present study, we applied a 2 × 2 factorial treatment arrangement in a 4 × 4 Latin square design to continuous culture fermenters (n = 4). Treatments were control (-NO3) versus nitrate (+NO3; 1.5% of diet DM), factorialized with control (faunated, FAUN) versus defaunated (DEF). Fermenters were fed once daily (40 g DM; 50:50 forage:concentrate diet); four periods lasted 11 d, with 3 d of sample collection. Buffer dilution and solids passage rate were maintained at 7.0%/h and 5.0%/h, respectively. There were no main effects of DEF or interaction of faunation status with addition of NO3 (P > 0.05). The main effect of +NO3 increased (P < 0.05) H2(aq) compared with –NO3 by 11.0 μM. The main effect of +NO3 also decreased (P < 0.05) daily CH4 production compared with –NO3 by 8.17 mmol CH4/d. Because there were no treatment effects on NDF digestibility (P > 0.10), the main effect of +NO3 also decreased (P < 0.05) CH4 production compared with –NO3 by 1.43 mmol CH4/g NDF digested. There were no effects of treatment (P > 0.10) on other nutrient digestibilities, N flow, or microbial N flow per gram of nutrient digested. These data support the existing literature that nitrate incorporation in the diet can decrease the methanogenesis by dairy cattle. More importantly, methanogens are not necessarily inhibited by defaunation in a highly reduced environment. DNA was extracted for Illumina sequencing of a subunit of the 16S rRNA gene that is common for both bacteria and archaea. Results are on-going.

Objective 3: To use 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

Project Title: Meta-analysis of Post-Ruminal Microbial Nitrogen Flows in Dairy Cattle. I. Derivation of Equations

Progress of work

The objective was to summarize the literature and derive equations that relate the chemical composition of diet and rumen characteristics to the intestinal supply of microbial nitrogen (MicN), efficiency of microbial protein synthesis (EMPS), and flow of non-ammonia non-microbial N (NANMN). In this study, 619 treatment means from 183 trials were assembled for dairy cattle sampled from the duodenum

or omasum. Backward elimination multiple regression was used to derive equations to estimate flow of nitrogenous components over a large range of dietary conditions. An intercept shift for sample location revealed that omasal sampling estimated greater MicN flow relative to duodenal sampling, but sample location did not interact with any other variables tested. The ruminal outflow of MicN was positively associated with dry matter intake (DMI) and with dietary starch percentage at a decreasing rate (quadratic response). Also, MicN was associated with DMI and rumen-degraded starch and neutral detergent fiber (NDF). When rumen measurements were included, ruminal pH and ammonia-N were negatively related to MicN flow along with a strong positive association with ruminal isovalerate molar proportion. When evaluating these variables with EMPS, isovalerate interacted with ammonia such that the slope for EMPS with increasing isovalerate increased as ammonia-N concentration decreased. A similar equation with isobutyrate confirms the importance of branched chained volatile fatty acids to increase growth rate and therefore assimilation of ammonia-N into microbial protein. The ruminal outflow of NANMN could be predicted by dietary NDF and crude protein percentages, which also interacted. This result is probably associated with neutral detergent insoluble N contamination of NDF in certain rumen-undegradable protein sources. Because NANMN is calculated by subtracting MicN, sample location was inversely related compared with the MicN equation, and omasal sampling underestimated NANMN relative to duodenal sampling. As in the MicN equation, sampling location did not interact with any other variables tested for NANMN. Equations derived from dietary nutrient composition are robust across dietary conditions and could be used for prediction in protein supply-requirement models. These empirical equations were supported by more mechanistic equations based on the ruminal carbohydrate degradation and ruminal variables related to dietary rumen degradable protein.

Project Title: Meta-analysis of Post-Ruminal Microbial Nitrogen Flows in Dairy Cattle. II. Approaches to and Implications of More Mechanistic Prediction

Progress of work

Several attempts have been made to quantify microbial protein flow from the rumen; however, few studies have evaluated tradeoffs between empirical equations (microbial N as a function of diet composition) and more mechanistic equations (microbial N as a function of ruminal carbohydrate digestibility). Although more mechanistic approaches have been touted because they represent more of the biology and thus might behave more appropriately in extreme scenarios, their precision is difficult to evaluate. The objective of this study was to derive equations describing starch, neutral detergent fiber (NDF), and organic matter (OM) total tract and ruminal digestibilities; use these equations as inputs to equations predicting microbial N (MicN) production; and evaluate the implications of the different calculation methods in terms of their precision and accuracy. Models were evaluated based on root estimated variance ($\hat{\sigma}e$) and concordance correlation coefficients (CCC). Ruminal digestibility of NDF was positively associated with DMI and concentrations of NDF and CP and was negatively associated with concentration of starch and the ratio of ADF to NDF (CCC=0.946). Apparent ruminal starch digestibility was increased by omasal sampling (compared with duodenal sampling), was positively associated with forage NDF and starch concentrations, and was negatively associated with wet forage DMI and total dietary DMI (CCC=0.908). Models were further evaluated by calculating fit statistics from a common dataset, using stochastic simulation, and extreme scenario testing. In the stochastic simulation, variance in input variables were drawn from a multi-variate random normal distribution reflective of input measurement errors and predicting MicN while accounting for the measurement errors. Extreme scenario testing evaluated each MicN model against a data subset. When compared against an identical dataset, predicting MicN empirically had the lowest prediction error, though differences were slight ($\hat{\sigma}e$ 23.3 % vs 23.7 or 24.3 %), and highest concordance (0.52 vs 0.48 or 0.44) of any approach. Minimal differences were observed between empirical MicN prediction ($\hat{\sigma}e$ 25.3 %; CCC 0.530) and MicN prediction ($\hat{\sigma}e$ 25.3 %; CCC 0.532) from rumen carbohydrate digestibility in the stochastic analysis or extreme scenario

testing. Despite the hypothesized benefits of a more mechanistic prediction approach, few differences between the calculation approaches were identified.

University of Minnesota

Station Investigator: Brian A. Crooker

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

Project Title: Regulation and integration of hepatic function with mammary and adipose metabolism in Holstein cows during the periparturient period

Investigators: Wanda J. Weber, Georgina Cousillas, Angel Rosales, and **Brian A. Crooker**.
Collaborators: Ricardo C. Chebel, Chi Chen, Yang Da, Ted H. Elsasser, Scott C. Fahrenkrug, John Garbe, David Kerr, John D. Lippolis, Cavan Reilly, Kevin Silverstein, Bruce Walcheck

Progress of work

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) 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. Liquid chromatography-mass spectrometry analyses of metabolite concentrations in milk and serum samples collected weekly have been completed. Milk lipid analysis has presented as an abstract presented and a manuscript drafted for publication. Serum analysis has been completed and the results are being analyzed. Hepatic tissue analysis is underway.

Study 1: Effect of milk yield genotype on hepatic metabolic gene expression during the transition period

Progress of work

Objectives were to determine effects of milk yield genotype on hepatic expression of genes related to the somatotropic axis and carbohydrate and lipid metabolism. Multiparous cows from unselected (stable milk

yield since 1964; UH; n = 10) and contemporary (CH; n = 10) Holsteins that differed in milk yield by more than 4,500 kg milk/305-d were housed together, fed the same diet ad lib, and milked 2X/d. Liver biopsies were collected at -14, 3, 14, and 42 days in milk (DIM). RNA was extracted and expression of 23 genes was determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 4 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with DIM as the repeated effect. Means differed when $P < 0.05$. Expression of 17 genes was altered by DIM. Liver expression of 8 genes was greater and 5 genes was less in CH than UH. There was a genotype by DIM interaction for STAT5A as it decreased at 3 DIM and recovered by 42 DIM in CH but did not change in UH. GHR-1A and IGF-1 were less and STAT3 greater in CH than UH but expression of JAK2 and STAT5B did not differ. There was a genotype by DIM interaction for PC1 as it increased at 3 DIM in both genotypes and remained increased by 42 DIM in CH; in UH PC1 returned to prepartum values at 42 DIM. INSR, PCK1 and PPARGC1A were greater in CH than UH, increased at 3 DIM and remained increased by 42 DIM. PPARa did not differ between genotypes, decreased at 3 DIM and returned to pre-partum values by 14 DIM. PPARd did not differ between genotypes or DIM. Results are consistent with 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.

Study 2: Effect of milk yield genotype on lipidomic profiles of multiparous Holstein cows during the first 9-weeks of lactation

Progress of work

Concentrations of individual fatty acids (FAs) are commonly measured to define the chemical composition and nutritional value of cow milk. However, the distribution of FAs in different milk triacylglycerols (TAGs) and the distribution of these TAGs in different cow milks are rarely examined due to the challenges of analyzing numerous TAGs in milk. In this study, cows (n = 12/genotype) from unselected (stable milk yield since 1964, UH) and contemporary (CH) Holsteins that differed by more than 4,500 kg milk/305-d (UH<CH) were fed the same diet ad libitum and housed together beginning 5 weeks prepartum. Milk samples were collected weekly from each Tuesday pm milking through 9 weeks of lactation. No differences in milk protein and lactose content (PRO%: 3.13% vs. 2.98%, P=0.49; LAC%: 4.69 vs. 4.75, P=0.39) were observed between UH and CH samples. However, compared to UH, CH milk had higher fat content (FAT%: 3.55% vs. 4.33%, P<0.01). Milk TAGs were further examined by high-resolution liquid chromatography and mass spectrometry and multivariate data analysis (MDA). The distribution patterns of weekly UH and CH samples in the MDA model indicated that TAGs profiles of UH and CH differed greatly in early weeks of lactation, but became much more comparable by week 9. The structures of TAGs markers that differed between UH and CH were elucidated by MSMS fragmentation. Hierarchical clustering analysis of these TAGs markers revealed that oleic acid-containing TAGs were enriched in CH milk while the TAGs containing palmitic acid, short-chain and medium-chain FAs existed in much higher abundance in UH milk. Overall, these observations indicated that, in early lactation, CH had greater incorporation of mobilized fatty acids than UH, which led to different milk TAG profiles between two genotypes.

Project Title: Effect of bovine genotype on immune-endocrine-metabolite interactions in dairy cows

Progress of work

A combination of immunological challenges of live animals and a dermal fibroblast model are being used to investigate potential genetic and epigenetic influences on the innate immune response of Angus calves and unselected and contemporary Holstein calves and cows. Different selection strategies and management practices exist between beef and dairy breeds that likely give rise to genetic and epigenetic factors that contribute to their different immune response phenotypes. Results from our Holstein milk

yield genotypes indicate expression of genes involved with cytokine production, inflammation, cell differentiation and activation were altered in both genotypes during the transition period and that there was a less robust response in the contemporary cow. We used a repeated LPS challenge model and determined that hepatic expression of genes related to the somatotropic axis, glucose and lipid metabolism were similar when these genotypes were exposed to LPS. In contrast, expression of genes involved with cytokine production, inflammation, cell differentiation and activation were altered in both genotypes during the transition period and there was a less robust response in the contemporary cow.

