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, 20013 to October 31, 2014
Date of This Report: October 22, 2014
Annual Meeting Dates: October 20-21, 2014

Participants present: Armentano, Louis (learment@wisc.edu) - University of Wisconsin; Bateman, Gale (gbateman@provimi-na.com) - Provimi; Donkin, Shawn (sdonkin@purdue.edu) - Purdue; Erdman, Richard (erdman@umd.edu) - University of Maryland; Firkins, Jeffrey (firkins.1@osu.edu) – The Ohio State University; Hackmann, Timothy (thackmann@ufl.edu), Secretary - University of Florida; Harvatine, Kevin (kharvatine@psu.edu), Chair - Pennsylvania State University; Hanigan, Mark (mhanigan@vt.edu) - Virginia Polytechnic Institute and State University; Hristov, Alex (anh13@psu.edu) - Pennsylvania State University; Kebreab, Ermias (ekebreab@ucdavis.edu) – University of California - Davis; Rossow, Heidi (harossow@ucdavis.edu) – University of California – Davis; Ruis, Agustin (arius@utk.edu) - University of Tennessee; VandeHaar, Michael (mikevh@msu.edu) - Michigan State University; Benfield, David (benfield.2@osu.edu), Administrative Assistant; Smith, Steve (sismith@nifa.usda.gov) - NIFA-USDA (on conference call).

Participants submitting a written report, but not present:

Bradford, Barry (bbradfor@k-state.edu)- Kansas State University; Crooker, Brian (crook001@umn.edu) – University of Minnesota; Eun, Jong-Su (jseun@usu.edu) – Utah State University; Fadel, James (jgfadel@ucdavis.edu) - University of California – Davis; Schroeder, J. W. (JW.Schroeder@ndsu.edu), North Dakota State University

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. Minutes

a. Monday, October 20th
   i. Remembrance of Brian Bequette
   ii. Remarks by administrative advisor, David Benfield
      1. Multistate projects are not restricted to US membership; we may want to invite Canadians especially
      2. Farm Bill authorized $25 m in funding for animal research, but funding has not yet been allocated
      3. NIFA has allocated large funds towards plant science and water research

4. We may wish to be nominated for an Experiment Station Section Award for Excellence in Multistate Research ($15 K)
5. Minimum requirement for project membership is submission of annual report, not meeting attendance, but committee can set additional criteria
    a. In 2012, committee had decided that members absent over 2 consecutive years should be removed
    b. Members absent since 2012 will be removed

iii. USDA update via conference call from Dr. Steve Smith
1. Personnel update
    a. New division director (Parag Chitnis)
    b. New National Program Leader for Aquaculture
    c. Vacancy for Program Manager in Animal Protection/Agrosecurity
2. NIFA budget for fiscal year 2015
    a. AFRI budget anticipated to increase from $316 m to $325 m, but sequestration may decrease anticipated budget by 7 to 8%
    b. No major changes in Hatch funds or other programs
    c. President's budget allocated $80 m for Opportunity, Growth and Security Initiative (some of which could have been used for animal research), but allocation removed in house and senate committee budgets
3. Competitive programs
    a. Foundational Program FY 2015 RFA should be released in Oct to Nov 2014
    b. RFAs should be released on a more predictable and shorter (<1 yr) cycle
    c. Exploratory program (introduced in 2014) is for high-risk, high-reward ideas that do not panel well
    d. Climate Variability and Change was skipped in 2014, but it is still considered high priority and has not been deleted from programs to be funded in future

iv. NRC Discussion
1. Rich Erdman (chair), overview
    a. Next edition should be released in 2016
    b. Budget is $400 K (for staff time and travel expenses)
        i. ADSA foundation provided $200 K seed funds
        ii. 11 industry partners provided $19 K each
        iii. 2 staff members are co-chairing project along with an assistant and consultant (4 staff in total)
    c. Committee members
        i. Selected from 80 nominations
        ii. List: Allen, Michael; Armantano, Louis; Erdman, Richard (chair); Firkins, Jeffrey; Hall, Mary Beth; Hanigan, Mark; Hristov, Alex; Kononoff, Paul; Lapierre, Hélène; Santos, Jose; Van Amburgh, Michael; VandeHaar, Michael; Weiss, William (vice-chair)
    d. Chapter assignments already made

e. Meetings: Sept 2014, Mar 2015, Jul 2015, Oct 2015
f. Draft for review will be sent out in early 2016, revised, and reviewed by National Academies Press, and final publication sometime in 2016

2. James Fadel (presented in absentia by Ermias Kebreab)
    a. Should the NRC (2016) be an evaluator (to determine if proposed ration meets requirements) or optimizer (to formulate least-cost rations)?
    b. Optimizer would be useful for producers and researchers
    c. Committee discussion: We should be cautious in developing NRC optimizer because 1) CNCPS, not NRC, is most heavily used by commercial nutritionists, 2) least-cost rations, if incorrect, would turn users off to 2016 and future editions of NRC
    d. Sensitivity analysis should be done because many input variables have low sensitivity
        i. Examples: rate of passage and digestion, amino acid composition
        ii. Variables that are insensitive should be removed from the model, but biologically important ones should still be discussed in the text
        iii. Mineral discussion should propose mineral mixes, as these mixes are used by producers (at least in CA)
    e. Barry Bradford (presented in absentia)
        i. Nutrient requirements should include an estimate of variance to reflect variation amongst animals in a herd
    f. Mike VandeHaar
        i. Comments on prediction of energy
            1. User should have option of making NDFD as a user input, as lignin/NDF ratio is sometimes unreliable
            2. Discount factor for DE with increasing intake (multiples of maintenance) is flawed and should be updated with more recent datasets
            3. Efficiency of DE use for ME and ME use for NE were not changed between NRC (1989) and NRC (2001), except for FA
        ii. Comments on prediction of MP
            1. Several aspects require re-examination
                a. Contribution of N recycling to microbial protein
                b. Digestibility of microbial true N (80% in NRC [2001])
                c. Size of endogenous N (4.75 g/kg DMI in NRC [2001])
                d. Intestinal digestibility of fraction C protein
            2. k_d and k_p may be removed from model

a. Animal responses are insensitive to values
b. Fixed values of RDP/RUP could be used instead
c. Committee discussion: they may still be biologically important

iii. Comments on prediction of DMI
1. Predicted DMI should be a starting point for formulating diets, and actual DMI may often be known
2. All functions that require energy should affect DMI, but currently DMI is affected only BW and milk, not body reserves, growth, pregnancy, or work
3. Empirical factors for adjusting DMI were proposed, such as diet NDF or dietary ingredients

g. Kevin Harvantine
i. Suggestions for improving fat section
1. Generate feed library of FA profiles
2. Committee discussion: Change from EE to FA system?
3. Discuss factors affecting rumen biohydrogenation (though exclude from model due to difficulty in mathematical representation)
4. Represent digestibility of fat
a. By source
b. By fatty acids
c. Other factors
5. Define omega-3 FA requirement
a. Calculate metabolic need in milk, gain, sloughed cells
b. Summarize duodenal flow and calculate balance
c. Summarize transfer efficiency of different FA into milk (to improve prediction of milk FA)

ii. Rumination
1. Define requirement or goal for rumination time
2. Describe variation in rumination and time to change
3. Exploit data from rumination monitors that increasingly are being used

h. Tim Hackmann
i. Microbial protein flow predicted poorly in NRC (2001)
ii. Underlying problem is assumption of (nearly) constant microbial efficiency
iii. Empirical equations predict microbial efficiency poorly

iv. Mechanistic model have greater potential for representing variation in efficiency
v. Committee discussion
1. Not enough data to parameterize a mechanistic model, though elements of a future model may be discussed in text
2. Microbial flow should be predicted with TTDOM or TDN as in NRC (2001) or DMI

v. Station reports
1. Rich Erdman, University of Maryland

b. Tuesday, October 21st
i. Election of new officers for 2014-5
1. Secretary: Agustin Rius
2. Chair: Timothy Hackmann

ii. Next year's meeting
1. Time: Oct 19th to 20th
2. Venue: Holliday Inn, 5615 N. Cumberland Avenue Chicago, Illinois 60631
3. Possible statics workshop
a. Brief introduction to R
b. Application of R or SAS to meta-analysis, emphasizing adjustment for study effect
c. Presented by student from lab of Mark Hanigan or Ermias Kebreab

iii. Station reports
1. Kevin Harvatine, Pennsylvania State University
2. Timothy Hackmann, University of Florida
3. Mark Hanigan, Virginia Polytechnic Institute and State University
4. Michael VandeHaar, Michigan State University

B. **Summary of station reports**

1. Louis Armentano (University of Wisconsin-Madison, Obj. 1)

High Oleic Acid Soybeans (Plenish) in Place of Control (High Linoleic Acid) Soybeans in Dairy Cow Diets

Sixty four Holstein cows were randomly assigned to each treatment. The treatments consisted of 2 diets containing different varieties of soybeans: a control soybean with a typical fatty acid profile and Plenish soybeans which contained lower linoleic acid and higher oleic acid. Diets were balanced to meet the requirements of the NRC for a 650kg multiparous cow producing 40kg milk/day with 3.75% fat and 3.00% protein (2001). The soybeans were added to provide .8 kg of ether extract/cow/day. There were no significant effects by treatment on any of the response variables that we measured. However, there was a small numeric increase in milk fat yield by the cows fed the Plenish diet (53g/day). Using a one tailed test on fat concentration, the residual degrees of freedom, the p value was just = 0.10. Based on our previous responses to free oil supplements differing in oleic and linoleic we would expect to see a difference favoring the plenish beans in fat concentration and yield. We did not detect this difference. This may be due to the use of whole beans and a smaller difference when oils are intrinsic to the bean. Also

the power of the trial design was not quite as strong as some designs we have used previously. Therefore this data does not contradict the effect of oleic vs. linoleic, but we cannot say that this effect is expressed when the fatty acids are substituted in the form of whole beans.

2. Gale Bateman (Provimi, Obj. 1)

Whole or ground oats in calf starters: Effects on rumen fermentation and rumen development

A series of 3 trials were conducted to determine effects of whole or ground oats in starter grain on rumen fermentation and development of preweaned calves. Male Holstein calves (43.1 ± 2.3 kg BW at birth; n = 8, 9, and 7 for trials 1, 2, and 3 respectively) were housed in individual pens in a heated facility; bedding was covered with landscape fabric to avoid any consumption of bedding. In trials 1 and 2 only, calves were fitted with a rumen cannula by wk 2 of life. Water was offered free choice, and milk replacer was fed to 12% of birth BW. In all trials, a fixed amount of starter (containing 25% oats either ground and in the pellet or whole; 18.7% CP, 12.7% NDF) was offered daily based on average intakes of calves on similar milk replacer diets; orts were fed through the cannula in Trials 1 and 2. Calves were randomly assigned to all pelleted starter (Ground, n = 11) or pellets plus whole oats (Whole, n = 13). Rumen contents (Trials 1 and 2) were sampled weekly at -8, -4, 0, 2, 4, 8, and 12 h after grain feeding for pH and VFA determination. Calves were euthanized 3 wk (Trial 1) or 4 wk (Trials 2 and 3) after grain was offered; organs were harvested, emptied, rinsed, and weighed to gauge digestive organ development. Experimental design was complete randomized block. Starter intake was not different between treatments by design ($P > 0.05$); weekly intakes were 481 ± 24, 1575 ± 30, 3176 ± 48, 4656 ± 143 g for wk 1 to 4 of grain feeding. Weekly measurements of rumen digesta pH and molar proportion of individual VFA did not change with diet. Molar proportion of butyrate and pH linearly decreased with age, while acetate proportion increased. Reticulorumen weight (569 ground vs. 503 whole ± 24 g) and papillae length (0.75 ground vs. 0.68 whole ± 0.03 mm) tended to be greater for ground ($P < 0.1$) while abomasum weight (240 ground vs. 274 whole ± 9 g) was greater for whole ($P < 0.05$). Liver and omasum weights were not different. Under the conditions of this study, physical form of oats in starter grain did not affect rumen fermentation parameters; greater rumen weight and papillae length in Ground may be a result of greater nutrient availability of ground oats.

Performance of and digestion in calves fed conventional, moderate, and aggressive milk replacer programs

Calves fed large amounts of milk replacer (MR) gain more BW pre-weaning than calves fed less MR; however, post-weaning growth may be reduced. Limited research suggests that impaired nutrient digestion may depress growth post-weaning. We compared growth and post-weaning digestion in 3-d old male Holstein calves fed one of three MR programs. Programs were Conventional (C, 0.45 kg/d of powder containing 21% CP, 21% fat (DM basis), fed for 42 d), Moderate (M, 0.68 kg/d of powder containing 27% CP, 17% fat (DM basis), fed for 42 d), and Aggressive (A, up to 0.91 kg/d of powder containing 27% CP, 17% fat, fed for 49 d). All calves were fed a 20% CP (DM basis) textured starter and water ad libitum for 56 d. The trial used 96 calves (initial BW = 41.4 ± 1.86 kg) received 5 wk apart in two replicates of 48 calves. During d 51 to 55, fecal samples were collected from five calves per treatment randomly selected from calves in the first replicate. Selected nutrients and acid insoluble ash (used as an internal marker) were analyzed in starter and feces to estimate nutrient digestibility. Data were analyzed as a

randomized complete block design with replicate as block. Repeated measures analysis was performed on overall (0 to 56 d) data. Means were separated with a protected LSD test. Pen was the experimental unit. Calves fed C had lower ($P < 0.05$) average daily BW gain (0.35, 0.51, and 0.55 kg/day, respectively, for C, M, and A; SEM = 0.018), gain/feed (0.35, 0.49, and 0.48; SEM = 0.016), and change in hip width (3.3, 4.1, and 4.1 cm; SEM = 0.20) compared to other calves. Calves fed A had greater ($P < 0.05$) change in body condition score and lower ($P < 0.05$) starter intake compared to other calves. Digestibility of OM was 79, 78, and 68% and NDF digestibility was 54, 51, and 26% for calves fed C, M, and A, respectively, and were lower ($P < 0.05$) in calves fed A. Results are similar to previous published results in calves and suggest that depressed post-weaning digestion may be related to reduced starter intake and impaired rumen development.

Performance of and digestion in calves fed two levels of milk replacer and functional ingredients

We compared growth and post-weaning digestion using 48 male Holstein calves (initial BW = 42.6 ± 1.50 kg; initial age = 2 to 3 d) fed to 56 d. Calves were fed diets in a 2 × 2 factorial arrangement of feeding rate (Low [L], 0.68 kg/d of milk replacer (MR) powder and High [H], up to 1.36 kg/d of MR powder) and inclusion of a functional ingredient (without [NT-] or with [NT+] NeoTec5 g, Provimi North America, Brookville, OH). The MR contained 27% CP and 17% fat (DM basis) and was fed to weaning at 49 d. The NT+ treatment was administered in MR before weaning and in calf starter (CS) from weaning to d 56. All calves were fed NT- CS before weaning. The CS were textured (pellets, oats, corn) and contained 20% CP (DM basis). Starter and water were available for ad libitum consumption throughout the study. During d 51 to 55, fecal samples were collected from five calves per treatment selected at random. Selected nutrients and acid insoluble ash (as an internal marker) were analyzed in CS and feces to estimate digestibility. Data were analyzed as a completely randomized design with a factorial arrangement of MR rate (L/H) and Neo-Tec5 g (NT+/NT-) using a repeated measures ANOVA. Pen was the experimental unit. There were no interactions of main effects. Average daily BW gain (ADG**)**, change in body condition score (BCS), and average fecal score from d 0 to 56 were greater ($P < 0.05$) in calves fed H vs. L. Calf ADG, hip height change, and BCS change were greater ($P < 0.05$) in calves fed NT+ vs. NT-. Intake of CS during the digestion period tended ($P < 0.10$) to be lower in calves fed H vs. L. Digestibility of DM, OM, NDF, and ADF was reduced by 7, 7, 65, and 58%, respectively, in calves fed H compared to L ($P < 0.05$). Feeding NT+ increased digestibility of DM, OM, NDF, and ADF by 4, 4, 65, and 74%, respectively ($P < 0.05$) compared to NT-. Feeding high rates of MR reduced ADG by 12% during the last 2 wk of the trial (0.58 vs. 0.65 kg/d for H and L, respectively), which was likely due to reduced intake and digestion of CS as calves transitioned from MR to CS. Feeding NeoTec5 g improved ADG, hip width change, and digestion of nutrients.

Effect of milk replacer solids content on intake, growth and fecal characteristics of Holstein calves

Increased energy intake during cold weather is required to maintain adequate calf growth. Many producers have limited ability to increase volume of liquid offered to calves; therefore, increasing solids content (SC) of the MR solution can increase energy content of milk replacer (MR). Common SC are 12 to 13%, but calves may be fed MR with SC up to 18% in some situations. It is unclear whether changing SC may affect performance, intake or health of young calves fed MR. Our objective was to compare different SC in calves fed MR to 56 d. Holstein

bull calves (n = 48; initial BW = 45.4 ± 4.0 kg; 2 to 3 d of age) were assigned randomly to receive a commercial MR (Nurture Plus EZ, Provimi North America, Brookville OH) at 0.625 kg of MR powder from 0 to 39 d, then 0.313 kg/d until weaning at d 42. The MR (23% protein and 21% fat, DM basis) was diluted to 10.0, 12.5, 15.0, or 17.5% SC and offered twice daily in open pails. Amount of reconstituted MR offered was 6.25, 5.00, 4.17 and 3.57 kg/d for 10.0, 12.5, 15.0 and 17.5% SC, respectively. Texturized calf starter (CS; 20% CP, DM basis) and water were available for ad libitum consumption throughout the study. Data were analyzed as a completely randomized design using a repeated measures ANOVA. Orthogonal polynomials were used to determine linear and quadratic effects of SC. Pen was the experimental unit. There was no effect (P > 0.10) of SC on average daily gain (0.57 ± 0.027 kg/d), CS intake (0.74 ± 0.06 kg/d), MR intake (0.603 kg/d to weaning), 812 J. Anim. Sci Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1 gain to feed ratio (0.48 ± 0.017 kg ADG/kg DM intake), or hip width change (0.1 ± 0.01 cm/d) from d 0 to 56. Number of abnormal fecal days and medical days preweaning declined linearly (P < 0.05) with increasing SC. Number of preweaning abnormal fecal days were 0.22, 0.13, 0.13 and 0.07 ± 0.039 and preweaning medical days were 0.32, 0.25, 0.19 and 0.08 ± 0.056, respectively, for calves fed 10.0, 12.5, 15.0, and 17.5% SC. Increasing milk replacer SC reduced abnormal fecal days and number of treatments.

Changes in serum IgG and total protein concentrations in calves fed differing amounts of colostrum replacer

It is unclear whether differences in serum IgG concentration of calves at 24 h (acquired passive immunity) influence the onset of active IgG production or the age at which serum IgG concentrations normalize. Our objective was to monitor changes in serum IgG and total protein (TP) concentrations in calves fed to achieve high (H) or low (L) passive immunity. Newborn Holstein calves (initial BW = 42.2 ± 4.7 kg) were individually fed a colostrum supplement (n = 23; 50 g of IgG/feeding) or a colostrum replacer (n = 22; 150 g of IgG/feeding) at 0.5 ± 0.22, 6.6 ± 0.46, and 12.9 ± 1.94 h of age. Blood was collected and serum analyzed for IgG using radial immunodiffusion and total protein using optical refractometer every 7 d from wk 0 (2–3 d of age) to wk 8. Calves were vaccinated with Inforce 3 (Zoetis) on arrival, Bovi-shield Gold 5 (Zoetis) at 2 and 6 wk and Presponse HM (Boehringer Ingelheim Vetmedica) at 5 and 8 wk, as prescribed by a veterinarian. Data were analyzed as a completely randomized design using repeated measures ANOVA. Serum IgG (g/L) and total protein (TP; g/ dl) concentrations at 24 h of age were 19.4 ± 0.63 and 4.07 ± 0.076, respectively, in calves fed H, and 8.52 ± 0.62 and 3.32 ± 0.074, respectively, in calves fed L. Serum IgG and TP were affected by a week ´ treatment interaction (P < 0.001). Serum IgG concentration in calves fed H declined to 13.7 g/L at wk 3, then increased to 20.7 g/L at wk 8. Serum IgG in calves fed L declined to 6.3 g/L at wk 1, then increased to 20.6 g/L at wk 8. By wk 7, serum IgG concentration were similar (P > 0.05). Serum IgG was < 10 g/L for 0.3 and 4.9 wk in calves fed H and L, respectively. Total protein concentrations were lower in calves fed L from wk 0 to 6 (P < 0.01); thereafter, differences were not significant. Temporal changes in serum TP and IgG concentrations were independent. Active IgG synthesis was dependent on age of calf and acquisition of passive immunity. J. Anim. Sci Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1 915 Calves with lower serum IgG concentrations at wk 0 began producing IgG at an earlier age and produced more IgG, so that by wk 7, circulating IgG concentrations were similar to those in calves with successful passive immunity.

Effect of Amaferm on digestion of diets containing forages with high or low neutral detergent fiber digestibility

Amaferm (AF), an extract obtained from fermenting *Aspergillus oryzae*, has been reported to stimulate fiber degrading ruminal fungi and bacteria. The objective of this study was to measure effects of AF on fermentation of typical lactation dairy cow rations containing forages with high NDF digestibility (NDFd) or low NDFd. Corn silage (CS) and alfalfa haylage (AH) with 30 h NDFd of 66.4 and 41.4% of NDF, respectively, were used as the only forages to formulate a high NDFd ration (HFd). A CS and AH with 30-h NDFd of 51.2 and 34.3% of NDF, respectively, were used as the only forages to formulate a low NDFd ration (LFd). Diets were formulated to contain (DM basis) 16.0% NDF from CS and 8.0% NDF from AH. Corn, soybean meal, urea, blood meal, Megalac and molasses were adjusted to equalize CP, soluble CP, starch, nonfiber carbohydrates and fat between diets. A completely randomized experimental design was used with a 2 × 2 factorial arrangement of diet forage NDFd (high or low) and level of AF (0.0 or 0.06% of DM). Diets were fermented in triplicate in continuous culture fermentors at the Rumen Fermentation Profiling Laboratory, West Virginia University, Morgantown, WV. Data on pH were reduced to daily means for each fermentor. Fermentation data were analyzed using the PROC MIXED of SAS with a repeated-measures model. Fermentor was treated as a random variable. First-order autoregressive structure type was selected as the appropriate covariance structure based on the goodness-of-fit criteria. Digestibility (%) of DM, NDF, and nonstructural carbohydrates (NSC) were, respectively, 67.8, 41.4, and 79.3 for HFd, 66.2, 34.8, and 77.5 for HFd + AF, 62.9, 34.9, and 79.3 for LFd and 65.8, 39.6, and 79.5 for LFd + AF. DM digestibility tended to be more for high NDFd vs. low NDFd treatments ($P = 0.08$). Digestibility of NSC was similar among treatments. Adding AF improved NDF digestion of LFd but reduced NDF digestion of HFd (forage NDFd x AF interaction; $P < 0.01$). Average fermentor pH for HFd, HFd + AF, LFd and LFd + AF were 6.03, 6.08, 6.31 and 6.24, respectively, with a main effect due to forage NDFd observed ($P < 0.01$). Adding AF improved NDF digestion of the LFd diet but not the HFd diet. The difference in response of HFd and LFd diets to AF may be related to differences in fermentor pH.