Study 1: Differential responsiveness of Holstein and Angus dermal fibroblasts to LPS challenge occurs without major differences in the methylome

Progress of work

We have previously found substantial animal-to-animal and age-dependent variation in the response of Holstein fibroblast cultures challenged with LPS. To expand on this finding, fibroblast cultures were established from dairy (Holstein) and beef (Angus) cattle and challenged with LPS to examine breed-dependent differences in the innate immune response. Global gene expression was measured by RNA-Seq, while an epigenetic basis for expression differences was examined by methylated CpG island recovery assay sequencing (MIRA-Seq) analysis. The Holstein breed displayed a more robust response to LPS than the Angus breed based on RNA-Seq analysis of cultures challenged with LPS for 0, 2, and 8 hours. Several immune-associated genes were expressed at greater levels (FDR < 0.05) in Holstein cultures including TLR4 at all time points and a number of pro-inflammatory genes such as IL-8, CCL20, CCL5, and TNF-α following LPS exposure. Despite extensive breed differences in the transcriptome, MIRA-Seq unveiled similar patterns of genome-wide DNA methylation between breeds, with an overall hypomethylation of gene promoters. However, by examining the genome in 3Kb windows, 49 regions of differential methylation were discovered between Holstein and Angus fibroblasts, and two of these regions fell within the promoter region (-2500 to +500 bp of the transcription start site) of the genes NTRK2 and ADAMTS5. Fibroblasts isolated from Holstein cattle display a more robust response to LPS in comparison to cultures from Angus cattle. Different selection strategies and management practices exist between these two breeds that likely give rise to genetic and epigenetic factors contributing to the different immune response phenotypes.

Study 2: Milk yield genotype impacts expression of hepatic innate immune genes during the transition period in Holsteins

Progress of work

Objectives were to determine effects of milk yield genotype on hepatic expression of genes related to innate immunity. Multiparous cows from unselected (stable milk yield since 1964; UH; n = 10) and contemporary (CH; n = 10) Holsteins that differed in milk yield by more than 4,500 kg milk/305-d were housed together, fed the same diet ad lib, and milked 2X/d. Liver biopsies were collected at -14, 3, 14, and 42 days in milk (DIM). RNA was extracted and expression of 44 genes was determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 4 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with DIM as the repeated effect. Means differed when P < 0.05. Expression of 19 genes was altered by DIM. Expression of 9 genes was greater and 9 genes was less in CH than UH. There were genotype by DIM interactions for CD14 and C/EBPd. Expression of CD14 was lower in CH than UH. In both genotypes, expression of CD14 decreased at 3 DIM, but remained lower in CH through 42 DIM, while CD14 expression recovered at 14 DIM in UH. C/EBPd expression was greater in CH than UH. In CH expression of C/EBPd increased at 3 DIM and returned to prepartum values at 42 DIM. Expression of C/EBPd in UH did not decrease until 42 DIM. LBP and XBP1 were greater in CH than UH, increased at 3 DIM and recovered by 14 DIM. TLR4 decreased at 3 DIM and although it was increasing, remained less than prepartum expression through 42 DIM for both genotypes.

TLR2 and ICAM1 were less in CH than UH, decreased at 3 DIM and remained decreased by 42 DIM. XDH was greater in CH than UH, increased at 14 DIM and remained increased by 42 DIM. IL-1b was greater in UH than CH, but its receptor (IL1bR1) was less in UH than CH and there was no effect of DIM. Results indicate expression of genes involved with cytokine production, inflammation, cell differentiation and activation were altered in both genotypes during the transition period and that there was a less robust response in the contemporary cow.

Study 3: Effect of milk yield genotype on hepatic metabolic gene expression and repeated lipopolysaccharide (LPS) administration. Objectives were to determine effects of milk yield genotype on hepatic expression of genes related to the somatotropic axis and glucose and lipid metabolism during an LPS challenge.

Progress of work
Multiparous cows (n = 12/genotype) from unselected (stable milk yield since 1964, UH) and contemporary (CH) Holsteins that differed by more than 4,500 kg milk/305-d were housed together and fed the same diet ad lib for more than 4 months before being blocked (2/genotype) by DIM and randomly assigned within genotype to receive saline or 0.25 μg/kg BW of LPS (Escherichia coli 055:B5). Cows were synchronized to be at day 8 of their estrous cycle for the first challenge (C1) at 70-84 DIM. Liver biopsies were collected at 0, 4 and 24 h relative to treatment. A second identical challenge (C2) and sampling was conducted 4 d later. RNA was extracted and expression of 23 genes associated with metabolism were determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 4 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with time as the repeated effect. Means differed when $P < 0.05$. Sixteen genes presented a time by treatment interaction due to changes in expression after LPS. Expression of INSR, INSR-b and IGFBP2 was greater and GHR-1A was less in CH than UH, expression of these genes decreased in response to LPS in both challenges, but the response did not differ between genotypes. There was a time by challenge interaction for IGFBP2 as it was decreased at 24 h in C1 but not in C2. There were challenge effects for IGF1 and GHR-1A due to greater expression in C1 than in C2 but the response did not differ between genotypes. There were time by treatment interactions for PC1, PCK1, PPARa and PPARd. In response to LPS expression of PC1 and PPARd increased, and PCK1 and PPARa decreased at 4 h in both challenges, but the response did not differ between genotypes. Results indicate that LPS administration altered hepatic expression of genes related to the somatotropic axis, glucose and lipid metabolism and that these responses were similar for the low and high milk yield genotypes.

Study 4: Milk yield genotype affects hepatic expression of innate immune genes when challenged with lipopolysaccharide (LPS)

Progress of work
Objectives were to determine effects of milk yield genotype on hepatic expression of genes related to innate immune response during an LPS challenge. Multiparous cows (n = 12/genotype) from unselected (stable milk yield since 1964, UH) and contemporary (CH) Holsteins that differed by more than 4,500 kg milk/305-d were housed together and fed the same diet ad lib for more than 4 months before being blocked (2/genotype) by DIM and randomly assigned within genotype to receive saline or 0.25 μg/kg BW of LPS (Escherichia coli 055:B5). Cows were synchronized to be at day 8 of their estrous cycle for the first challenge (C1) at 70-84 DIM. Liver biopsies were collected at 0, 4 and 24 h relative to treatment. Acute innate immune responses were assessed in C1. A second identical challenge (C2) and sampling was conducted 4 d later to assess the impact of a repeated challenge. Expression of 44 genes associated with immunity was determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 4 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with time as

the repeated effect. Means differed when $P < 0.05$. There were time by treatment interactions for 37 genes due to changes in expression after LPS. At 4 h, LPS increased expression of 15 genes and reduced expression of FASLG in UH relative to CH. The 15 genes included TLR4, CD14, ICAM-1, IRF-1, MYD88, IL-6 and TNF. During C2, expression of these genes was less than in C1 and was not affected by genotype. There was a genotype by treatment by challenge interaction for CCL20 as its expression at 4 h in C2 was greater in CH than UH. IL-10 increased at 4 h for both genotypes, but expression during C2 was less than C1. TGFB1 increased more in UH than CH at 4 h in both challenges. During the acute phase (C1), UH cows had a more robust expression of genes related with immune cell activation, cytokine production and chemoattractant production and activation than CH. Responses during C2 were diminished in both genotypes which indicate compensatory mechanisms invoked by C1 were still affecting the response to LPS. Results indicate milk yield genotype impacts the response to LPS and contributes to a less robust response in the contemporary cow.

Virginia Polytechnic Institute and State University

Station Investigators: Mark Hanigan and Robin White

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

Project Title: Absorbed Supply of Amino Acids

Progress of work

We adapted an isotope method previously reported by Lapierre and coworkers which utilized a bolus infusion of a labeled amino acid mixture to determine amino acid clearance rates. We completed analyses of a trial designed to look at amino acid absorption from 1) abomasally infused casein (positive control), 2) abomasally infused Ile, Leu, and Met, 3) soybean meal, 4) blood meal, 5) feather meal, and 6) a test ruminally protected AA. The study used a Latin Square design with six animals in six periods. Feed and fecal samples were taken to determine apparent nitrogen digestibility of the different diets. Samples of the non-infused ingredients were ruminally incubated in a 72 hour time series to determine protein degradation rates. On the last day of each period, the steers received a 2 hour, constant infusion of a complete mixture of isotopically labeled AAs and blood was collected over a 4 hour period. Plasma was assessed for isotopic enrichment and plasma AA entry rates were estimated for each diet by fitting a 3-pool dynamic model to the temporal observed enrichment data. The entry rates for each ingredient were derived from the observed dietary rates by solving a matrix that included each ingredient and a term for ruminally degradable protein from non-test ingredients as a proxy for microbial growth driven by rumen fermentable nutrient supply.

Derived plasma AA entry rates for abomasal infustions of Ile, Leu, and Met were 93, 94, and 93% of the infused amount of each, respectively. Plasma EAA entry rates for abomasally infused casein ranged from 73 to 92% of the infused amount. The infused results were consistent with expected appearance rates given known rates of use of EAA during absorption. Plasma EAA entry rates calculated for blood meal, feather meal, and soybean meal ranged from a low of 30% for Leu from blood meal to a high of 96% for Lys from soybean meal. Mean bioavailabilities were estimated from averaged EAA entry rates and found to be 93.4% for the infused AA, 86.7% for infused casein, 41.6% for soybean meal, 48.3% for feather meal, and 56.2% for blood meal. Coefficients of variation for the estimates 10% for the ingredients and infused AA and 5% for the casein estimates indicating the technique is of value in assessing EAA supplies to the animal.

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

Project Title: Volatile Fatty Acid (VFA) Production

Investigators: Mark Hanigan and Robin White with collaborators from Ohio and Maryland

Progress of work

Current models for predicting VFA production in the rumen have large errors of prediction which are due to incomplete understanding of the underlying mechanisms driving VFA production, interconversions, and absorption. Net production is dictated by the combination of de novo synthesis and interconversions which can both be affected by rumen thermodynamic state. Rumen thermodynamics are controlled in part by rumen pH and VFA concentration. Understanding how the thermodynamic state affects the rumen fermentation pathways (and thus the production and interconversion of VFA) will allow for a more accurate and precise predictions of VFA production in the rumen. The objective of this study was to identify how VFA production, interconversion, and absorption rates change in response to decreased pH and increased propionate concentration. Six ruminally cannulated Holstein steers (294 ± 33 kg initial BW) were used in a replicated 3 x 3 Latin Square design. The trial consisted of 3, 10 day periods, with 3 treatments applied across each period. Treatments were continuous ruminal infusion of 1) a dilute blend of HCl and H3PO4 to reduce rumen pH by 0.5 (LowpH), 2) propionate (HighVFA), and 3) distilled water as a control (CON). Each period included a 7 day adjustment period followed by 3 days of sampling. The 3 sampling days were used for sequential ruminal infusions of Na-2-[13C] acetate, Na-2-[13C] propionate, and Na-2-[13C] butyrate for 6 hours each in addition to the treatment infusion. A bolus of polyethylene gycol was injected into the rumen 1 h prior to the start of stable isotope infusions and Co-EDTA was infused continuously during the stable isotope infusions to estimate rumen liquid volume. Rumen fluid was sampled before the start of the lable infusions and hourly for 12 hours to assess VFA concentrations and enrichment, Co concentration, polyethylene glycol concentration, and pH. A non-linear, 3 pool dynamic model was fitted to the concentration and isotopic enrichment data to estimate de novo VFA production, VFA interconversion, and VFA absorption rates for each animal within treatment. Numerical analyses of the data is ongoing. However, the isotopic data show significant transfer of carbon from propionate to valerate, isobutyrate, and isovalerate; butyrate to isovalerate; and acetate to isovalerate and valerate. This carbon exchange likely explains the failure to recover all of the infused label observed in prior experiments. Thus the standard 3-pool model used for prior work is inadequate and a 6-pool, exchanging model is required, and experiments must include infusions of labelled isobutyrate, valerate, and isovalerate to allow derivion of the additional entry and exchange rates.