3. Barry Bradford (Kansas State University, Obj. 1 and 2)

Yeast product supplementation influences feeding behavior and measures of immune function in transition dairy cows

Forty multiparous Holstein cows were blocked by expected calving date and randomly assigned within block to 1 of 4 treatments (10 cows per treatment) from 21 days before expected calving to 42 days postpartum. Rations were top-dressed with yeast culture plus enzymatically hydrolyzed yeast (YC-EHY; Celmanax®, Vi-COR®, Mason City, IA) at the rate of 0, 30, 60, or 90 g/day throughout the experiment. Pre- and postpartum DMI and water intake did not differ ($P > 0.10$) among treatments. There were quadratic dose effects ($P < 0.05$) for prepartum feeding behavior, reflecting decreased meal size, meal length, and intermeal interval, and increased meal frequency for cows received 30 and 60 g/d of YC-EHY. Postpartum feeding behavior and milk yields were unaffected ($P > 0.10$) by treatments. However, tendencies for increased ($P \leq 0.10$) percentages of milk fat, protein, and lactose were detected for cows receiving YC-EHY. Furthermore, YC-EHY tended to increase the proportion of total energy supply secreted in milk nutrients ($P \leq 0.10$). Increasing YC-EHY dose linearly increased ($P < 0.01$) plasma anti-ovalbumin IgG levels following 3 ovalbumin challenges, suggesting that treatments enhanced

humoral immunity. Increasing YC-EHY dose also quadratically increased fecal IgA concentrations in early lactation (P = 0.03), suggesting that 30 and 60 g/d doses enhanced mucosal immunity. These results provide some intriguing evidence that YC-EHY shift meal patterns, resulting in cows eating smaller, more frequent meals. By accounting for energy derived from release of stored tissue (i.e. body fat), we found that YCEHY tended to improve efficiency of energy utilization for milk production. Humoral immunity (antibodybased protection) is thought to be suppressed during the transition to lactation, so the ability of a feed additive to enhance this capacity is encouraging. Feeding a yeast culture product with enzymatically-hydrolyzed yeast did not affect milk production or DMI during the transition to lactation, but modulated feeding behavior and several aspects of immunity. In the current study, a 60 g/day dose of YC-EHY resulted in favorable changes in feeding behavior, mucosal and humoral immunity, and supported the numerically greatest energy efficiency and milk yield postpartum. Future studies with larger numbers of animals may provide more insight into production implications of these biological responses.

Effect of post-partum treatment with non-steroidal anti-inflammatory drugs on milk production and culling risk in dairy cattle

Multiparous cows (n = 51 per treatment) from a commercial dairy were enrolled in the study 12-36 hours after calving. Cows receiving SS treatment (SS) received a placebo bolus on day 1 of treatment and an oral drench containing 125/g/day of SS in 375 mL of water for 3 consecutive days beginning on day 1 of treatment. Meloxicam treated cows (M) received 675 mg of meloxicam as a bolus on day 1 of treatment in combination with 3 consecutive daily drenches of 375 mL of water. Control animals (CON) received a placebo bolus on day 1 and water drenches. Adjusted 305 d mature equivalent yields of milk, fat, and protein through DHIA testing revealed significant whole lactation milk and protein responses to both M and SS treatments, representing 6 to 9% advantages for the NSAID treatments. Numerical differences in fat yield were of similar magnitude (5 – 6%), but were not statistically significant. In addition, daily milk yield data from the farm management system were analyzed to assess treatment responses over time. The overall treatment effect determined by this analysis was again significant for both NSAID treatments ($P < 0.05$), with a slightly larger mean response of 10 – 12%. These results represent the fourth study demonstrating that short-term early lactation treatment with SS can enhance peak milk yield, and the first to demonstrate similar results with M. More importantly, this study is the first such finding with a relatively large sample size and with daily milk yield data to allow for accurate analysis of the lactation curve following treatment. Both M and SS increased 305-day milk and protein yields compared to CON with no effect on 305-d milk fat. These responses were primarily due to increased peak milk yield and sustained differences through late lactation, and did not appear until the 2nd month of lactation. Furthermore, neither treatment affected body condition score, and M tended to improve retention in the herd compared to CON. The long-term benefits of early lactation NSAID use are surprising and will require further research to understand the underlying mechanisms. Although it is not currently legal to use these approaches commercially, ongoing research may allow for nutritional or pharmaceutical approaches to take advantage of these findings in the future.

4. **Brian Crooker (University of Minnesota, Obj. 2)**

Effect of milk yield genotype on gene expression in liver and adipose tissue from periparturient Holsteins

Multiparous cows from unselected (stable milk yield since 1964; UH; n = 5) and contemporary CH; n = 6) Holsteins that differed in milk yield (6,200 and 11,100 kg milk/305 d) were fed the same diet ad lib, milked 2X/d, and exposed to the same management and environmental conditions. Liver and adipose biopsies were collected at -14, 3, 14, and 35 days in milk (DIM). RNA was extracted and expression of 38 genes (focused on the somatotropic axis, glucose and lipid metabolism) and 12 possible internal control genes determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 5 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 23 genes in liver and 20 in adipose was altered by DIM. Liver and adipose expression of 6 and 8 genes was greater in CH and of 8 and 7 genes was greater in UH, respectively. There were line by day interactions for IGFBP2 and IGF-ALS in liver and DGAT2, HNF4a, IGF2 in adipose. Hepatic GHRtot and GHR-1A were greater in UH than CH. GHR-1A decreased at 3 but recovered by 14 DIM. Adipose GHRtot was greater in CH. Hepatic IGFBP2 was greater in CH than UH, increased at 3 DIM and although decreasing, remained increased through 35 DIM. Hepatic IGF-ALS was greater in UH, decreased at 3 DIM in both lines and returned to prepartum values by 14 and 35 DIM in UH and CH, respectively. Hepatic FGF21 was greater in CH than UH and peaked at 3 DIM in both lines. FGF21 was not detected in adipose. DGAT1 was similar in UH and CH liver and greater in CH adipose. Liver DGAT1 increased at 3 DIM but was not altered by DIM in adipose. DGAT2 was similar in UH and CH liver and greater in UH adipose. Liver DGAT2 decreased through 35 DIM in both lines. Adipose DGAT2 decreased at 3 DIM and recovered by 35 DIM in UH but not in CH cows. Results are consistent with a prolonged postpartum reduction in hepatic sensitivity to somatotropin and in triglyceride synthesis from de novo fatty acids in adipose of contemporary Holsteins.

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

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. Next Generation Sequencing technology (RNA-Seq) will be used to determine transcript expression in liver, adipose, and mammary biopsies collected at -14, 3, 14, and 42 days postpartum. Traditional and liquid chromatography-mass spectrometry analysis of these tissues and serum and milk samples collected weekly will be used to determine metabolite concentrations. Bioinformatic analysis of differences between our divergent Holsteins will be used to identify networks, pathways, and specific mechanisms associated with regulating and coordinating nutrient use among tissues. 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 and we are currently processing and analyzing the samples that were collected.

Effect of milk yield genotype on immune-endocrine-metabolite interactions in dairy cows

Specific objectives of this study are 1) to determine hepatic inflammation and innate immune status and their correlation with circulating profiles of hormones and metabolites of the two genotypes during the transition from pregnancy to lactation and 2) to determine response (inflammation and circulating profiles) of the two genotypes to LPS administered during early lactation. Blood samples will be collected weekly from -5 to 5 weeks postpartum and processed immediately to determine leukocyte populations and neutrophil phagocytic activity, and oxidative burst. Metabolic status will be determined by targeted metabolite analysis for glucose, triglycerides, cholesterol, and NEFA and untargeted metabolomics analysis of lipids, amino acids, organic acids. Hormonal signaling will be assessed from plasma concentrations of insulin, growth hormone, insulin-like growth factor-I, and leptin. Inflammation biomarkers (haptoglobin, serum amyloid A, and xanthine oxidase) in plasma will be used to assess inflammation status. At -28 and 1 day postpartum, neutrophils from 5 cows per genotype will be collected for proteomic analysis by mass spectrophotometry. Milk samples will be collected weekly from 1 to 5 weeks postpartum and analyzed for protein, fat, lactose, and xanthine oxidase. Hepatic biopsies will be collected at -2, 1, 2, and 6 weeks postpartum and analyzed for triglyceride and lipid components, nitrated proteins, and cytochrome C oxidase activity, general histology, neutrophil and macrophage content and gene expression. The animal portion of this study has been completed and we are currently processing and analyzing the samples that were collected.

Effect of cattle genotype on innate immune response

Individual immune responses to pathogens can be variable, depending on environmental, genetic, and possibly epigenetic influences. Holstein and Angus cattle are selectively bred and managed for different traits, which could impact disease susceptibility between these breeds. Selection of dairy cows over the past 50 years has dramatically altered their genome and genome wide association studies have indicated SNPs in regions that are associated with immune response. 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 UMN unselected and contemporary Holstein calves (n = 4/genotype). Heifers were challenged with 0.5 ug LPS/kg BW and blood samples collected from -1 to 24 h post LPS administration for determination of inflammatory response components. Cryopreserved fibroblasts from skin biopsies were revived and challenged with LPS (100 ng/ml) for subsequent determination of inflammatory response components in the culture media 24 h after LPS administration. Samples have been collected and analysis is underway.

5. **Shawn Donkin (Purdue University, Obj. 2)**

Effect of postruminal infusion of fructose on hepatic steatosis

The objective of this study was to examine the effects of postruminal fructose supply on accumulation of liver triglycerides (TG) and other parameters of fatty liver disease in lactating

dairy cattle. Eighteen multiparous late-lactation (241.7 ± 28.5 d in milk) Holstein cattle were assigned to either control (CON), postruminal fructose infusion (INF), or a pair-fed (PAIR) group. INF cows were previously fitted with rumen cannulae and received 1,000 g/d D-fructose for 7 d as a 16.67% w/v fructose solution (1,000 g in 5 L water) delivered postruminally. As expected, milk production decreased (26.2, 23.1, 23.3 ± 0.79 kg for CON, INF, PAIR, respectively; $P < 0.05$) in both INF and PAIR groups during infusion period and was accompanied by decreased feed intake (23.5, 20.1, 19.4 ± 1.21 kg/d dry matter intake; $P < 0.05$. Data do not support the use of 7 d postruminal infusion of D-fructose at 1000 g/d as a model to study hepatic steatosis in lactating dairy cattle.

Regulation of bovine hepatic gene expression of gluconeogenic enzymes by nutrients and hormones: Differential regulation of bovine PEPCKC promoter by propionate and hormones

The objectives were to 1) study the control of bovine PCK1 promoter by propionate and major hormonal cues and 2) identify the potential propionate-responsive elements within PCK1 promoter. Genomic and mRNA sequence of bovine PCK1 was obtained through the NCBI database. Bovine PCK1 putative promoter from -1238 to +221 relative to Transcription Start Site (TSS) and the series of 5′-deletions, termed -815, -409, -251, -85 bp PCK1 promoter were synthesized using specific 5' primers and the same 3' primer. Mutations were made within the -1238 to +221 region using the GENEART Site-Directed Mutagenesis System kit (Invitrogen). The following mutations were generated: Mutation 1: deletion of the core sequence of the putative HNF4α site (from −1078 to − 1074bp), Mutation 2: deletion of the core sequence of the putative HNF4α site (from +68 to +72 bp), Mutation (1+2): deletion of the core sequence of both putative HNF4α sites (from −1078 to − 1074bp sequence of 5'-CAAAG-3' and from +68 to +72 bp). Each promoter-luciferase construct was transiently transfected into rat hepatoma (H4IIE) cells. Cells were exposed to various concentrations of propionate and hormones indicated in the figures below. Luciferase activity was determined in the cell homogenate 23 h later. Activity of PEPCKC promoter was normalized to expression of Renilla luciferase. Experiments were repeated in at least 3 separate cell preparations and 3 replicates within each preparation. Data were analyzed using the Proc Mixed procedure of SAS. Propionate linearly increases) the transcriptional activity of the bovine PCK1 promoter. The effects of propionate are greater than and additive to the effects of cAMP and DEX to induce the bovine PCK1 promoter. The primary induction of PCK1 is contained in two regions within the promoter, -1238/-409 and -85/+221. Site-directed mutagenesis on the core sequence of two separate HNF4α binding sites indicate that only 40% of PCK1 induction by propionate involves either, or both sites. These results indicated HNF4α binding sites are involved in, but not fully responsible for the propionate-induced transcription from the bovine PCK1 gene promoter.

6. Richard Erdman and Brian Bequette (University of Maryland, Obj. 1 and 2)

Changes in Diet Digestibility in U.S. Dairy Cows over the last 40 years

To account for the depression in digestibility with level of feeding, previous NRC reports had applied a constant 8% discount on maintenance energy values of feeds and predicted energy values at 3X maintenance feeding. The 2001 Dairy NRC adopted a system of continuous discounting of diet energy concentration with level of feed intake. In that system, diets that have higher maintenance TDN are discounted more than diets with lower maintenance digestibility and digestibility of diets is predicted to continue to decline as cows eat more and more feed.

Some researchers have questioned whether diet digestibility continues to decline with feeding levels above 3X or 4X maintenance feeding. Perhaps digestibility plateaus at a certain level of feed intake? Anticipating this question, this summer I had an undergraduate student (Melissa Shaughness) collect data from the literature on total tract digestibility in lactating dairy cows. We used data from reports published in Journal of Dairy Science since 1970. For a paper to be selected for possible inclusion, the study had to include the word "digestibility" in the title or abstract. Studies had to be conducted at U.S. institutions since we wanted to focus on dairy cattle in the U.S. Finally, only papers where digestibility data measured by either total collection or iNDF were included since we felt that other marker methods such as chromic oxide or AIA were not precise enough to provide accurate values of diet digestibility. Sarah Potts (VandeHaar former student) has conducted a preliminary analysis of the data is shown in the Figures 1 and 2 below. Figure 1 shows the general trends in measured feed intake and diet digestibility studies reported since 1970. First, the data is noisy and secondly, these studies were conducted at a relatively small number of institutions (15). Noise in the data would be expected since there is no way to correct for study effects or differences due to experimental treatments and diet other than gross composition of the diet (CP and NDF concentrations). As expected feed intake increased with year of study while and DM digestibility has continued to decrease over time. Over the last 44 years, diet digestibility has declined by 5.28 percentage units (.12 units per year) while DMI increased by 8 kg/cow/d. These results were consistent with the use of continuous adjustment of diet digestibility with level of feeding. Using that reasoning, we further adjusted the digestibility data for level of feeding effects using intake as a percentage of BW as a regression factor. While addition of DMI per unit BW in the regression analysis reduced the slope of the trend line for year effects, it was clear that increasing feed intake is not the only factor associated with the trend for reduced digestibility over time. Perhaps there are other adjustments due to diet effects (NSC, starch?) that need to be made? Also it might be possible that we are selecting cows that are just not as efficient at digesting feed but are able to compensate by eating greater and greater amounts of feed. Alternatively, even if today's cows appear to be less efficient at digesting feed, they might also compensate by being more efficient at converting apparently digested nutrients into milk.

Meta-analysis of Buffer Feeding Studies

We have been interested in the effects of DCAD and feed efficiency. There have been previous meta-analysis done on the effects of dietary DCAD on feed intake and milk production and acid-base status in lactating dairy cows (Hu and Murphy, 2004) which showed an increase in feed intake, milk fat percentage, and FCM with increasing DCAD in studies where measured DCAD ranged from -100 to over 500 mEq/kg DM. Prior to the use of the DCAD concept in dairy cattle feeding, numerous studies had been published on the use of buffers such as sodium and potassium carbonates or bicarbonates which would inherently alter DCAD. However, many of those studies could not be included in previous meta-analysis since they lacked complete cation composition (Na, K, or Cl) to calculate DCAD. In order to overcome that hurdle, Marie Iwaniuk looked at using the 2001 Dairy NRC software to estimate missing cation data using the diet ingredients reported in those studies. In general, the NRC software predicted DCAD in good agreement with measured reported values published in the literature. She then incorporated the previous studies with dietary buffers published from 1960 to 1990 to develop relationships between DCAD and performance and digestibility. As expected feed intake and milk production increased with increasing DCAD (Na + K – CL –S) up to ~ 350 mEq/kg DM. However, FCM continued to increase with increasing DCAD up to ~500 mEq/kg DM. This was due to the linear

relationship (data not shown) between DCAD and milk fat percent where fat percent increased at a constant 0.1 percentage units per 100 mEq DCAD over the entire range of reported DCAD (up to 800 mEq/kg DM). There is was also a linear relationship between digestibility of DM, and NDF and DCAD where digestibility increased by 0.7 and 1.6 units per 100 mEq/kg for DM and NDF digestibility, respectively. This suggests that the majority of the digestibility responses to DCAD were due to changes in fiber digestibility. We believe that many dairy producers are not feeding diets with adequate DCAD to maximize milk fat concentration and diet digestibility.

Comparative Glucose and Krebs Cycle Metabolism of the Bovine and Murine Mammary Gland

The compositions of bovine and murine milk differ significantly with respect to the proportions of lactose, protein, and fat. To better understand the metabolic origins of this difference, we focused on interrogating the crossroads of glycolysis and the TCA cycle in cows and mice using a glucose stable isotope tracer. We collected mammary tissue from mid-lactation dairy cows (n=4) and day 15 lactation mice (n=6) then sliced the tissue forming explants approximately 0.5mm thick (100-150mg). The tissue slices were incubated for 3 h (5% CO_2) at 37^0C in DMEM containing a 50:50 mix of unlabeled and [$^{13}C_6$] labeled glucose at one of four concentrations: 10mM, 7.5mM, 5mM, or 2.5mM. Following incubation, tissue slices were collected and stored at -80^0C until processing. Intracellular metabolites were extracted and derivatized for the determination of isotopomer enrichments via gas chromatography-mass spectrometry. The alanine, glutamate, and aspartate [$^{13}C_6$] enrichments were used as representatives of their TCA cycle counterparts pyruvate, α-ketoglutarate, and oxaloacetate respectively. These data provided the inputs to calculate glycolytic and TCA cycle fluxes. In dairy cows, increasing glucose concentration was reflected by increasing glycolytic rates seen in the contribution of glucose carbons to the pyruvate pool. However, glucose contributions to pyruvate reached a plateau at 44-46% for the two highest glucose concentrations, 7.5mM and 10mM. The mouse mammary also increased glycolytic rates with higher glucose reaching 43% glucose contribution to pyruvate at the highest glucose concentration though no plateau was observed. TCA cycle flux was established by the relative activity of pyruvate dehydrogenase (PDH) to pyruvate carboxylase (PC) based on [$^{13}C_6$] tracer kinetics. The dairy cow transitions towards higher PDH activity from the lowest glucose level, 2.5mM, to the three higher levels as displayed by a higher relative flux. In contrast, the mouse shows no change in relative activity with changing glucose concentrations. These data suggests the dairy cow shifts away from anapleurotic flux into the TCA cycle and towards energy producing activities with higher glucose availability while the mouse does not adapt in this way.

7. Jong-Su Eun (Utah State University, Obj. 1)

Effects of altering alfalfa hay quality when feeding steam-flaked versus high-moisture corn grain on ruminal fermentation and lactational performance of dairy cows.

This experiment was performed to test a hypothesis that nutritive benefits of feeding highmoisture corn (HMC) would be different when fed with different qualities of alfalfa hay (AH) due to associative effects on ruminal fermentation and nutrient utilization efficiency. Eight multiparous lactating Holstein cows were used; 4 were surgically fitted with ruminal cannula. Days-in-milk averaged 184 ± 10.7 at the start of the experiment. 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; fair quality AH [FAH; 39.6% neutral detergent fiber (NDF) and 17.9% crude protein (CP)] or high quality AH (HAH; 33.6% NDF and 21.9% CP) was combined with steam-flaked corn (SFC) or HMC to form 4 treatments: FAH with SFC, FAH with HMC, HAH with SFC, and HAH with HMC. The AH was fed at 32% dry matter (DM), whereas SFC or HMC was included at 17% DM. Quality of AH did not affect DM intake, whereas feeding HMC decreased DM intake regardless of quality of AH. Digestibility of DM was greater for cows fed HAH compared to those fed FAH (70.1 vs. 67.6%). Digestibility of NDF increased by feeding HMC (67.6 vs. 58.4%), but not by quality of AH. Under FAH, starch digestibility decreased by feeding HMC compared with SFC (85.7 vs. 95.0%), but it was similar under HAH, resulting in an interaction between quality of AH and type of corn grain (CG). Feeding different qualities of AH did not affect milk yield; however, feeding HMC decreased milk yield in FAH diet, causing an AH × CG interaction. Efficiency of milk yield/DM intake was improved due to feeding HMC regardless of quality of AH. In addition, dietary N utilization for milk N tended to increase by feeding HMC, but it was not influenced by quality of AH. Yield of microbial protein increased by feeding HAH diets compared with FAH diets, whereas feeding HMC diet increased microbial protein yield under HAH diet, leading to an interaction between and AH and CG.

*Birdsfoot trefoil (*Lotus corniculatus *L.) pasture affects in vitro ruminal fermentation characteristics and methanogenesis in continuous cultures due to condensed tannins.*

This study evaluated the effects of feeding 2 different pasture forages [orchardgrass (OG) vs. birdsfoot trefoil (BFT)] combined with 3 supplements [no supplement, ground barley (GB), and forage-concentrate mixture (FCMX)] on in vitro fermentation characteristics. The experiment was performed in a 2 (source of pasture forage) × 3 (supplement) factorial design with 3 independent runs of continuous cultures (n = 3). Culture pH averaged 6.15 and was not different across treatments. Total VFA concentration averaged 39.5 mM and did not differ among treatments. Feeding different pasture forages did not influence acetate and propionate concentrations. While acetate concentration was similar across treatments, propionate concentration increased with supplementing GB or FCMX, resulting in a decreased acetate-topropionate ratio due to the supplementations. Ammonia-N concentration tended to decrease ($P < 0.06$) with BFT compared with OG (9.40 vs. 13.5 mg/100 mL), whereas supplementation resulted in no difference in the ammonia-N concentration, regardless of source of pasture forage. Due to condensed tannins (CT) in BFT, density of cellulolytic bacteria was affected, but it was species-specific; *Ruminococcus albus* was not influenced due to CT, whereas *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* were reduced (Table 1). Likewise, *Methanobrevibacter* spp, were reduced, while *Methanomicrobium* spp. was not responded to CT. Methane production decreased when fermentors were offered BFT compared with OG (8.50 vs. 10.9 mmol/d), but supplementation did not affect the methane production under OG as well as BFT (Figure 1).