Objective 3: To use 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

Investigators: Mark Hanigan and Robin White

Project Title: Postabsorptive Amino Acid Model (Copyright of all elements retained by VT)

Progress of work

The **first objective** of this work was to evaluate an integrated model of portal drained viscera (PDV) and liver (LIV) utilization of essential amino acids (EAA). Predictions of utilization using a previously derived model and constants for PDV resulted in concordance correlation coefficients (CCC) ranging from 0.26 to 0.75. Rederivation of rate constants on an extended dataset using the original model resulted in CCC from 0.74 to 0.87. Modification of the model using variable rate constants improved CCC from 0.75 to 0.91. Using the previously derived fixed constants for the LIV model resulted in CCC from -0.03 to 0.30.

Rederivation of the constants yielded CCC of -0.03 to 0.29. Modification of the model to utilize variable rate constants resulted in CCC of 0.14 to 0.56. The newly derived models accurately predicted EAA use by PDV and LIV tissue, and can be used for calculating post-splanchnic EAA availability.

Our **second objective** was to quantitatively describe absorbed essential amino acid (EAA) conversion to milk protein. A system of equations (Eqn. 1 to 6) was derived for each EAA and fit to data from 53 previously published studies using nonlinear least squares regression (R Statistical Software, v. 3.1.0). Mammary uptake (*Um*; mol/d; Eqn. 1) was calculated based on mammary blood flow (*BFm*; L/d), arterial (*A*; mol/L) and mammary venous EAA concentrations (*Vm*; mol/L):

$$Um = (A - Vm) \times BFm \qquad 1$$

Mammary venous EAA concentrations (Eqn.2) were predicted as functions of *A*, *BFm*, milk protein demand (*MPD*, g/g) and fitted rate constants *K1* and *K2*:

$$Vm = \frac{A \times BFm}{(K1 + K2 \times MPD) + BFm} \qquad 2$$

The incorporation of *MPD* into Eqn. 2 replicated a saturating response when mammary uptake exceeded *MPD*. For each EAA, *MPD* was calculated as a function of *Um* and milk EAA output (AA_{milk}, mol/d):

$$MPD = \frac{Um - AA_{milk}}{AA_{milk}} \qquad 3$$

AA_{milk} was calculated for each study using an assumed milk EAA profile (AA_{prof}, g/g), measured milk protein production (MTPrt, g/d), and EAA molecular weights (g/mol):

$$AA_{milk} = \frac{AA_{prof} * (MT\,\mathrm{Pr}\,t)}{MolecularWeight} \qquad 4$$

When compared to literature data, *Um* was predicted with concordance correlation coefficients ranging from 0.48 to 0.94. Mammary uptake in response to increased absorbed EAA was linear over the available range of data. Mean predicted efficiencies of EAA intake (AA_{in} mol/d) to milk protein (Eff_{in}; %; Eqn. 4) and AA_{abs} to milk protein (Eff_{abs}; %; Eqn. 5) were calculated for each study and the average across all studies for each EAA is presented in Figure 1. The AA_{in} was calculated based on the NRC (2001) feed AA profiles and AA_{abs} was calculated using the NRC (2001) model. EAA intake was calculated based off the given diets for each study and AA_{abs} was measured based off NRC predictions for each given diet.

$$Eff_{MilkAAdietary} = \frac{AA_{milk}}{AA_{in}} \times 100 \qquad 5$$

$$Eff_{milkAA} = \frac{AA_{milk}}{AA_{abs}} \times 100 \qquad 6$$

The mean efficiency of absorbed N use for milk N was 31%; however, the predicted efficiency of absorbed EAA use for milk ranged from 40% to 76%. Although potentially due to averaging high EAA use efficiency with low NEAA use efficiency, the discrepancy between N use efficiency and EAA use efficiency is more likely due to under-prediction of absorbed EAA supply (Lapierre et al., 2006) because when efficiencies are expressed per unit of EAA intake, they more closely resemble N use efficiency. Although the predicted efficiencies are high on average, the model gives a good indication of the variance of milk protein for each EAA.

University of Florida

Station Investigator: Timothy Hackmann

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

Project title: Comparing the responses of rumen ciliate protozoa and bacteria to excess carbohydrate

Investigators: César R. V. Teixeira, Rogério de Paula Lana, Junyi Tao, and **Timothy J. Hackmann**

Progress of work

The aim of this study was to determine how ciliate protozoa from the rumen would respond to excess glucose and how these responses compare to those for bacteria. We hypothesized that ciliates would respond by synthesizing more reserve carbohydrate and spilling less energy than would bacteria. Ciliates and bacteria were isolated from rumen fluid using filtration and centrifugation, respectively, resuspended in N-free buffer to limit growth, and dosed with 5 mM glucose. Compared to bacteria, ciliates consumed glucose >3-fold faster and synthesized reserve carbohydrate 4-fold faster. They eventually incorporated 53% of glucose carbon into reserve carbohydrate—double the value for bacteria. Energy spilling (dissipation of excess ATP energy as heat) was not detected for ciliates, as all heat production (104%) was accounted by synthesis of reserve carbohydrate and endogenous metabolism. For bacteria, reserve carbohydrate and endogenous metabolism accounted for only 68% of heat production, and spilling was detected within 11 min of dosing glucose. This spilling accounted for >50% of heat production at the time of glucose exhaustion. Ciliates formed lactate >4-fold faster than bacteria, and ciliates and bacteria fermented hexose at equal rates. Isotrichid ciliates lysed and appeared to do so after exhaustion of glucose. Together with earlier studies, these results suggest that ciliates alter the course of carbohydrate metabolism in the rumen by outcompeting bacteria for excess carbohydrate, maximizing reserve carbohydrate synthesis, and minimizing energy spilling.

Project title: Use of a fluorescent glucose analog (2-NBDG) to identify uncultured rumen bacteria that use glucose

Investigators: Junyi Tao, Rebecca K. Diaz, Cesar Teixeira, Halima Sultana, and **Timothy J. Hackmann**

Progress of work

Most rumen bacteria are uncultured, making their niche in the rumen difficult to identify. Fluorescent substrates could potentially identify the substrates preferences and niche of these uncultured bacteria, but uptake of these substrates by rumen bacteria has not been evaluated. Our objective was to determine if a fluorescent substrate analog of glucose (2-NBDG) would be taken up by cultured and mixed rumen bacteria. When we incubated cultured species of rumen bacteria in 2-NBDG (0 to 100 μM) and monitored uptake with fluorimetry, we could detect uptake by the five of the twelve glucose-utilizing species tested. Across these five strains, Vmax (maximal velocity of transport) was 3.19 (0.98 SEM) nmol mg protein-1 min-1 and Km (Michaelis constant) was 1.24 (0.41 SEM) μM. These five species possessed the same type of glucose transporter (mannose phosphotransferase system [PTS]), and experiments with genetic mutants confirmed that this transporter was responsible for 2-NBDG uptake. When we incubated mixed rumen bacteria prepared from rumen fluid with 2-NBDG, we could detect uptake with fluorimetry, but Vmax (0.180 [0.05 SEM] nmol mg protein-1 min-1) was relatively low and Km (6.37 [4.86 SEM] μM) was relatively high compared to the pure cultures. Uptake could also be detected by flow cytometry, with 18.5% of cells positive. Ongoing work is attempting to 1) separate 2-NBDG positive cells by cell sorting and subject them with DNA sequencing and 2) develop new analogs to target glucose-utilizing bacteria that do not take up 2-NBDG. In sum, 2-NBDG is taken up by some rumen bacteria that use glucose (those

with a mannose PTS), and in combination with other fluorescent glucose analogs, could be used to identify uncultured rumen bacteria that use glucose.

Project title: Novel mechanisms of ATP synthesis for glucose fermentation to acetate and succinate

Investigators: **Timothy J. Hackmann**, Jeffrey Firkins, Halima Sultana, and Gabrielle Aiello

Progress of work
Fermentation pathways appearing in microbiology textbooks were thought to have been delineated decades ago, but a genomic analysis from our lab showed that glucose fermentation to butyrate may generate 50% more ATP by novel mechanisms. We continue this work by examining pathways for fermentation of glucose to acetate and succinate. All analyses were completed with publicly-available genomes on IMG. For the type-strain *Prevotella albensis* M384, two mechanisms raise the ATP yield by 40% above textbook values. First, the organism generates 0.5 ATP via a novel mechanism involving electron transport phosphorylation. In detail, the oxidoreductase NifJ generates reduced ferredoxin during pyruvate conversion to acetyl-CoA; the ion pump Rnf oxidizes ferredoxin and generates an electrochemical potential ($\Delta\mu H+$ or $\Delta\mu Na+$); and ATP synthase uses the electrochemical potential to drive synthesis of ~0.5 ATP. Importantly, Rnf balances the fermentation by also generating reduced NAD, which is used during fumarate reduction to succinate. Second, the organism saves 1.5 ATP with the unique glycolytic enzyme Pfp. This enzyme is similar to phosphofructokinase but uses pyrophosphate, not ATP, to phosphorylate fructose-6-P. Both mechanisms are possessed many (though not all) *Prevotella spp*. from the rumen and *Bacteroides* spp. from the human gut, the most prevalent groups from those habitats. The mechanism involving electron transport phosphorylation is also possessed by *Selenomonas ruminantium*, the other cultured group in the rumen that forms succinate from fumarate. This and earlier analyses suggests ATP yields of fermentation are underestimated. Biochemical experiments are underway to test genomic predictions.

Pennsylvania State University

Station investigators: Alexander N. Hristov and Kevin J. Harvatine

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

Project title: Effect of rumen-protected Capsicum oleoresin on milk production, total tract digestibility, and responses to glucose and LPS challenge in lactating dairy cows

Investigators: Oh, J., M. Harper, F. Giallongo, D. Bravo, and E. H. Wall, and A. N. Hristov.