8. Ermias Kebreab (University of California, Davis, Obj. 3)

Quantifying Body Water Kinetics and Fecal and Urinary Water Output from Lactating Holstein Dairy Cows

The objective of the present study was to construct a mechanistic, dynamic, and deterministic mathematical model to quantify urinary and fecal water outputs (kg/d) from individual lactating dairy cows. The model contains four body water pools: reticulo-rumen (QRR), post-reticulo-rumen (QPR), extracellular (QEC), and intracellular (QIC). Dry matter (DM) intake (DMI), dietary forage, DM, crude protein, acid detergent fiber and ash contents, milk yield and milk fat and protein contents, days in milk, and body weight were input variables to the model. A set of linear equations were constructed to determine drinking, feed and saliva water inputs to QRR, and fractional water passage from QRR to QPR. Water transfer via the rumen wall was subjected to changes in QEC and total water input to QRR. Post- reticulo-rumen water passage was adjusted for DMI. Metabolic water production and respiratory cutaneous water (RCW) losses were estimated with functions of heat production in the model. Water loss in urine was driven by absorbed N left after being removed via milk. Model parameters were estimated simultaneously using observed fecal and urinary water output data from lactating Holstein cows (n = 670). The model was evaluated with data that were not used for model development and optimization (n = 377). The observations in both data sets were related to thermoneutral conditions. The model predicted drinking water intake, fecal, urinary and total fresh manure water output with root mean square prediction errors as a percentage of average values of 18.1, 15.6, 30.7, and 14.6%, respectively. In all cases > 96% of the prediction error was due to random variability of data. The model can also be used to determine saliva production, heat and metabolic water production, RCW losses, and size of major body water pools in lactating Holstein cows , particularly under thermoneutral conditions. Details are published in Appuhamy et al. (2014a).

Development of mathematical models to predict volume and nutrient composition of fresh manure from lactating Holstein cows

A set of empirical and deterministic models were developed for predicting fecal and urinary water, carbon (C), nitrogen (N), ADF and NDF output (kg/d) from lactating Holstein cows. Dietary nutrient contents, milk yield and composition, body weight, age and days in milk were used with or without dry matter intake (DMI) as potential predictor variables. Multicollinearity, goodness of fit, model complexity, and random study and animal effects were taken into account during model development, which used 742 measured fecal and urinary nutrient output observations (kg/d). When evaluated with independent data (n = 364), the models predicted fecal and urinary nutrient output successfully with root mean square prediction error as a percentage of average observed values (RMSPE%) ranging from 10 to 21%. All the predictions except urine output had RMSPE% ranging from 18 to 25%, even when DMI was not used as model input. Total fresh manure volume, C: N ratio, and hemicellulose and cellulose output predictions were appreciable, particularly if DMI was used (RMSPE% = 9 to 21%). Nutrient output predictions were in reasonable agreement with observed values throughout the data range (systematic bias < 14% of total bias). The models can be integrated successfully with process based manure or soil models to assess nutrient transformation in dairy production systems. Further details will be published in Appuhamy et al. (2014b).

9. James Fadel (University of California, Davis, Obj. 3)

Bayesian analysis of energy balance data from growing cattle using parametric and nonparametric modelling

The objective of this study was to analyze a large database of indirect calorimetry records on RE and HP in growing cattle. Maintenance requirements and partial efficiencies were estimated with parametric and nonparametric models under a Bayesian setting. Estimated NE_M and ME_M values were close in the piecewise linear and nonparametric models. Specifically, NE_M estimates (FCAT) were 0.50 MJ/kg $BW^{0.75}$d in the piecewise linear model and 0.49 MJ/kg $BW^{0.75}$d in the nonparametric regression model. Furthermore, ME_M was 0.52 MJ/kg $BW^{0.75}$d in the piecewise linear model and 0.53 MJ/kg $BW^{0.75}$d in the nonparametric model. The efficiencies were also similar between the piecewise linear and nonparametric regression models. Specifically, k_m was 0.94 and 0.92 in the piecewise linear and nonparametric models, respectively. Additionally, k_g was 0.54 and 0.61 in the piecewise linear and nonparametric models respectively. The estimate from the modified Lofgreen and Garrett model of 0.37 MJ/kg $BW^{0.75}$d was practically the same as the estimate from the logarithmic regression. The NE_M in the linear model was smaller than the one from modified Lofgreen and Garrett model (0.29 MJ/kg $BW^{0.75}$d). In particular, the 95% Credible Interval [0.25, 0.33] MJ/kg $BW^{0.75}$d contained the NE_M of 0.32 MJ/kg $BW^{0.75}$d proposed by Lofgreen and Garrett (1968) but does not contain the NE_M estimates from the logarithmic regression in this database. The NE_M estimate from the nonparametric model, was substantially larger than estimates from the other HP models (0.49 MJ/kg $BW^{0.75}$d). Its 95% Credible Interval [0.39, 0.61] MJ/kg $BW^{0.75}$d did not include the NE_M proposed by Lofgreen and Garrett (1968) and from any of the other HP models utilized in our study. DIC suggests that the nonparametric model was the best choice although the nonparametric model, by design, has a greater ability to fit to the data. Consequently, the DIC in the nonparametric model is expected to be substantially smaller than in the parametric models because of its greater flexibility even though it is expected to be penalized more severely for model complexity.

10. Jeffrey Firkins (The Ohio State University, Obj. 1)

Effect of lowered pH and increased solid passage rate on hydrogen escape and methane production from continuous culture

A recent theory implicates physiological conditions and rumen aqueous hydrogen (H2(aq)) concentration as drivers of methane emission and volatile fatty acid production. The objective of this research was to directly test two proposed mechanisms for methane mitigation: low pH inhibiting methanogens and cellulolytics, or high solids passage rate (kp) increasing H2(aq) concentrations. The present study was conducted as a 2×2 factorial treatment arrangement in a Latin Square design using continuous culture fermenters (n = 4, volume = 1.71L). Treatments were control pH (CpH; ranging 6.3 to 6.9) or low pH (LpH; 5.8 to 6.4) factorialized with either low kp (Lkp; 2.5%/hr) or high kp (Hkp; 5.0%/hr); total dilution by buffer was constant at 7.0%/hr. Fermenters were fed once daily (40 g DM; 50:50 concentrate:forage) and periods lasted 10 d with 3 d of sampling. The main effect of LpH decreased ($P < 0.001$) H2(aq) by 3.82 µM, but there was no effect nor interaction ($P > 0.10$) of kp. The main effect of LpH also decreased ($P < 0.001$) escaped hydrogen gas (H2(g)) 60.1 µmol/L×d but there was no effect ($P > 0.10$) of kp. A significant treatment × time interaction was explained in that CpH/Hkp had greater ($P < 0.05$) H2(g) from 15 to 24 h post-feeding compared with CpH/Lkp. Also, both CpH/Hkp and CpH/Lkp were greater ($P < 0.05$) than both LpH treatment combinations from 6 to 23 h post-feeding. There was no main effect ($P > 0.10$) of pH on total methane production, but Hkp tended ($P = 0.08$) to decrease total methane production compared with Lkp by 880 mmol/L×d. A

treatment × time interaction ($P < 0.01$) was explained in that CpH/Lkp had the greatest ($P < 0.05$) methane production from 11 to 23 h post-feeding, whereas LpH/Lkp was not different ($P > 0.10$) from CpH/Lkp at 24 h post-feeding. This was likely because the main of effect of Hkp decreased ($P = 0.02$) methane production rate 45.1 µmol/L×d compared with Lkp. Further, CpH/Lkp had greatest ($P < 0.05$) methane production rate from 2 to 11 h post-feeding. The main effect of LpH decreased ($P = 0.002$) A:P ratio from 2.61 to 2.34 compared with CpH. Also, Hkp decreased ($P = 0.002$) A:P ratio from 2.62 to 2.34 compared with Lkp. The results indicate increased kp and low pH decreased A:P ratio independent of changing the current diet. Low pH decreased hydrogen escape but not methane production. High solids passage rate tended to decrease methane production and decreased methane production rate.

Kinetics of Microbial Methionine Metabolism in Continuous Cultures Administered Different Methionine Sources

The Met precursor, 2-hydroxy-4-(methylthio) butanoic acid (HMB) is expected to be more extensively degraded in the rumen than its isopropyl ester (HMBi). A control and 3 isomolar treatments—0.097% DL-methionine (DL-Met), 0.11% HMBi (HMBi), and 0.055% HMBi plus 0.048% Met (Met+HMBi)—were dosed every 8 h simultaneously with the 3-time-daily feeding into continuous cultures. For 6 consecutive doses, both [1-^{13}C]-L-Met M1) and [methyl-$^{2}H_3$]-LMet (M3) replaced part of the unlabeled DL-Met; [$^{13}C_5$]-DL-HMBi (M5) replaced a portion of the unlabeled DL-HMBi; and [1-^{13}C]-L-Met and [$^{13}C_5$]-DL-HMBi replaced a portion of the respective unlabeled doses for the Met+HMBi treatment to evaluate kinetics of HMBi turnover and transfer into free Met followed by transfer into bacterial Met. The label in the carboxyl group (M1) recycled more and was recovered in bacterial Met to a lower extent than the label in the methyl group (M3) of Met. Increasing HMBi inclusion (0, 50, and 100% of the exogenously dosed Met molar equivalent) tended to increase its escape from 54.7 to 71.3%. Despite less Met available from HMBi and from less dosage of Met, increasing HMBi increased accumulation of free Met in fermenter fluid. Because HMBi (after de-esterification of the isopropyl group) produces Met through the intermediate α-ketomethylthyiobutyrate with an aminotransferase that also has high affinity for branched chain AA, we provide evidence that the HMBi-derived Met is likely released from bacterial cells and accumulates rather than being degraded, potentially as a result of D-stereoisomer metabolism. More research is needed to evaluate racemization and metabolism of stereoisomers of Met and other AA in ruminal microbes.

11. Timothy Hackmann (University of Florida, Obj. 3)

Accumulation of reserve carbohydrate by rumen protozoa and bacteria in competition for glucose

The aim of this study was to determine if rumen protozoa could form large amounts of reserve carbohydrate compared to bacteria when competing for glucose in batch cultures. We separated large protozoa and small bacteria from rumen fluid by filtration and centrifugation, recombined equal protein masses of each group into one mixture, and subsequently harvested (re-separated) these groups at intervals after dosing glucose. This method allowed us to monitor reserve carbohydrate accumulation of protozoa and bacteria individually. When mixtures were dosed with a moderate concentration of glucose (4.62 or 5 mM; n = 2 each), protozoa accumulated large amounts of reserve carbohydrate (Fig. 1c); 58.7 (2.2 SEM) % of glucose carbon was recovered in protozoal reserve carbohydrate at time of peak reserve carbohydrate. Only 1.7 (2.2

SEM) % was recovered in bacterial reserve carbohydrate, which was less than for protozoa ($P < 0.001$) (c.f., Fig. 1c,d). When provided a high concentration of glucose (20 mM; n = 4 each), 24.1 (2.2 SEM) % of glucose carbon was recovered in protozoal reserve carbohydrate, which was still more ($P = 0.001$) than the 5.0 (2.2 SEM) % of glucose carbon recovered in bacterial reserve carbohydrate. Our results suggest that protozoa can sequester sugar from bacteria by accumulating reserve carbohydrate, giving protozoa a competitive advantage and perhaps stabilizing fermentation in the rumen.

Uptake of a fluorescent analog of glucose (2-NBDG) by rumen bacteria: Specificity and kinetics compared to [14C]-glucose

Most rumen bacteria cannot be cultured, making their niche in the rumen difficult to identify. Fluorescent substrates have potential identify substrates preferences and thus the niche of these uncultured bacteria, but specificity and kinetics of their uptake have not been thoroughly evaluated. The aim of this ongoing study is to determine if cultured strains of rumen bacteria would take up a fluorescent analog of glucose (2-NBDG) with the same specificity and kinetics as [14C]-glucose. To determine specificity, we are screening 2-NBDG uptake by 12 bacterial species that ferment glucose and 4 species that do not. Thus far, we have found that 2 glucose-fermenting species (Streptococcus bovis JB1, Selenomonas ruminantium HD4) indeed take up 2-NBDG, but 1 species (Megasphaera elsdenii T81) does not. For S. bovis JB1, Vmax for 2-NBDG uptake was 3.5-fold lower than that for [14C]-glucose, and Km was 8.5-fold lower. Based on these results with cultured bacteria, 2-NBDG would not be expected to identify some, but not all, glucose-fermenting bacteria in the rumen.

12. Mark Hanigan (Virginia Polytechnic Institute and State University, Obj, 2 and 3)

Rapamycin, AICAR, and individual non-essential amino acids on mTOR signaling and casein synthesis rates

Additional work was completed with our lactogenic, tissue slice model to assess the effects of rapamycin, AICAR, and individual non-essential amino acids (NEAA) on mTOR signaling and casein synthesis rates. The level of inclusion of individual NEAA had no impact on cell signaling or rates of casein synthesis demonstrating that regulation of the mTOR pathway is restricted to the essential amino acids (EAA). AICAR, which stimulates phosphorylation of AMPK and thus simulates an energy deficiency in the cell, had very marginal effects on mTOR phosphorylation, but almost completely abated casein synthesis. This suggests that mTOR is not the only regulatory pathway controlling energy signaling. The cells were quite resistant to rapamycine requiring a dose 10 fold greater than normally used to have any effect on cell signaling or casein synthesis, and then only minor reductions.

Varying volatile fatty acid concentrations and pH on hydrogen and methane production

A dual continuous flow fermentor study was conducted at Ohio in collaboration with Firkins and Kohn of Maryland with the objective of determining the effect of varying volatile fatty acid concentrations and pH on hydrogen and methane production. The experiment constituted 4 treatments applied in 4 periods. Treatments were: control, acetate infusion (20 mmol/d), propionate infusion (7 mmol/d), and low pH (0.5 units lower than control). The fermenters were fed 40 g of a pelleted 50:50 alfalfa: concentrate diet once daily. Aqueous methane

concentrations were significantly higher ($P<0.05$) for low pH and propionate treatments compared to control and acetate treatments. Headspace hydrogen production was significantly higher for the acetate treatment and lower for the pH treatment. Acetate treatment resulted in greater cumulative hydrogen production than the other treatments, whereas propionate treatment had no effect. The effect of treatment on aqueous hydrogen concentration was not significant ($P>0.05$). These results reveal that aqueous methane concentration is affected by low pH and propionate infusion, and acetate infusion increases the headspace hydrogen production in continuous ruminal fermenters.

Including thermodynamic driven interconversions among the VFA in Molly

The objective of this work was to modify the model to include thermodynamic driven interconversions among the VFA, rederive parameter estimates with and without VFA interconversion, and compare prediction errors to test the hypothesis. Prediction accuracy for all 3 VFA was improved by simply refitting the model to the data, as expected. Although further improvements in predictions of concentrations of VFA were observed with inclusion of interconversions, the change in prediction errrors were very small. Surprisingly, prediction accuracy was slightly less for net production rates after refitting Molly, and errors increased slightly more for acetate and propionate with inclusion of the interconversions. Based on this work, there appears to be little value in considering the effects of thermodynamics on VFA production in existing models of rumen function. However, only a couple of the studies used for the work were designed to test such effects, and thus additional data are needed to fully test the hypothesis.

Modelling predictions of grass digestibility and CH_4 predictions from fresh temperate grass diets

Ruminants convert forage into human edible products thus representing a vital contribution to food sufficiency. However, they also generate large quantities of methane (CH_4) and excrete more than 70% of the nitrogen they consume. Maximizing human edible food production while minimizing production costs and environmental impact requires a robust representation of animal responses to a wide range of inputs. The Molly cow model represents key elements of digestion and metabolism and thus is potentially useful in such an exercise. However, it has not been sufficiently evaluated on fresh grass diets. The objective of this work was to assess model predictions of grass digestibility and CH_4 predictions from fresh temperate grass diets. Ruminal degradation rates for protein, starch, and fiber are scaled in the model using observed dietary *in situ* rates which have been previously calibrated to North American diets. The approach worked well for grass protein; however, fiber digestibility was dramatically under predicted when using observed *in situ* measurements necessitating recalibration of the model. After calibration, root mean square prediction errors expressed as a percent of the observed values (RMSPE) were 11.8, 12.9, 39.5, 12.6, 8.2, and 26.2% for NDF passage, ADF passage, nonammonia, nonmicrobial nitrogen (NANMN) passage, fecal NDF output, fecal ADF output, and fecal N output, respectively. More than half of the NANMN error was associated with slope bias indicating that the representation of ruminal N degradation did not fully capture the true process. The model predicted CH_4 production for dry and lactating cows fed alfalfa pellets and fresh grass with concentrate, respectively with an RMSPE of 25%. There was no prediction bias with respect to predicted CH4 production, intake rate, or dietary NDF content.

Expand an existing model of EAA effects on mTOR phosphorylation

The mammalian target gene of rapamycin, mTOR, regulates rates of mRNA translation, initiation and elongation. Specific essential amino acids (EAA) increase mTOR phosphorylation in mammary epithelial cells and this has been correlated with increased rates of casein synthesis. The objective of the present work was to expand an existing model of EAA effects on mTOR phosphorylation, described by Appuhamy and Hanigan (2010), to include individual EAA and to incorporate the effect of mTOR phosphorylation on synthesis rates of α-S1-casein ($SR_{(Csn)}$) in the mammary gland of lactating dairy cows. Isoleucine and phenylalanine were the EAA with the most explanatory power for mTOR phosphorylation (RMSPE, 20% of the observed mean). Casein synthesis, which was originally represented as a function of EAA, had reduced prediction errors when represented as a mass action function of predicted mTOR phosphorylation. Additional work is underway to extend the cell signaling model to include signaling proteins involved in mediating energy and insulin signaling and to assess the independent effects of EAA as regulators of protein synthesis and as substrates.

Modelling of phosphorus excretion of cattle

Eutrophication caused by excessive phosphorus (**P**) and nitrogen (**N**), released into aquatic system impairs growth and survival of aquatic species. Continuous land application of animal manures based on crop N demand has resulted in a buildup of P because the N to P ratio in manure typically does not match crop requirements. Excessive P is transported to surface water aggravating eutrophication. Numerous studies reported that fecal P excretion is positively correlated with dietary P intake in dairy and beef cattle (Wu et al., 2000, Knowlton and Herbein, 2002, Geisert et al., 2010). Thus it is important that P requirement models are accurate so that dietary P is closely matched to animal requirements if excretion of P is to be minimized. It was hypothesized that consideration of the form of P within dietary ingredients would yield better predictions of P availability to the animal. The P digestion and metabolism model of Hill et al. (2008) was fitted to data from six studies (Feng et al., 2011, Ray et al., 2012b, Feng et al., 2013, Ray et al., 2013, Feng et al., 2014, Jarrett et al., 2014) that measured ruminal and intestinal digestion of phytate, inorganic, and non-phytate organic forms of P. All digestive parameters in the model were well described by the data. Prediction errors were relatively large ranging from 22% to over 100%, however, the model exhibited no slope or mean bias after fitting. Variables with large error of prediction were generally those representing flows of P fractions that were quite small due to high digestibility in the digestive tract. Thus it was concluded that the model was an unbiased representation of the data. Key findings were that ruminal digestion of phytate is greater than 85%, intestinal digestion of non-phytate, organic P was very minimal, a portion of the inorganic P is absorbed from the large intestine, and inorganic P absorption from the small intestine was highly regulated based on post-absorptive need.

13. Kevin Harvatine (Pennsylvania State University, Obj. 2)

Effect of lysolecithin on fatty acid biohydrogenation and milk fat synthesis.

Thirteen multiparous Holstein cows (> 70 DIM) were used in a cross-over design that tested the effect of lysolecithin under diets differing in fermentability and polyunsaturated fatty acid (FA) concentration. Experimental periods were 20 d and included two 10 d phases. During phase 1, a standard fiber and low fat diet was fed (32% NDF, no added oil) and during phase 2 a lower NDF higher oil diet was fed (30.5% NDF and 2% oil from whole soybeans and soybean oil). A 14 d washout period between experimental periods allowed milk fat recovery. Treatments were

control and lysolecithin (10 g/d/cow of LYSOFORTE™, Kemin Industries, Des Moines, IA) extended in a ground corn carrier. Milk was sampled on d 0, 5, and 10 of each phase for determination of fat and protein concentration and FA profile. There was no effect of treatment or treatment by time interaction for DMI or milk yield, however on d 5 of phase 2 lysolecithin tended to decrease DMI ($P < 0.10$). There was a treatment by time interaction for milk fat concentration and yield ($P < 0.05$). Milk fat concentration was higher in lysolecithin on d 5 of phase 1, but decreased progressively in both treatments during phase 2. Milk fat yield was not different among treatments during phase 1, but was lower in lysolecithin on d 5 and tended to be lower on d 10 of phase 2 ($P < 0.10$). There was no effect of treatment or treatment by time interactions for milk protein concentration or yield. No treatment by time interactions were detected for the concentrations of milk de novo (< 16 C) or preformed (> 16 C) FA. Concentrations of de novo FA decreased, but preformed FA increased during phase 2 ($P < 0.001$) and no treatment differences were detected at any time point. There was an effect of time, but no treatment by time interactions for milk trans FA isomers ($P < 0.05$). Briefly, trans 11 C18:1 and cis-9, trans-11 conjugated linoleic acid (CLA) decreased progressively during phase 2 as trans 10 C18:1 and trans-10, cis-12 CLA increased progressively. Lysolecithin increased milk fat concentration when feeding a higher fiber and lower fat diet, but decreased milk fat yield when feeding a lower fiber and higher fat diet, although biohydrogenation pathways were not modified.

Time-course of select ruminal microbes during induction and recovery from diet induced milk fat depression in dairy cows.

Diet induced milk fat depression (MFD) results from bioactive fatty acids produced in the rumen during altered rumen biohydrogenation, and concurrent changes in the rumen microbial population are commonly proposed as a key factor in development of the condition. An experiment was conducted to characterize the changes in select rumen microbial populations during induction and recovery from dietinduced MFD. Eight ruminally cannulated cows were used in repeated design and fed a low fiber, high PUFA diet (Induction; 29.5% NDF and 3.7% PUFA; DM basis) for a period of 21 d, and then switched to a high fiber, low PUFA diet (Recovery; 36.9% NDF and 1.1% PUFA) for 21 d. The control was the high fiber, low PUFA diet. We have previously reported decreased milk fat yield by d 7 and near maximal MFD by d 13 during induction and a progressive increase in milk fat yield with full recovery by d 15 during recovery. Ruminal digesta samples were collected 8 h after feeding on days 0, 4, 8, 12, and 20 and select ruminal microbial populations were quantified by Real-time PCR. Data were analyzed by PROC MIXED with the repeated statement and treatments compared at each time point. Treatment by time interactions were observed for most taxa ($P < 0.05$). Megasphaera eldesnii and S. ruminantium (lactate using bacteria) increased progressively >170% until d 12 of induction and decreased progressively during recovery. Streptococcus bovis (amilolytic bacteria) peaked at 350% higher than control on d 4 of induction ($P < 0.01$) and rapidly decreased during recovery. Prevotella bryantii (amilolytic bacteria) decreased 66% from d 8 to 20 of induction compared with the control and increased to control levels by d 12 of recovery. Ruminococcus albus (fibrolytic bacteria) and P. ruminicola (fibrolytic bacteria) were nearly constant during induction and recovery. However, F. succinogenes (fibrolytic bacteria) decreased 97% compared to control by d 4 of induction and increased progressively to an equal extent during recovery. The Butyrivibrio/Pseudobutyrivibrio group (C18:1 trans-11 producer) decreased progressively during induction and increased during recovery, whereas the Butyrivibrio hungatei group (C18:1 trans11 producer) was not affected by treatment. Both ciliate protozoa and total fungi decreased

progressively by >90% during induction and increased during recovery. Rapid adaptation of most of the observed microbial species occurred during both induction and recovery from diet-induced MFD, and the time-course of adaptation matches the time-course of changes in biohydrogenation intermediates and inhibition of milk fat.