Progress of work
This study investigated the effects of rumen-protected Capsicum oleoresin (RPC) on productivity and responses to a glucose tolerance test and a lipopolysaccharide (LPS) challenge in dairy cows. Nine multiparous Holstein cows (100 ± 9.1 days in milk; 665 ± 83.3 kg BW) were used in a replicated 3 × 3 Latin square design experiment balanced for residual effects with 3, 28-d periods. Treatments were 0 (control), 100, and 200 mg RPC/cow/d. RPC was mixed with a small portion of the total mixed ration and top-dressed. DMI (29.5 kg/d; SEM = 0.74) was not affected ($P = 0.72$) by RPC. Milk yield tended to quadratically increase ($P = 0.08$; SEM = 1.27) with RPC: 42.8, 44.7, and 43.9 kg/d, respectively. Feed efficiency was linearly increased ($P < 0.01$; SEM = 0.056) by RPC supplementation: 1.48, 1.52, and 1.57 kg/kg, respectively. Concentrations of fat, true protein, and lactose in milk were not affected ($P \geq 0.69$) by RPC. On the day of the glucose challenge, glucose was intravenously administered at 0.25 g/kg BW and

blood samples were collected at 0, 5, 10, 15, 20, 30, 40, 50, 65, 80, and 110 min following administration. Serum glucose concentration peaked 5 min post glucose administration. RPC did not affect serum glucose concentration during the glucose tolerance test. Insulin concentration at 5, 10, and 40 min and the area under the insulin concentration curve were lower ($P \leq 0.04$) for both RPC application rates compared with the control. Peak concentration of insulin tended to be decreased ($P = 0.07$) by RPC. Concentration of NEFA in serum was linearly increased ($P = 0.03$) by RPC at and after 65 min following glucose administration. Concentration of beta-hydroxybutyrate in serum was not affected ($P = 0.17$) by RPC during the glucose tolerance test. In summary, milk yield and feed efficiency were increased by RPC in this experiment. RPC increased serum NEFA and decreased insulin concentration during the glucose tolerance test whereas glucose concentration was not affected by treatment. Data suggest that dietary supplementation of RPC increased insulin sensitivity and likely redirected glucose for lactose synthesis and milk production and also slightly enhanced fat mobilization in lactating dairy cows. In the 2nd part of the experiment, bacterial LPS was intravenously administered at 1.0 μg/kg BW and blood samples were collected at 0, 2, 4, 8, and 24 h after administration. DMI, milk yield, and white blood cells including neutrophils, lymphocytes, monocytes, and eosinophils were decreased ($P < 0.01$) and rectal temperature, hemoglobin, and serum concentration of cortisol and haptoglobin were increased ($P < 0.01$) by LPS. Plasma concentration of thiobarbituric acid reactive substances, red blood cells, and platelets were not affected ($P \geq 0.13$) by LPS. Post-LPS challenge DMI (25.7 kg/d; SEM = 1.73), milk yield (35.7 kg/d; SEM = 2.44), and milk composition were not affected ($P \geq 0.25$) by RPC. Rectal temperature, white blood cells, red blood cells, hemoglobin, and platelets were also not affected ($P \geq 0.20$) by RPC, except lymphocyte counts were quadratically increased ($P = 0.02$) by RPC at 0 h. Compared with the control, RPC decreased ($P \leq 0.04$) serum concentrations of cortisol and haptoglobin, and increased ($P < 0.01$) concentration of thiobarbituric acid reactive substances in plasma following LPS challenge. Collectively, feed intake, milk yield, rectal temperature, white blood cells, and red blood cells were not affected by RPC in dairy cows challenged by LPS. However, RPC increased concentration of thiobarbituric acid reactive substances in plasma and decreased cortisol and haptoglobin concentrations in serum. Data suggest that dietary supplementation of RPC increased oxidative stress in plasma and alleviated acute phase responses induced by LPS in lactating dairy cows.

Project title: Effects of feeding sorghum, oat, triticale, or wheat silages on feed intake, milk production and composition, and methane production to in lactating dairy cows

Investigators: Harper, M., J. Oh, F. Giallongo, J. C. Lopes, G. Roth, and A. N. Hristov

Progress of work

The objective of the first experiment was to evaluate the production effects of replacing corn silage (serving as the control) with either sorghum or oat silage in the total mixed ration fed to lactating dairy cows. Twelve Holstein cows (days in milk 81 d ± 24; BW 615 kg ± 49.6) were used in a replicated 3 × 3 Latin square design experiment with 3, 28 d periods. Feeding was ad libitum for 5 to 10% refusals. The control diet consisted of (DM basis): 44% corn silage, 11% ground corn, 7.5% alfalfa haylage, 4% hay/straw mixture, 7.5% SoyPLUS (West Central Cooperative, Ralston, IA), 7.5% whole roasted soybeans, 7% canola meal, 4.5% molasses, 4% cottonseed hulls, and 3% mineral premix. For the sorghum diet, 22.7% (DM basis) of the corn silage in the diet was replaced with sorghum silage. Similarly, 22.7% of the corn silage in the oat diet was replaced with oat silage. The MP balance of the control, sorghum, and oat diets was 199, 238, and 290 g/d, respectively, whereas the balance of NEl was 2.8, 2.4, and 2.8 Mcal/d. The forage sorghum (Alta AF 7202) was harvested on November 11, 2014 with the harvester set to 1 inch total chop length. The oats (Forage Plus) were mowed in a vegetative state and harvested on November 14, 2014. Sorghum and oat silages had DM of 30.5 and 30.8% and (DM basis): lactic acid, 2.89 and 7.27%; NDF, 62.7 and 54.7%; and CP, 9.5 and 11.7%, respectively. Enteric methane emission measurements were collected with the GreenFeed system. Control and oat diets resulted in

higher DMI and milk yield than sorghum: 26.7, 27.1, vs 26.0 kg/d (SEM = 1.68, P = 0.02) and 39.6, 40.2, vs 38.7 kg/d (SEM = 3.57, P < 0.01), respectively. Methane emission (502 g/d; SEM = 26.7, P = 0.59) and milk fat yield (1.41 kg/d; SEM = 0.07, P = 0.78) were not affected by diet. The sorghum silage diet decreased milk true protein concentration (P = 0.03) compared with the control or oat silage (2.78 vs 2.85 and 2.83%, respectively). Similarly, milk protein yield was decreased (P = 0.05) by the sorghum diet (1.04 vs 1.13, and 1.13 kg/d, respectively). The results indicate that a 22.7% replacement of corn silage DM with oat silage is a viable alternative for dairy producers in the Northeast U.S. The second experiment was of a similar design and evaluated production effects of replacing corn silage (serving as control) with either triticale or wheat silage in a total mixed ration fed to lactating dairy cows. Twelve Holstein cows (days in milk 38 ± 5.6 , BW 632 ± 101.6kg) were used in a replicated 3 × 3 Latin square design experiment with 3, 28 d periods. For the triticale diet, 22.7% (DM basis) of the corn silage was replaced with triticale silage. Similarly, 22.7% of the corn silage in the wheat diet was replaced with wheat silage. Diets met or exceeded the MP and NEl requirements of the cows. The triticale (Hyoctane) and wheat (Malabar) were harvested May 13 and 20, 2015, respectively, at the boot stage. The silages had DM of 30.7 and 40.7%, pH 4.48 and 4.46 and (DM basis): lactic acid, 7.03 and 6.43%; NDF, 51.1 and 51.0%; and CP, 17.3 and 14.6%, respectively. Diet did not affect DMI (27.5 kg/d; SEM = 1.8, P = 0.37), enteric methane emission (470 g/d; SEM = 23.4, P = 0.16), or milk fat yield (1.55 kg/d; SEM = 0.11, P = 0.35). Milk yield was higher in control versus triticale or wheat diets (42.7, 41.2, and 41.4 kg/d, respectively; SEM = 5.2, P = 0.01). Energy corrected milk yield was also higher (P = 0.05) in control (40.9 kg/d) versus triticale (38.6 kg/d) or wheat (38.5 kg/d) diets. Triticale and wheat diets increased (P < 0.001) milk urea nitrogen concentration compared with the control (12.7 and 13.1 vs 10.8 mg/dL, respectively). Milk true protein and lactose yields were lower for both triticale and wheat diets compared with the control: 1.20, 1.20, and 1.27 kg/d (SEM = 0.096, P = 0.02) and 2.00, 1.98, and 2.14 kg/d (SEM = 0.173, P = 0.01), respectively. The results indicate that a 22.7% replacement of corn silage DM with either triticale or wheat silage in the diet of lactating dairy cows did not affect enteric methane emission or DMI, but decreased milk yield.

Project title: Effects of feeding a histidine-deficient diet on lactational performance of dairy cows

Investigators: Giallongo, F., M. Harper, J. Oh, C. Parys, I. Shinzato, and A. N. Hristov

Progress of work

A 10-wk randomized complete block design study with 24 Holstein cows (days in milk, 87 ± 22 d; BW, 630 ± 56 kg) was conducted to determine the effects of feeding a His-deficient diet on lactational performance of dairy cows. Following a 2-wk covariate period, cows were blocked by days in milk, milk yield, and parity, and randomly assigned to one of the following 2 treatments: His-adequate diet [HAD; digestible His (dHis) supply of 75 g/d, or 2.8% of MP requirements] and His-deficient diet (HDD; dHis supply of 50 g/d, or 2.0% of MP requirements). Both HAD and HDD were supplemented with rumen-protected (RP) Met (Mepron; Evonik Nutrition & Care GmbH) and RPLys (AjiPro-L; Ajinomoto Co., Inc.) supplying dMet and dLys at 2.5 and 2.4% and 7.5 and 7.2% of MP requirements, respectively. At the end of the study, HDD was supplemented with RPHis (an experimental product supplying 9.3 g/d of dHis; total dHis supply of 62 g/d, or 2.5% of MP requirements) for 9 d. The diets consisted of (DM basis): 45% corn and 20% alfalfa silages and 35% concentrate feeds. Diets contained 16.2 and 16.0% CP, respectively and supplied MP and NEL in excess of cow requirements. DMI, yields of milk, energy-corrected milk, and milk protein were decreased (P ≤ 0.02) by HDD (25.4, 37.6, 34.4, and 1.07 kg/d) compared with HAD (27.1, 40.5, 37.4, and 1.18 kg/d, respectively). Milk urea nitrogen was decreased (P < 0.01) by HDD versus HAD. Feed and energy-corrected milk feed efficiencies, milk nitrogen efficiency, milk fat and protein concentrations, milk fat yield, and BW and BW change of the cows were not affected by treatments (P ≥ 0.12). Blood hemoglobin concentration was 5.4% lower (P < 0.01) in cows fed HDD compared with HAD, suggesting a provision of about 24 g of His from this endogenous depot during the

8-wk experimental period. Plasma His concentration was decreased ($P < 0.01$) by HDD versus HAD. Supplementation of RPHis increased DMI (26.6 vs. 25.1 kg/d, $P < 0.01$), but did not affect milk yield (36.0 vs. 34.8 kg/d, $P = 0.28$) compared with HDD. Overall, feeding a diet deficient in His but supplying adequate MP, Met, and Lys had negative effects on DMI and lactational performance of dairy cows. The effect on DMI was reversed when RPHis was supplemented.