Effect of 2-hydroxy-4-(methylthio)butanoate (HMTBa) on risk of diet-induced milk fat depression.

Dietary polyunsaturated fatty acids (FA) and diet fermentability are key risk factors for diet-induced milk fat depression (MFD). A role for HMTBa in increased milk fat yield has been proposed, but the interaction of HMTBa and dietary risk factors for MFD have not been investigated. The objective was to evaluate the effect of HMTBa (ALIMET® feed supplement, Novus International, Inc., St. Charles, MO, USA) on milk fat synthesis when feeding diets with increasing risk for MFD. Thirty multiparous Holstein cows [227 ± 88 DIM, producing 38 ± 17 kg milk/d (Mean ± SD)] were used in a randomized block design. Treatments were control (corn carrier) and HMTBa (HMTBa fed at 0.1% of the diet DM provided with a corn carrier). The experiment was 70 d and included a 14 d covariate period followed by three phases that fed diets with increasing risk of MFD. During the low-risk phase (28 d) the base diet was balanced to 33.5% NDF and had no exogenous oil, during the moderate-risk phase (14 d) the diet was balanced to 31% NDF and contained 0.75% soybean oil, and during the high-risk phase (14 d) the diet was balanced to 28.5% NDF and contained 1.5% soybean oil. Milk yield, DMI, and BW were measured daily. Milk was sampled every 7 d and analyzed for fat and protein concentration. Data were analyzed using PROC Mixed with repeated measures and the effect of treatment was tested at each time point. There was no overall effect of treatment or treatment by time interaction for DMI, BW, milk yield, and milk protein concentration and yield. A treatment by time interaction was observed for milk fat concentration ($P = 0.02$) and yield ($P = 0.01$). HMTBa increased milk fat percent during the high-risk phase on d 63 (2.83 vs 3.55, $P < 0.0001$) and d 70 (2.91 vs 3.43%, $P = 0.005$) and increased milk fat yield on d 63 (821 vs 1093 g/d, $P = 0.002$) and d 70 (771 vs 951 g/d, $P = 0.018$). In conclusion, HMTBa increased milk fat yield when cows were fed a diet with a high risk of diet-induced MFD.

13. Alex Hristov (Pennsylvania State University, Obj. 1)

Effects of rumen-degraded protein and rumen-protected amino acids on performance of dairy cows fed metabolizable protein-deficient diets.

The main objective of this experiment was to investigate the effects of slow-release urea and rumenprotected (RP) Met and His supplementation of a metabolizable protein (MP)-deficient diet on lactation performance of dairy cows. Sixty Holstein cows (87 ± 40 DIM and 640 ± 70 kg BW) were used in a 10-wk randomized complete block design trial. After a 2-wk covariate period, cows were blocked by parity, DIM, and milk yield, and randomly assigned to 1 of 5 dietary treatments: MP-adequate diet [AMP; 107% of MP requirements (NRC, 2001)]; MP-deficient diet [DMP; 95% of MP requirements]; DMP supplemented with Optigen (Alltech Inc.; DMPO); DMPO supplemented with RPMet as Mepron (Evonik Industries AG; DMPOM); and DMPOM supplemented with RPHis (Balchem Corp.; DMPOMH). The basal diet consisted of (DM basis): 43% corn silage, 8% grass hay, 4% cottonseed hulls, and 45% concentrate and contained 16.7, 15.8, and 14.8% CP for AMP, DMPO, and DMP, respectively. Total-tract apparent digestibility of nutrients, and urinary N and urea excretions were decreased ($P < 0.01$)

by DMP compared with AMP. Relative to AMP, milk N secretion as a proportion of N intake tended to be higher (P = 0.07) for DMP. DMI was not affected by MP level but tended to be higher (P = 0.09) for the RPHissupplemented diet (28.4 kg/d) compared with DMPOM (27.0 kg/d). Yields of milk and milk fat were not affected by treatment, averaging 44.0 kg/d and 1.56 kg/d, respectively; milk fat content tended to be lower (P = 0.06) for DMPOMH (3.36%) than DMPOM (3.78%). Milk true protein content was increased (3.26 vs. 3.16%, P = 0.04) and milk protein yield was numerically increased (1.49 vs. 1.39 kg/d, P = 0.14) by RPHis, compared with DMPOM. Cows fed DMP gained 14 g/d BW whereas cows on all other treatments gained on average 267 g/d ($P \leq 0.10$). Supplementation of the DMPO diet with RPAA increased (P = 0.03) plasma glucose and numerically increased (P = 0.12) plasma insulin. In conclusion, feeding a 5% MP-deficient diet did not decrease DMI and yields of milk and milk components, despite the reduction in nutrient digestibility. Supplementation of the DMPOM diet with RPHis tended to increase DMI and increased milk protein content. These results confirm previous data and suggest that His may have a positive effect on voluntary feed intake in high-yielding dairy cows.

Effect of dietary supplementation of Capsicum extract on immune responses, blood cell counts, blood chemistry, and oxidative stress markers in lactating dairy cows.

The objective of this experiment was to investigate the effects of dietary Capsicum extract (CE) on T-cell phenotypes, phagocytotic and oxidative burst activity of neutrophils, blood cell counts, blood chemistry, and oxidative stress markers in lactating dairy cows. Eight multiparous Holstein cows (DIM, 50 ± 9.6 d; BW, 591 ± 32.6 kg), including 3 ruminally-cannulated, were used in a replicated 4 × 4 Latin square design with 25-d periods. Treatments were 0 (CON), 250, 500, and 1,000 mg CE/cow/d, in which the principal active compounds were capsaicin and dihydrocapsaicin. The CE was mixed with a small portion of the TMR and topdressed. Compared with CON, CE did not affect concentration of cluster of differentiation antigen (CD) 4 positive, CD8+, CD25+, and γδ+ cells. The phagocytosis and oxidative burst activity of neutrophils were also not affected by CE. Relative to CON, total white blood cells, neutrophils, and eosinophils were linearly increased (P = 0.04, 0.01, and 0.03, respectively) with CE supplementation. Treatments had no effect on lymphocytes, monocytes, and basophils. Red blood cells quadratically increased (P = 0.04) with CE. Hemoglobin was higher (P < 0.01) for CE than CON and responded quadatically to CE level of supplementation. Platelets were lower for CE than CON and linearly decreased (P = 0.04) with CE supplementation. Glucose, creatinine, albumin, and total protein in blood plasma were not affected by CE. Blood urea N was increased (P = 0.02) by CE relative to CON and blood plasma P concentration tended to be lower (P = 0.09) for CE than CON. Although there was no effect of CE on oxygen radical absorbance capacity (ORAC) and thiobarbituric acid reactive substances (TBARS), CE tended to decrease (P = 0.09) 8-isoprostane relative to CON (14.7 vs. 16.5 pg/mL, respectively). In conclusion, dietary supplementation of CE did not affect T cell phenotypes and neutrophil activities in this study. However, CE increased total white blood cells, neutrophils, and eosinophils, and tended to decrease 8-isoprostane. It is suggested that CE may facilitate cells with function in innate immunity and reduce blood oxidative stress markers in lactating dairy cows.

Extruded Soybean Meal Increases Feed Intake and Milk Production in Dairy Cows

Extruded soybean meal (ESBM) has higher fat content and lower ruminal protein degradability than solvent-extracted soybean meal (SSBM), but information on its nutritive value for dairy

cows is scarce. A replicated 3 × 3 Latin square design trial with 9 Holstein cows (parity, 3.1 lactations; DIM and BW at the beginning of the trial, 161 ± 21 d and 637 ± 20.3 kg, respectively) and 28-d experimental periods was conducted to evaluate the effect of ESBM processed at 2 extruder temperatures, 149°C (LTM) and 171°C (HTM), on milk production and composition and blood plasma amino acid profile in dairy cows. The control diet contained 13% SSBM [53.8% crude protein (CP) with 71.4% ruminal degradability and 1.8% ether extract (EE)], which was replaced with equivalent amount (DM basis) of LTM (46.8% CP, 59.8% degradability, 10.0% EE) or HTM (46.9% CP, 41.1% degradability, 10.9% EE) ESBM in the 2 experimental diets (LTM and HTM, respectively). Other ingredients in the diets were (DM basis): 40% corn silage, 20% alfalfa haylage, 5% grass hay, 9% ground corn grain, 5% cottonseed hulls, 5% molasses, salt, urea (LTM and HTM diets only), and mineral-vitamin premix. The diets had 16% CP and met or exceeded the NEL and metabolizable protein requirements of the cows (NRC, 2001). Both LTM and HTM tended to increase (P = 0.06) DMI compared with the control diet (28.3, 28.2, and 26.8 kg/d, respectively). This resulted in increased (P < 0.001) milk yield by both ESBM diets: 40.2 and 40.8 vs. 37.5 kg/d, respectively. Milk fat (3.38 to 3.60%) and milk true protein (2.86 to 2.95%) contents and milk fat yield were not affected by treatment. Milk protein yield tended to be increased (on average by 60 g/d; P = 0.09) by the ESBM diets. Plasma urea N and MUN were increased (P < 0.03) 18 and 13%, respectively, by the ESBM diets compared with the control. Blood plasma concentrations of His, Leu, Val, were increased (P ≤ 0.03) by HTM compared with the control and LTM. Concentration of plasma Met was decreased (P = 0.05) and that of carnosine was increased (P = 0.02) by the ESBM diets compared with the control. This study demonstrated that replacement of SSBM with ESBM in the diet of lactating dairy cows increased feed intake, which resulted in increased milk yield, and increased milk protein yield.

Effect of Cashew Nut Shell Liquid on Lactation Performance and Rumen Methane Production in Dairy Cows.

Technical cashew nut shell liquid (CNSL) is a by-product of the cashew nut industry in tropical countries, and is known to exhibit a wide range of biological activities, including inhibitory effect against grampositive bacteria. This study was conducted to investigate the effects of CNSL (73.3% cardanol, 16.4% cardol, and 3.0% metilcardol) on DMI, milk yield and composition, rumen fermentation and CH4 and CO2 production, and nutrient digestibility in dairy cows. Eight multiparous Holstein cows (DIM, 140 ± 14 d; BW, 669 ± 47.8 kg) were used in a crossover design trial with two, 21-d periods. The TMR was based on corn silage and alfalfa haylage, and was formulated to meet or exceed the energy and metabolizable protein requirements of the cows (NRC, 2001). The diet contained (DM basis): 15.5% CP, 32.0% NDF, and 1.53 Mcal/kg NEL. Treatments were control (CON, no CNSL supplementation) or 30 g/cow/d CNSL. The daily dose of CNSL was mixed with about 2 kg of TMR and top-dressed. Dry matter intake (average 26.6 ± 1.0 kg/d), 3.5% FCM (38.8 ± 1.6 kg/d) and milk composition (fat 3.32 ± 0.28 and protein 3.09 ± 0.05) were not affected by CNSL. Milk yield was numerically increased (P = 0.13) by CNSL (40.9 kg/d) compared with the control (39.0 kg/d). Rumen CO2 production, measured using GreenFeed (C-Lock Inc., Rapid City, SD), was not affected by CNSL. Compared with the control, CNSL numerically decreased (P = 0.12) rumen CH4 production (534 vs. 505 ± 39.6 g/cow/d, respectively) and CH4 emission intensity (P = 0.16; 13.3 vs. 12.3 ± 1.05 g/kg milk) and tended to decrease (P = 0.08) CH4 production per kg of DMI (19.1 vs. 20.3 ± 0.83 g/kg). CNSL did not affect total tract apparent digestibility of nutrients, except NDF digestibility tended to be increased (P = 0.09; 36.8 vs. 34.2 ± 1.47%, respectively).