Project title: Effect of high-oleic acid whole, heated soybeans or extruded soybean meal on production performance, milk fatty acid composition, and enteric methane emission in dairy cows

Investigators: Lopes, J. C., M. Harper, F. Giallongo, J. Oh, D. M. Kniffen, R. A. Fabin, and A. N. Hristov

Progress of work
The objective of this study was to investigate the effect of 3 soybean sources differing in fatty acid profile and grain processing method on productivity, milk composition, and enteric methane emission in lactating dairy cows. The soybean sources were: extruded conventional soybean meal (SBM; 48% CP and 8.7% ether extract; 22% oleic acid), extruded Plenish® (DuPont Pioneer, Johnston, IA), high-oleic acid variety SBM (51.4% and 8.4%, respectively; 75% oleic acid), and whole, heated Plenish® soybeans (40.0% and 20.2%, respectively). The study involved 15 Holstein cows (54 ± 8.3 days in milk) in a replicated 3 x 3 Latin square design experiment with 3, 28-d periods. The inclusion rate of the 3 soybean sources in the diet was (all data are on DM basis): 17.1, 17.1, and 7.4%, diets CESBM, PESBM, and WHPSB, respectively, providing 1.4 to 1.5% soybean oil. The rest of the dietary ingredients were: corn silage, 41%; alfalfa haylage, 16%; grass hay/straw mix, 4%; ground corn grain, 10%; cottonseed hulls, 4%; molasses, 4.9%; and a mineral/vitamin premix, 3%. The WHPSB diet also contained 9.7% solvent-extracted SBM. The diets had similar content of CP (17.0-17.6%), NDF (31.0-32.0%), ether extract (3.8-4.0%), and NEl (1.53-1.54 Mcal/kg). Compared with CESBM, the Plenish® diets tended to increase ($P = 0.09$) DMI (27.1, 27.8, and 27.8 kg/d, CESBM, PESBM, and WHPSB, respectively). Milk yield was not affected ($P \geq 0.10$) by treatment (average of 42.2 kg/d; SEM = 1.41). The Plenish® diets increased ($P < 0.01$) milk fat content (3.55, 3.74, and 3.76%, respectively). Feed efficiency was decreased ($P < 0.001$) by the Plenish® diets, compared with CESBM (1.50-1.51 vs. 1.57 kg/kg, respectively). Treatments had no effect ($P \geq 0.13$) on enteric methane (average of 463 g/d, SEM = 29.7) or carbon dioxide (average of 12,113 g/d, SEM = 241.5) emissions and methane emission yield (16.6 to 17.2 g/kg DMI). Diets had a marked effect on milk fatty acid profile. Generally, the Plenish® diets increased ($P \leq 0.01$) mono-unsaturated and cis-9 18:1 and decreased ($P \leq 0.01$) poly-unsaturated, total trans-, and conjugated linoleic fatty acids concentrations in milk fat. In this study, compared with conventional extruded SBM, the Plenish® soybean treatments had no effect on milk yield, increased milk fat concentration, decreased feed efficiency, and modified milk fatty acid profile in a manner expected from the greater concentration of oleic acid in Plenish® soybean oil.

Project title: Trends in milk urea nitrogen, milk composition, and milk yield in dairy farms in the Northeast

Investigators: Hristov, A. N., M. Harper, J. Oh, F. Giallongo, J. C. Lopes, G. Cudoc, J. Clay, and L. E. Chase

Progress of work
The main objective of this survey was to examine trends in milk urea nitrogen (MUN) in DHI herds (all dairy cattle breeds were included) in the Northeast U.S. Data for milk fat and protein concentrations, milk yield, days in milk (DIM) on test day, and lactation number of the cows were also collected. Close to 11

million historical (2004 to 2015) records from the Dairy Records Management Systems (Raleigh, NC) for 11 states (CT, DE, MA, MD, ME, NC, NH, NJ, NY, PA, RI, VA, VT, and WV) were included in the analysis. Average (across states and years) MUN, milk fat, milk true protein, milk yield, DIM, and lactation number were (mean and SD): 13.3 (0.65) mg/dL, 3.85 (0.07)%, 3.13 (0.04)%, 31.6 (0.86) kg/d, 178 (19.2) d, and 2.3 (0.04) lactations. MUN was 13.3 mg/dL in 2004, decreased to 12.4 to 12.6 mg/dL in 2009-10, steadily increased to 14.6 mg/dL by 2013, and then decreased to 13.0 and 12.4 mg/dL in 2014 and 2015, respectively. Milk fat concentration steadily increased from 3.69 in 2004 to 3.92-3.93% in 2013-14 and decreased to 3.87% in 2015. Milk true protein was 3.01% in 2004, increased to 3.15-3.16% in 2008-09 and declined to 3.13-3.11% thereafter. Except for 2004 (33.5 kg/d), milk yield steadily increased from 30.7 in 2005 to 32.3-32.8 kg/d in 2014-15. The likely explanation for the higher average milk yield in 2004 was the lower average test day DIM (119 d) in that year vs. all other years (184 d, SD = 4.3). In an effort to explain the observed trends in MUN, we investigated variability in dairy feed cost in PA and the U.S. (Northeast data were not available). Average dairy feed cost in PA (for a cow producing 29.5 kg milk/d) increased from $3.08 in 2005 to $5.22 in 2008, declined to $4.01 by 2010, increased again to $6.03 in 2012, and then declined to $5.07/d in 2015. Dairy feed cost for the U.S. followed similar trends. It was apparent that high MUN coincided with high feed cost and vice versa. Therefore, our conclusion from this survey is that MUN in Northeast dairy herds fluctuated following trends in feed cost; however, ration data are not available to better define the reasons for the variations in MUN levels.

Project title: Changes in Milk Odd and Branched-Chain Fatty Acids During Induction and Recovery from Biohydrogenation-Induced Milk Fat Depression.

Investigators: Elizabeth Palmer, Michel Baldin, Daniel Rico, and Kevin Harvatine

Progress of work

We have observed that the concentration of odd and branched-chain fatty acids (OBCFA) in milk fat markedly changes during biohydrogenation (BH) induced milk fat depression (MFD). The objective was to characterize the time course of changes in milk OBCFA during induction and recovery of BH-induced MFD. Nine Holstein cows were randomly assigned to a treatment sequence in a repeated design that allowed analysis of recovery from a MFD diet. A 36.9% NDF and 1.1% PUFA diet was fed during the control and recovery periods, and a 29.5% NDF and 3.7% PUFA diet was fed during the induction period. Treatment periods were 21 d long and milk was sampled every other day. Data were analyzed using the MIXED procedure of SAS with repeated measures, time was the repeated variable, and cow by treatment was the subject. Preplanned contrasts were control versus induction and control versus recovery at each time point. The production data has been previously published (Rico and Harvatine, 2013 JDS 96:6621). Briefly, milk fat percentage and yield decreased progressively during induction and were lower than control by d 3 and 5, respectively. Milk fat concentration and yield increased progressively when cows were fed the recovery diet and were not different from control on d 19 and 15, respectively. During induction of MFD milk fat content of iso-14:0, iso-15:0, anteiso-15:0, 15:0, iso-16:0, anteiso-17:0, 17:0, and total OBCFA decreased rapidly (3.8 to 3.0% of total FA; $P < 0.01$ for all) and generally the concentration of these fatty acids was lower than control by d 3 ($P < 0.05$ for all). Contrarily, during recovery milk fat content of iso-14:0, iso-15:0, anteiso-15:0, 15:0, iso-16:0, anteiso-17:0, 17:0, and total OBCFA increased rapidly and the concentration of these fatty acids was not different from control on d 3 ($P > 0.1$ for all). In conclusion, the changes in milk OBCFA during induction and recovery of MFD occur rapidly, suggesting that these fatty acids could be used as markers of altered rumen biohydrogenation during milk fat depression.

Project title: Biohydrogenation Kinetics of Oleic, Linoleic and Alpha-Linolenic Acids In Vivo.

Investigators: M. Baldin, N.L. Urrutia, J.G. de Souza, Y. Ying, and K.J. Harvatine

Progress of work
Biohydrogenation (BH) of unsaturated fatty acids (FA) has been extensively studied in vitro; however, in vitro BH rates and extents may not parallel BH pathways in vivo. The objective was to assess rate and extend of oleic (OA), linoleic (LA) and alpha-linolenic acid (ALA) biohydrogenation in vivo. Each FA was characterized in a separate experiment (EXP.1 – oleic, EXP.2 – linoleic, and EXP.3 – alpha-linolenic) using 4 ruminally cannulated lactating Holstein cows in each experiment. A single bolus consisting of 200 g of an oilseed (EXP.1 87% OA sunflower, EXP.2 70% LA safflower, and EXP.3 54% ALA flaxseed) and 12 g of heptadecanoic acid (17:0) was mixed with rumen contents. Rumen digesta was collected at -1, -0.25, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 h relative to the bolus. Samples were immediately placed on dry ice, stored at -20oC, freeze-dried, methylated and analyzed by GC-FID. On the day of infusion, cows were fed at a rate of 4.2%/h of expected daily DMI. The geometric mean of the 4 cows was calculated and the disappearance of 17:0, OA, LA, and ALA was fit to a single exponential decay model using the nonlinear procedure of JMP Pro. Overall, the boluses increased total fat in the rumen from 4.1 to 7.4% and enriched 17:0 from 0.4 to 2.5% of FA. The bolus enriched OA from 9.0 to 30.1% of FA in EXP. 1, LA from 12.5 to 35.9% of FA in EXP.2, and ALA from 1.9 to 19.8% of FA in EXP.3. The fractional rate of 17:0 disappearance was 10.9, 8.5, and 6.7%/h in EXP.1, 2 and 3, respectively, and was used as a marker of FA passage. The fractional rate of disappearance of OA was 55%/h, LA was 61.2%/h, and ALA was 93.9%/h in EXP.1, 2 and 3, respectively, and all three unsaturated FA reached pre-bolus concentration within 4 h. Based on kd/(kd+kp), the extent of BH was 83.4% for OA, 87.8% for LA, and 93.3% for ALA in EXP.1, 2 and 3, respectively. Assuming that BH equals disappearance minus passage, the BH rates were 44.0, 52.7, and 87.1%/h for OA, LA, and ALA in EXP.1, 2 and 3, respectively. In conclusion, the extent of oleic, linoleic, and alpha-linolenic biohydrogenation was near expected values, but the rate of ruminal biohydrogenation was higher than that commonly observed in vitro for these three unsaturated FA.

The University of Tennessee

Station investigators: Agustin Rius

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

Project title: Effect of feeding different concentrations of rumen degradable protein and rumen undegradable protein to heat stressed lactating dairy cows

Progress of work
Feeding low CP diets may improve performance of lactating dairy cows during summer. A study was conducted to evaluate the effect of feeding low RDP and RUP levels in cows during summer. Forty-eight midlactation Holstein cows were assigned to treatments using a complete randomized block design in a 2 × 2 factorial arrangement of treatments (n = 12/treatment). Treatments included two levels of RDP (10 and 8%) and two levels of RUP (8 and 6%). A common diet (10% RDP and 8% RUP) was fed from d 1 to 21 followed by the respective treatment diets from d 22 to 42. Milk samples were collected from d 36 to 42. Cows were housed in a freestall barn and exposed to the prevailing temperature and humidity of July and August with no supplemental cooling. Main effects and their interaction were tested using the Mixed procedure of SAS and reported as least squares means ± SEM. Rectal temperatures and respiration rates were recorded before noon and after noon during the treatments. Compare with before noon, after noon increased temperature and respiration rates (38.9–39.7 ± 0.07°C [$P < 0.001$] and 64.0–87.1 ± 1.4

breaths/min [P < 0.001]). Compared with the 10% RDP, the 8% RDP treatment increased DMI and milk protein yield in the 6% RUP treatment (19.0 vs. 18.4 ± 0.32 kg/d and 1.02 vs 0.96 ± 0.02 kg/d) but decreased DMI and milk protein yield in the 8% RUP treatment (19.4 vs. 20.1 ± 0.32 kg/d [interaction, P < 0.01] and 1.02 vs. 1.08 ± 0.02 kg/d [interaction, P < 0.01]). There was a trend (P < 0.07) for an interaction such that the 8% RDP treatment increased energy-corrected milk (ECM) yield compared with 10% RDP in the 6% RUP treatment (31.7 vs. 29.4 ± 0.76 kg/d) but reduced ECM yield in the 8% RUP treatment (32.5 vs. 33.0 ± 0.76 kg/d). The 10% RDP treatment increased (P < 0.001) milk-urea nitrogen compared with the 8% RDP treatment (10.2 vs. 6.9 ± 0.28 mg/ dL). The 8% RUP treatment increased (P < 0.001) milk-urea nitrogen compared with the 6% RUP treatment (9.8 vs. 7.2 ± 0.28 mg/dL). The 8% RDP treatment increased (P < 0.001) NUE compared with 10% RDP (35.1 vs. 31.6 ± 0.76%). The 6% RUP treatment increased (P < 0.001) NUE compared with 8% RUP (35.1 vs. 31.6 ± 0.76%). Therefore, lower RDP diets can be fed with 6% RUP diets without compromising milk production, whereas the combination of low RDP with 8% RUP depressed productivity. Lower RDP and RUP diets increase NUE in heat-stressed cows.

University of Wisconsin – Madison

Station investigator: Heather White

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

Project title: The methyl-donor choline reduces reactive oxygen species in a linear fashion in primary bovine hepatocytes

Investigators: Tawny Chandler and Heather White

Progress of work

The objective of this experiment was to expose bovine primary hepatocytes to increase doses of choline chloride (CC), in the presence of fatty acids, in order to identify the dose response to choline exposure. Cells were exposed to CC (0, 5, 10, 35, 105, 180, 360, and 2000 uM) and a fatty acid cocktail (1mM) that mimicked the profile in circulation at the time of calving for 24 h. Media was collected for quantification of reactive oxygen species (ROS) and very low density lipoproteins (VLDL), and cell lysates were collected for mRNA isolation for gene expression. Thus far, ROS secreted into the media has been quantified and data are shown below. Overall, there was a linear decrease (P=0.0003) in the concentration of ROS as CC dose increased. There was also a decrease in ROS when “high” (105uM and greater) were contrasted to “low” (0 to 35 uM) doses (P=0.0016). Further analysis still needs to be completed on VLDL secretion and gene expression.

Project title: Supplementation of conjugated linoleic acid during the prefresh period improved metabolic status postpartum

Investigators: Rafael Oliveira and Heather White

Progress of work

This experiment was a followup to an experiment reported in last year’s station report where we supplemented CLA during the entire transition period. The objective of this study was to examine the effect of CLA supplementation prefresh on lactation and metabolic health of dairy cows. The study was conducted on a commercial dairy herd in Wisconsin, USA. Cows were moved from the far off dry cow diet to the prefresh cow pen once per week. One load of TMR was offered once daily in the afternoon to

all cows. Multiparous cows were randomly assigned to a treatment at d -17 to -25 of the predicted calving date. Treatments were a mixture of 10 g/d of each trans-10 cis-12 and cis-9 trans-11 CLA (100 g/d of Lutrell® Pure; BASF, Ludwigshafen, Germany; 75% FA: 20% of the mix CLA) or source of saturated lipids as Control (75g of Energy Booster 100; Milk Specialties Global, Eden Prairie, MN; 96.1% FA: 46.2% C18:0, and 37.0% C16:0). The CLA methyl esters in Lutrell are lipid-encapsulated to be insoluble and resistant to ruminal biohydrogenation processes. Treatments were mixed with 200 g of corn gluten feed and provided on the clean bunk floor before TMR being offered to individual cows locked in the prefresh pen headlocks, daily from start of the prefresh period until calving. The intake of the treatments were observed visually and recorded daily. For the 284 cows (141 and 143 on Control and CLA treatments) the mean lactation was 3.1±1.13 and 2.9±0.98 and duration of prepartum transition period were 16.1±4.53 and 16.4±4.33 d for Control and CLA treatments, respectively. Cows were milked three times per d starting at 0530, 1330, and 2130 h. Milk yield of each cow was recorded daily from 1 to 60 DIM and averaged weekly. One milk sample was obtained weekly for solids and SCC. Milk samples were preserved in 2-bromo-2-nitropropane-1,3-diol, and the concentrations of solids and SCC were measured by fourier transform infrared spectrometry using a FOSS MilkoScan FT6000 (AgSource Laboratories, Verona, WI) for determination of milk composition. Body condition score for the 284 cows were measured on the first day of supplementation (-16.2 ± 4.43 d prepartum), and at d 1 and 30 of lactation. Health disorders from calving to 60 DIM were defined by the manager and veterinarian, and were subsequently retrieved from Dairy Comp 305 (Valley Ag Software). Blood samples from the coccygeal vessels were obtained from 50 randomly selected cows per treatment (only 34 and 30 cows on Control and CLA treatments, respectively, consumed the treatments for 14 d or more and were utilized in the final data set), for BHBA and NEFA analysis, on the first day of supplementation, between d −4 and −15 relative to calving (-9.6±3.08 d prepartum), and at d 1, 7, 14, and 30 of lactation. Analysis for NEFA was performed using the Wako NEFA-HR(2) Microtiter Procedure kit (Wako Diagnostics, Richmond, VA) and BHBA was analyzed using the Stanbio BHBA LiquiColor kit (Procedure No. 2440-058, Stanbio Laboratory, Boerne, TX). Continuous variables were analyzed with the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with a model containing the fixed effects of treatment (control, CLA), time (week of lactation or day of blood sampling), the interaction of treatment and time, and the random effect of cow within treatment. Body condition score change from the d -16 to +1, +1 to +30, and -16 to +30 relative to calving were analyzed separated in a model containing treatment as fixed effect and cow within treatment as random effect. Frequency distributions were analyzed with the GENMOD procedure of SAS using logistic regression for binomial data. Mean days of supplementation for cows in the Control and CLA treatment groups was 15.6±4.65 and 15.6±4.77 d, respectively. Body condition score at d 16 before calving and previous corrected 305 d milk and fat yield were not different between treatments. Overall, supplementation of CLA during the prefresh period tended (P=0.08) to increase milk production, increase milk protein, and tended to increase (P=0.07) milk fat yield although there was no change in milk fat percent across nine weeks postpartum. Supplementation of CLA decreased blood BHBA and NEFA postpartum.

Project title: DL-methionine increases anti-oxidative capacity and prevents inflammatory responses in primary bovine hepatocytes

Investigators: Q. Zhang and H. M. White

Progress of work:
Supplementation of rumen-protected methionine (Met) to dairy cows during the peripartal period improves postpartal performance and may decrease oxidative stress. The aim of the present study was to elucidate the effects of increasing doses of DL-Met on hepatic inflammatory responses and oxidative status. Hepatocytes isolated from 4 calves less than 7 days old were maintained as monolayer cultures for 24h prior to addition of treatments. Treatments included 0, 10, or 40 μM DL-Met added to Met-free

media containing 100 μM Lys (0MET100LYS, 10MET100LYS, or 40MET100LYS), and 10 μM DLMet added to Met-free media containing 25 μM Lys (10MET25LYS). Both 40MET100LYS and 10MET25LYS had a Met:Lys of 1:2.5. Cells were exposed to each treatment in triplicate for 16h and then challenged with either 0 or 100 ng/mL lipopolysaccharide (LPS) for 8 h. Cell lysates were collected for quantification of glutathione (GSH) by fluorometic assay and quantification of gene expression by quantitative PCR. Abundance of mRNA was normalized to the mean of 3 reference genes. Cell media was collected for quantification of reactive oxygen species (ROS) by fluorometric assay. Data were analyzed using PROC MIXED of SAS 9.3. The model included treatment, LPS, their interaction, and random effect of calf. Data are reported as LSMEANS±SE. There was an interaction (P<0.05) of treatment and LPS on intracellular GSH concentration which was increased (P<0.01) as Met concentration increased (107.5, 114. 5 vs. 131. 5±23.5 μM), and 40MET100LYS had greater (P<0.01) GSH than 10MET25LYS (131. 5 vs. 97.5±23.5 μM), in the absence of LPS challenge. With LPS challenge, GSH concentration was not different (P>0.10) among treatments. Hepatocytes challenged by LPS showed an inflammatory response with increased (P<0.001) expression of tumor necrosis factor (1.425 vs. 2.257±0.344 arbitrary unit (AU)) independent of treatment. However, there was an interaction (P<0.01) of treatment and LPS on interleukin *(IL)-6* expression, which was increased by LPS in cells receiving10MET100LYS (1.086 vs. 3.851±0.643 AU) and 10MET25LYS (0.918 vs. 2.296±0.643 AU), but was not increased by LPS in cells receiving 40MET100LYS (0.912 vs. 1.770±0.643 AU). Cell culture media ROS level was not different (P>0.10) among treatments with or without LPS. The data suggest that a stress model can be established using primary bovine hepatocytes with LPS challenge. Increasing Met concentration enhances intracellular antioxidant production and alleviates inflammatory responses, although ROS released from the cells was not affected. The treatment effects were attributed to increase in Met concentration, not the Met:Lys.

Project title: The effect of increasing concentrations of different methionine forms and 2-hydroxy-4-(methylthio) butanoic acid on hepatic oxidative status and genes controlling methionine metabolism and transmethylation flux.