Total urinary N, urea N, and urinary purine derivatives excretions were not affected by treatment. MUN concentration was numerically increased ($P < 0.13$) in cows receiving CNSL (8.57 vs. 7.50 ± 0.62 mg/dL, respectively). Plasma urea and glucose concentrations were not affected by CNSL. In this study, CNSL tended to decrease rumen CH4 production per kg DMI and numerically increased milk yield without affecting DMI in dairy cows.

Successful Mitigation of Rumen Methane Production in Dairy Cows Using an Inhibitor

Livestock is a major contributor to methane, a potent greenhouse gas, emissions in the U.S. and globally. This experiment was undertaken to test the effect of a methane inhibitor on rumen methane production, feed intake, and milk yield and composition in lactating Holstein cows. The experiment was a randomized block design with a 2-wk covariate period and 12 wks of data collection. The trial involved 48 primi- and multiparous cows (77 ± 3.9 DIM and 2.2 ± 0.16 lactations, at the beginning of the trial) allocated to 4 treatments: control (no additive) and a methane inhibitor applied at 40 (Low), 60 (Medium), and 80 (High) mg/kg feed DM. The inhibitor was mixed with the TMR as a premix. A placebo premix was added to the control TMR. Methane, carbon dioxide, and hydrogen gas production in the rumen was measured using two methods: GreenFeed and the SF6 tracer method. Rumen gas production was measured during wks 2, 6, 9, and 12 of the trial and one wk after the treatment was discontinued. Feed intake and milk production and composition data for the first 2 wks of the experiment were discarded. Dry matter intake (average of 27.5 ± 0.42 kg/d), milk yield (45.5 ± 1.06 kg/d), and milk fat and protein concentrations were not affected by treatment. Milk protein yield was quadratically increased ($P = 0.006$) by the inhibitor. Body weight of the cows was not different among treatments, but BW gain tended ($P = 0.10$) to linearly increase for the inhibitor treatments (17.6 vs. an average of 32 kg/12 wks). Rumen carbon dioxide production was not affected by treatment. Methane production was linearly decreased ($P < 0.001$) by the inhibitor as measured by both GreenFeed and SF6 methods. The average methane production for the inhibitor treatments was about 30% lower than the control (338 vs. 481 g/d, SEM = 15.9, respectively, for GreenFeed and 367 vs. 485 g/d, SEM = 29.8 for SF6). Methane production per unit of DMI or per unit of milk were also about 30% lower ($P < 0.001$; both measurement methods) for the inhibitor treatments vs. the control. Hydrogen production (GreenFeed only) was linearly increased ($P < 0.001$; SEM = 0.116) by the inhibitor treatments (0.48, 0.96, and 1.27 g/d for Low, Medium, and High, respectively) compared with the control (0.02 g/d). The effect on methane production occurred within 2 wks of treatment and disappeared within a wk after the treatment was discontinued. This experiment demonstrated that the inhibitor compound used decreased methane production in the rumen of high-producing dairy cows by 30% without negatively affecting DMI or milk production and composition. The effect persisted after 12 wks of treatment. The decrease in methane production was accompanied by an increase in hydrogen gas production.

15. Heidi Rossow (University of California, Davis Obj. 1 and 3)

Updating Molly with new oxidation rates and ATP stoichiometry

Using estimates of mitochondrial respiration and oxidation, equations within Molly describing oxidation rates of glucose, fat, acetate, propionate and butyrate have been updated (based on information learned from Human model publication-see below). Stoichiometric coefficients

representing ATP yield per NADH and FADH have also been changed to reflect lower PO ratios and lower energetic efficiencies i.e., 32 ATP yield instead of 38 ATP from glucose oxidation.

Development of methods to monitor feed management and cow performance on commercial dairies

Developing methods to monitor feed management and cow performance on commercial dairies including assessing variability in nutrient supply and mixer wagon function using Chloride, lignin or fat content in the TMR, assessing calcium intake and excretion in dry cows, identifying blood markers of fatty liver and using mitochondrial flexibility to indicate future performance and longevity in the herd.

16. J.W. Schroeder (North Dakota State University, Obj. 1)

Evaluating lactating dairy cow diets containing varying levels of mustard bran

The aim is to determine the nature of hemolysis in dairy cows and its effects on animal health. The objective is to determine 1.) The S-methyl-cysteine sulfoxide content in mustard bran; 2.) The rate of conversion of S-methyl-cysteine to the toxic S-methyl cysteine dimethyl disulfide in the rumen; 3.) The nutrient digestibility of mustard bran (in vitro) and 4.) To determine milk production and composition, rumen fermentation, and intake responses of lactating Holstein cows. A lactation trial utilizing 40 Holstein cows in a randomized complete block design of treatments will compare a control diet replacing a portion of soybean meal and beet pulp with graded levels of mustard bran (0, 2.5, 5, and 8%) based on the S-methyl-cysteine sulfoxide content.

17. Michael VandeHaar (Michigan State University, Obj. 1, 2, and 3)

Relationships of digestibility and efficiency in cows fed high or low starch diets

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Data from four separate cross-over experiments were used to determine the relationship between RFI and digestibility. Cows were fed high (HS) or low (LS) starch diets, which were formulated to distinctly differ in starch content. High starch diets resulted in similar MilkE (P=0.34), but greater BodyE (P<0.01) than LS diets, and energy captured as milk output plus body tissue gain was greater for HS than LS (P<0.01). Estimated NEL intake was greater for HS than LS (44 vs. 42 Mcal/d; P<0.01), but GE intake was similar (~107 Mcal/d; P=0.16). Multiple of maintenance based on intake (MMI) and multiple of maintenance based on requirements (MMR) were both greater when cows were fed HS diets (P<0.01). Gross efficiency was greater when cows were fed HS compared with LS diets (31 vs. 29%; P<0.01), but milk:feed was not different (P=0.87) between HS and LS. RFI was not different between HS and LS diets. Digestibilities of starch and DM were greater for HS diets (P<0.01), but NDF digestibility was greater for LS diets (P<0.01). High RFI and LRFI cows had similar MilkE, MBW, and BodyE, but LRFI cows had significantly lower DMI, and significantly greater milk:feed, gross efficiency, and apparent DietNEL than HRFI cows for both diets. Digestibility of DM was correlated negatively with RFI (r=-0.30; P<0.01) when cows were fed LS diets, but not when cows were fed HS diets (r=-0.01; P=0.90). Starch digestibility correlated negatively with RFI when cows were fed both HS and LS (r=-0.31 and r=-0.23; P<0.01 and P=0.02, respectively). Digestibilities of NDF and CP correlated negatively to RFI when cows were fed LS diets (r=-0.23 and r=-0.23; P=0.02 and P=0.02, respectively), but not when cows were fed HS diets (P>0.5). Starch digestibility was

significantly greater for LRFI cows than HRFI cows (P<0.05) for HS diets but not LS diets. Digestibilities of NDF and DM were significantly greater for LRFI cows than HRFI cows for LS diets (P<0.05) but not HS diets. Digestibility of CP was similar among HRFI, MRFI, and LRFI cows for both HS and LS diets (P>0.05). When DM digestibility and the interaction of DM digestibility and diet were incorporated as covariates in the linear model used to estimate DMI and determine RFI, both were significant (P=0.04 and P=0.03, respectively). Multiple of maintenance based on intake was negatively related to digestibilities of DM (r=-0.47; P<0.01), starch (r=-0.25; P=0.01), and NDF (r=-0.43; P<0.01) when cows were fed LS diets. In contrast, when cows were fed HS diets, MMI was not related to DM or NDF digestibilities (P>0.2), but correlated negatively with starch digestibility (r=-0.35; P<0.01). We conclude that digestibility accounts for 9 to 32% of the variation in RFI for mid-lactation cows fed low starch diets, but no variation in RFI when cows were fed high starch diets. However, because high RFI cows ate at a higher multiple of maintenance, digestibility depression related to increases in feed intake might have accounted for most of the differences in DM digestibility when cows were fed low starch diets. Digestibility likely accounts for little of the direct variation in RFI for mid-lactation cows eating high and low starch diets.

C. Publications of NC-2040 Committee members during 2014 reporting year

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2. Abstracts

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D. Impacts

1. Discovery that high oleic acid soybeans may increase milk fat yield.

2. Improved understanding of 1) nutrient requirements of neonatal calves, especially as related to feed processing effects on growth and tissue maturation, and 2) rumen modifiers, especially *Aspergillus oryzae* extract.

3. Inflammatory signaling in early lactation has impacts on milk production throughout the entire lactation. Dietary yeast products can modulate both humoral and innate immune system function.

4. Improved understanding of genotype on liver, adipose tissue, and immune responses
5. Improved understanding of gluconeogenesis and CA cycle activity, as revealed by fructose infustions and experiments using the PCK1.

6. Discovery that diet digestibility has decreased over the last 40 years and DCAD increases FCM and fiber digestibility.

7. Feeding high-moisture corn in alfalfa hay-based diets can be a good approach to formulate an optimal dairy feeding program for improving feed efficiency and nutrient utilization. Condensed tannin in bird's foot trefoil could have positive effects on ammonia-N and methane concentrations.

8. Several models were developed to quantify manure volume and composition in lactating dairy cattle fed various types of diets. Evaluation of models showed that the equations predicted nutrient excretion well. A dynamic, mechanistic model of water kinetics in the dairy cow was also developed that traced inputs into the animal and excretion of water under thermo neutral conditions.

9. Maintenance requirements and partial efficiencies of utilizing dietary ME were estimated in various models with different strategies in specifying prior distributions and also with varying energy responses (RE, retained energy vs. HP, heat production). Biological principles associated with each model differ; consequently estimates of NE_M and k_m were spread over a wide range. In particular, two main classes of models were fitted: energy retention and HP models.

10. Improved mechanistic understanding of protozoal metabolism and their interaction with other microbes to manipulate microbial populations.

11. Protozoal persistence may be explained by greater reserve carbohydrate accumulation of protozoa. 2-NBDG has potential to identify some uncultured, glucose-fermenting species and thus better define microbial niches in the rumen

12. Modeling of VFA interconversions, grass digestibility, essential amino acids on mTOR phosphorylation, and phosphorous excretion by cattle.

13. Improved understanding of milk fat synthesis and milk fat depression

14. Improved understanding of metabolizable protein deficient diets, Capsicum extract, extruded soybean meal, cashew nut shell liquid, and methane inhibitors

15. Updating Molly with new oxidation rates and ATP stoichiometry. Development of methods to monitor feed management and cow performance on commercial dairies

16. Improved understanding of mustard bran on animal performance and health.

17. Digestibility accounts for some variation in RFI for mid-lactation cows fed low starch diets, but no variation in RFI when cows were fed high starch diets.

Members have been successful in the past year in receiving private and federal resources to leverage work of the committee. Six committee members have been appointed to write the latest edition of the NRC. Committee members remain active in speaking at regional and international conferences.