Investigators: Q. Zhang and H. M. White

Progress of work

The D-isomer of methionine (Met) cannot be utilized directly by the mammary gland in dairy cows; instead, it is transformed into L-Met, the proteogenic isomer, in liver and other extramammary tissues. It remains unclear whether different Met forms and a Met hydroxy analog, 2-hydroxy-4- (methylthio) butanoic acid (HMB), are metabolized and function similarly in liver. The objective of the present study was to examine the regulation of key genes in methionine regeneration, transulfuration, and transmethylation pathways and hepatic oxidative status, in response to increasing doses of different Met forms. Hepatocytes isolated from 4 calves less than 7 days old were maintained as monolayer cultures for 24h prior to addition of treatments. Treatments of (0, 10, 20, 40 μM) of D-Met, L-Met, DLMet, DL-HMB, or a 1:1 mixture of DL-Met and DL-HMB were added to Met-free media in triplicate. After 24h, cell lysates were collected for RNA isolation and quantification of gene expression by quantitative PCR, and mRNA abundance was normalized to the mean of 3 reference genes. Cell culture media were collected for quantification of reactive oxygen species (ROS) by fluorometric assay. Data were analyzed with PROC MIXED of SAS 9.3. Analyses of covariance confirmed equivalent slopes of Met form and the final model included form, dose, and random effect of calf within form. Data are reported as LSMEANS±SE. Neither Met form nor concentration affected (P>0.10) ROS released from the cells. There was no main effect of Met form (P>0.10) for any genes examined. The enzymes encoded by betaine-homocysteine methyltransferase (BHMT) and 5-methyltetrahydrofolatehomocysteine methyltransferase (MTR) utilize betaine and 5-methyltetrahydrofolate, respectively, to regenerate Met from homocysteine. Increasing concentration of Met did not alter (P>0.10) MTR expression (1.274,

1.269, 1.264, 1.255±0.257 arbitrary units (AU)), but decreased (P<0.05) BHMT expression (1.308, 1.223, 1.138, 0.968±0.234 AU). Expression of glycine N-methyltransferase, the enzyme that controls transmethylation flux from S-adenosyl-methionine, was not affected (2.205, 2.157, 2.108, 2.011± 0.735 AU; P>0.10) by Met concentration. There was no effect (P>0.10) of Met concentration on expression of cystathionine β-synthase (0.958, 0.972, 0.985, 1.012±0.168 AU), a key enzyme for the transulfuration pathway. The decrease in BHMT expression indicates decreased need for cellular Met regeneration with increasing Met concentration independent of Met form. The lack of differences among Met forms on regulating genes examined indicates that all Met forms were metabolized similarly within primary bovine hepatocytes, and had similar sparing effects on Met regeneration in liver.

C. Publications

Peer-reviewed articles

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

Catterton, TL, and RA Erdman. 2016. The effect of cation source and dietary cation-anion difference on rumen ion concentrations in lactating dairy cows. J. Dairy Sci. 99:6274-6284. http://dx.doi.org/10.3168/jds.2016-10853

Potts, S. B., J. P. Boerman, A. L. Lock, M. S. Allen, and M. J. VandeHaar. 2016. Relationship between residual feed intake and digestibility for lactating Holstein cows fed high and low starch diets. J. Dairy Sci. 99 (in-press).

Yang, S. Y., R. W. S. Ningrat, J.-S. Eun, and B. R. Min. 2016. Effects of supplemental virgin coconut oil and condensed tannin extract from pine bark in lactation dairy diets on ruminal fermentation in a dual-flow continuous culture system. Adv. Dairy Res. DOI: 10.4172/2329-888X.1000160.

Martineau, R., D. R. Ouellet, E. Kebreab, and H. Lapierre. 2016. Casein infusion rate influences feed intake differently depending on metabolizable protein balance in dairy cows: A multilevel metaanalysis. J. Dairy Sci. 99:2748-2761.

Yáñez-Ruiz D.R., A. Bannink, J. Dijkstra, E. Kebreab, D.P. Morgavi, P. O´Kiely, C. K. Reynolds, A. Schwarm, K.J. Shingfield, Z. Yu, and A.N. Hristov. 2016. Design, implementation and interpretation of in vitro batch culture experiments to assess enteric methane mitigation in ruminants – a review. Anim. Feed Sci. Technol., 216:1-18.

Moraes, L. E., S. A. Burgos, E. J. DePeters, R. Zhang, and J. G. Fadel. 2016. Short Communication: Urea hydrolysis in dairy cattle manure under different temperature, urea, and pH conditions. Journal of Dairy Science. Submitted.

Sorge, U. S., M. Henriksen, A. Bastan, N. Cremers, K. Olsen, and B. A. Crooker. 2016. Short communication: Iodine concentrations in serum, milk, and tears after feeding Ascophyllum nodosum to dairy cows - A pilot study. J. Dairy Sci. 99:8472-8476.

Jiang Y, Ogunade I, Qi S, Hackmann T, Staples C, Adesogan A. 2016. Effects of the dose and viability of Saccharomyces cerevisiae. I. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and qPCR. J Dairy Sci. In press.

Artiaga BL, Yang G, Hackmann TJ, Liu Q, Richt JA, Salek-Ardakani S, Castleman WL, Lednicky JA, Driver JP. 2016. α-Galactosylceramide protects swine against influenza infection when administered as a vaccine adjuvant. Sci Rep 6:23593.

Hammond, K. J., L. A. Crompton, A. Bannink, J. Dijkstra, D. R. Yáñez-Ruiz, P. O'Kiely, E. Kebreab, M. A. Eugenè, Z. Yu, K. J. Shingfield, A. Schwarm, A. N. Hristov, C. K. Reynolds. 2016. Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. Anim. Feed Sci. Technol. 219:13-30.

Lopes, J. C., L. F. de Matos, M. T. Harper, F. Giallongo, J. Oh, D. Gruen, S. Ono, M. Kindermann, S. Durval, and A. N. Hristov. 2016. Effect of 3-nitrooxipropanol on ruminal fermentation, methane and hydrogen emissions, and methane isotopic signature in dairy cows. J. Dairy Sci. 99:5335-5344. doi: 10.3168/jds.2015-10832.

Harper, M. T., J. Oh, F. Giallongo, J. C. Lopes, H. L. Weeks, J. Faugeron, and A. N. Hristov. 2016. Short communication: Preference of flavored concentrate premixes by dairy cows. J. Dairy Sci. 99:6585-6589.

Giallongo, F., M. Harper, J. Oh, J. Lopes, H. Lapierre, R. A. Patton, I. Shinzato, C. Parys, and A. N. Hristov. 2016. Effects of rumen-protected methionine, lysine and histidine on lactation performance of dairy cows. J. Dairy Sci. 99:4437-4452.

Hristov, A. N., J. Oh, F. Giallongo, T. Frederick, M. T. Harper, H. Weeks, A. F. Branco, W. J. Price, P. J. Moate, M. H. Deighton, S. R. O. Williams, M. Kindermann, and S. Duval. 2016. Short communication: Comparison between the GreenFeed system and the sulfur hexafluoride tracer technique for measuring enteric methane emissions from dairy cows. J. Dairy Sci. 99:5461–5465.

Herrero, M., B. Henderson, P. Havlík, P. K. Thornton, R. T. Conant, P. Smith, S. Wirsenius, A. N. Hristov, P. Gerber, M. Gill, K. Butterbach-Bahl, H. Valin, T. Garnett, and E. Stehfest. 2016. Greenhouse gas mitigation potentials in the livestock sector. Nature Climate Change 6:452–461. doi:10.1038/nclimate2925.

Yáñez-Ruiz D. R., A. Bannink, J. Dijkstra, E. Kebreab, D. Morgavi, P. O´Kiely, C. K. Reynolds, A. Schwarm, K. Shingfield, Z. T. Yu, and A. N. Hristov. 2016. Design, implementation and interpretation of in vitro batch culture experiments to assess methane mitigation in ruminants – a review. Anim. Feed Sci. Technol. 216:1-18.

Church, C. D., A. N. Hristov, R. B. Bryant, P. J. A. Kleinman, and S. K. Fishel. 2016. A novel treatment system to remove phosphorus from liquid manure. Appl. Eng. Agric. 32:103-112. DOI 10.13031/aea.32.10999.

Ramirez-Ramirez, H.A., E.C. Lopez, C.J. Jenkins, N.D. Aluthge, C. Anderson, S.C. Fernando, K.J. Harvatine, and P.J. Kononoff. 2016. Reduced-fat dried distillers grains with solubles reduces the risk for milk fat depression and supports milk production and ruminal fermentation in dairy cows. J Dairy Sci. 99:1912-28.

Ramirez-Ramirez, H.A., K.J. Harvatine, and P.J. Kononoff. 2016. Short communication: Forage particle size and fat intake affect rumen passage, the fatty acid profile of milk, and milk fat production in dairy cows consuming dried distillers grains with solubles. J Dairy Sci. 99:392-398.

Rico, D.E., A.W. Holloway, and K.J. Harvatine. 2015. Effect of diet fermentability and unsaturated fatty acid concentration on recovery from diet-induced milk fat depression. J. Dairy Sci. 98:7930-43.

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

Bradford, B.J., K. Yuan, and C. Ylioja. 2016. Managing complexity: Dealing with systemic crosstalk in bovine physiology. J Dairy Sci. 99:4983–96.

VandeHaar, M. J., L. E. Armentano, K. Weigel, D. M. Spurlock, R. J. Tempelman, and R. Veerkamp. 2016. Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency. J. Dairy Sci. 99:4941-4954

Manzanilla-Pech, C., R.F. Veerkamp, R.J. Tempelman, M.L. van Pelt, K.A. Weigel, M.J. VandeHaar, T.J. Lawlor, D.M. Spurlock, L.E. Armentano, E.E. Connor, C.R. Staples, M. Hanigan, Y. De Haas. 2016. Genetic parameters between feed intake-related traits and conformation in 2

separate dairy populations-the Netherlands and United States. J. Dairy Sci. 99(1):443-57. doi: 10.3168/jds.2015-9727.

Tye, B. M., S. Y. Yang, J.-S. Eun, and J. O. Hall. 2016. Lactational performance, energy partitioning, and nitrogen utilization of dairy cows fed with high-moisture corn grain with slow-release urea in high-forage lactation diets. J. Dairy Sci. (In press; manuscript ID: JDS-16-11379.R2)

Acetoze G, R. Kurzbard, K.C. Klasing, J.J. Ramsey, H.A. Rossow. 2016. Oxygen Consumption, Respiratory Control Ratio (RCR) and Mitochondrial Proton Leak of broilers with and without growth enhancing levels of minerals supplementation challenged with *Eimeria maxima (Ei).* J. Anim. Physol. Anim. Nutr. In Press

Benjamin, A., B. Green, B. Crooker, S. McKay, and D. Kerr. 2016. Differential responsiveness of Holstein and Angus dermal fibroblasts to LPS challenge occurs without major differences in the methylome. BMC Genomics. 17: 258.

Tao J, Diaz RK, Teixeira CR, Hackmann TJ. 2016. Transport of a fluorescent analogue of glucose (2-NBDG) versus radiolabeled sugars by rumen bacteria and Escherichia coli. Biochemistry 55:2578-2589.

Sadri, H., F. Giallongo, A. N. Hristov, J. Werner, C. Lang, C. Parys, B. Saremi, and H. Sauerwein. 2016. Effects of slow-release urea and rumen-protected methionine and histidine on mTOR signaling and ubiquitin proteasome-related gene expression in skeletal muscle of dairy cows fed a metabolizable protein-deficient diet. J. Dairy Sci. 99:6702-6713.

Ticiani, E., M. Urio, R. Ferreira, K.J. Harvatine, and D.E. De Oliveira. 2016. Transcriptional regulation of acetyl-CoA carboxylase α isoforms in dairy ewes during conjugated linoleic acid induced milk fat depression. Animal. IN PRESS.

White, H. M., E. R. Carvalho, S. L. Koser, N. S. Schmelz-Roberts, L. M. Pezzanite, A. C. Slabaugh, P. H. Doane, and S. S. Donkin. 2016. Short Communication: Regulation of hepatic gluconeogenic enzymes by dietary glycerol in transition dairy cows. J. Dairy Sci. 99:812-817.

Walker, C., M. A. Crookenden, K. M. Henty, R. R. Handley, B. Kuhn-Sherlock, H. M. White, S. S. Donkin, R. G. Snell, S. Meier, A. Heiser, J. J. Loor, M. D. Mitchell, and J. R. Roche. 2016. Epigenetic regulation of pyruvate carboxylase gene expression in the postpartum liver. J. Dairy Sci. 99:5820-5827.

Zhang, Q., S. Bertics, D. Luchini, and H. White. The effect of increasing concentrations of DL methionine and 2-hydroxy-4-(methylthio)butanoic acid on hepatic genes controlling methionine regeneration and gluconeogenesis. J. Dairy Sci. 99:8451-60.

Objective 3: To use 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

Reed, K.F., G.B. Arhonditsis, J. France, E. Kebreab. 2016. Technical Note: Bayesian calibration of dynamic ruminant nutrition models. J. Dairy Sci., 99:6362–6370.

Jayasundara, S., J. A. D. R. N. Appuhamy, E. Kebreab and C. Wagner-Riddle. 2016. Methane and nitrous oxide emissions from Canadian dairy farms and mitigation options: An updated review. Can. J. Anim. Sci., 96: 306–331.

Caro, D., Kebreab, E. and F. Mitloehner. 2016. Mitigation of enteric methane emissions from global livestock systems through nutrition strategies. Climatic Change, 137:467-480.

Appuhamy, J. A. D. R. N., J. France, and E. Kebreab. 2016. Models for predicting enteric methane emissions from dairy cows in North America, Europe, and Australia and New Zealand. Global Change Biol., 22:3039–3056.

Appuhamy, J. A. D. R. N., J. V. Judy, E. Kebreab, and P.J. Kononoff. 2016. Prediction of drinking water intake by dairy cows. J. Dairy Sci., 99:7191–7205.

Johnson, A.C., K.F. Reed, and E. Kebreab. 2016. Short Communication: Evaluation of nitrogen excretion equations from cattle. J. Dairy Sci., 99:7669–7678.

Bougouin, A., A. Leytem, J. Dijkstra, R.S. Dungan and E. Kebreab. 2016. Ammonia emissions from dairy cattle barn: A meta-analysis. J. Environ. Quality, 45:1123–1132.

Santiago-Juarez, B., L.E. Moraes, J.A.D.R.N. Appuhamy, W.F. Pellikaan, D.P. Casper, J. Tricarico and E. Kebreab. 2016. Prediction and evaluation of enteric methane emissions from lactating dairy cows using different levels of covariate information, Anim. Prod. Sci. 56: 557-564.

Boerman, J.P., J.L. Firkins, N.R. St-Pierre, and A.L. Lock. 2015. Intestinal digestibility of long chain fatty acids in lactating dairy cows: A meta analysis and meta regression. J. Dairy Sci. 98:8889-8903.

Roman-Garcia, Y., R.R. White, and J.L. Firkins. 2016. Meta-analysis of Post-Ruminal Microbial Nitrogen Flows in Dairy Cattle. I. Derivation of Equations. J. Dairy Sci. 99:7918-7931.

White, R.R., Roman-Garcia, Y., and J.L. Firkins. 2016. Meta-analysis of Post-Ruminal Microbial Nitrogen Flows in Dairy Cattle. II. Approaches to and Implications of More Mechanistic Prediction. J. Dairy Sci. 99:7932-7944.

Ellis, J. L., C. K. Reynolds, L. A. Crompton, M. D. Hanigan, A. Bannink, J. France, and J. Dijkstra. 2016. Prediction of portal and hepatic blood flow from intake level data in cattle. J. Dairy Sci. 99:9238-9253.

Castro Marquez, J., S. I. Arriola Apelo, J. A. D. R. N. Appuhamy, and M. D. Hanigan. 2016. Modeling mTOR signaling and its effects on milk protein synthesis in bovine mammary tissue. J. Dairy Sci. 99:6714-6736.

Manzanilla-Pech, C. I. V., R. F. Veerkamp, R. J. Tempelman, M. L. van Pelt, K. A. Weigel, M. VandeHaar, T. J. Lawlor, D. M. Spurlock, L. E. Armentano, C. R. Staples, M. Hanigan, and Y. De Haas. 2016. Genetic parameters between feed-intake-related traits and conformation in 2 separate dairy populations: the Netherlands and United States. J. Dairy Sci. 99: 443-457.

R. R. White, and M. D. Hanigan. 2016. Modeling cross-species feed intake responses to thermal stress. J. Agric. Sci. 154: 136-150.

Feng, X., J. P. Jarrett, K. F. Knowlton, R. E. James, and M. D. Hanigan. 2016. Short communication: Comparison of predicted dietary phosphorus balance using bioavailabilities from the NRC (2001) and Virginia Tech model. J Dairy Sci 99: 1237-1241.

Abstracts submitted to national and international scientific meetings

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

Ylioja, C. M., C. Schulte, R. A. Stock, and B. J. Bradford. 2016. Effects of dietary fat source on performance of lactating dairy cows fed a pre-mixed concentrate. J Dairy Sci. 99 (E-Suppl. 1):630-1 (Abstr.)

Ardalan, M., C. F. Vargas Rodriguez, G. I. Zanton, M. Vázquez-Añón, E. C. Titgemeyer, and B. J. Bradford. 2016. Relative availability for lactating dairy cattle of methionine from two sources of ruminally protected methionine. J Dairy Sci. 99 (E-Suppl. 1):752-3 (Abstr.)

Fagundes, M. A., S. A. Blaser, S. Y. Yang, J.-S. Eun, and J. O. Moon. 2016. Effects of supplementing rumen-protected methionine on lactational performance of dairy cows during early and mid-lactation. J. Dairy Sci. 99 (E-Suppl. 1):342. (Abstr.)

Ghelich Khan, M., S. Y. Yang, J.-S. Eun, and J. W. MacAdam. 2016. Nitrogen excretion of lactating dairy cows fed alfalfa hay- or birdsfoot trefoil hay-based high-forage diet. J. Dairy Sci. 99 (E-Suppl. 1):763. (Abstr.)

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D. Impacts

Several members of the group have been awarded competitive funding for research; collaboration with other investigators and industry has helped advanced our understanding of fed technologies, ruminant metabolism and environmental impact. All members of the group have national and international recognition for their work. Training of future leaders in agriculture continues to be a strong focus for all members of the group. Many masters and doctorate degrees have been awarded as result of the extensive research, addressing the 3 objectives of the project, represented by the NC2040 committee. The incorporation of undergraduate research components greatly enhances the academic training of students. Below are specific outcomes and impacts created by this committee.

1. In vitro data demonstrated that source and level of cobalt can have impacts on nutrient digestibility.
2. Demonstrated that sources of sodium do not interact negatively with other feed additive called monensin. Both are commonly fed to high producing dairy cows.
3. Refinement of genetic architecture of feed efficiency in dairy cows. So far 4,000 have been analyzed. Also, the Genomic Breeding Values for Feed Saved index is being developed for Holstein bulls, this has potential to aid in breeding and selection decisions for more efficient animals.

4. Supplementation with rumen protected methionine is a way of promoting more milk fat synthesis in dairy cows. If paid on component basis, having more milk in fat would be a positive economic impact.
5. Demonstration that testing for ketosis during week 1 is the best time to detect the disorder. Early detection can reduce the negative effects of this disorder.
6. Variation in mitochondrial enzyme activity occurs in dairy cattle as they age and that this data could be used as an indicator in calves of future performance on a dairy
7. Refinement of mathematical models to predict methane emissions. This information may be used in the new dairy NRC.
8. The study on water intake offers a set of empirical models that can assist in estimating the drinking water intake by dairy cows in commercial dairy herds
9. 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. Decision makers such as producers can benefit from knowledge of associated error while predicting outcomes such as milk production.
10. The rapid loss of urea in the first few hours indicates that careful experiments need to be conducted in the future to account N through mass balance experiments.
11. Investigations on feed additives and methane mitigation in Jersey cattle showed promising results in lowering methane production but reduced feed intake. Further development of strategies to overcome feed intake depression would be helpful in maintaining milk production while reducing methane emissions.
12. Equations derived from dietary nutrient composition are robust across dietary conditions and could be used for prediction in protein supply-requirement models.
13. Development of 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.
14. Estimates of individual essential amino acids bioavailability for soybean meal, blood meal, and feather meal were derived.
15. Development of a model that predicts flow through the system with reasonable accuracy and defines postabsorptive efficiencies of transfer of AA to milk protein. The equations can be used within a static ration balancing environment to predict EAA availability for milk protein synthesis
16. Most rumen bacteria are uncultured and thus have unknown niches in rumen fermentation. Along with other fluorescent analogs, 2-NBDG has potential to identify some uncultured, glucose-fermenting species and thus better define microbial niches in the rumen
17. Discovery of new mechanisms of ATP synthesis that could increase yields by 40% for glucose fermentation to acetate and succinate (or propionate). Some models predict production of microbial protein using ATP yield from fermentation, and these models may need to be revised.
18. Discovery of the importance of histidine for proper nutrition of dairy cows and subsequent milk production.
19. Discovery of new markers for altered biohydrogenation using odd and branched chain fatty acids during milk fat depression and further understanding of fatty acid metabolism in the rumen.
20. Heat stress decreases production of milk and milk proteins and increases protein catabolism and urinary nitrogen excretion resulting in reduced nitrogen-use efficiency (NUE) in livestock. Overcoming these challenges can have positive financial and environmental impancts.
21. Increasing methionine concentration enhances intracellular antioxidant production and alleviates inflammatory responses.