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, 2012 to October 31, 2013
Date of This Report: December 19, 2013
Annual Meeting Dates: October 20-21, 2013

Participants present: Armentano, Louis (learment@wisc.edu), University of Wisconsin; Boisclair, Yves (yrb1@cornell.edu), Chair, Cornell University; Bateman, Gale (gbateman@provimi-na.com), Provimi; Beitz, Donald (dcbeitz@iastate.edu) Iowa State University (Represented by Matt O'Neal); Bradford, Barry (bbradfor@k-state.edu), Kansas State University; Casper, David (david.casper@sdstate.edu) South Dakota State University; Davidson, Jill (jadavidson@landolakes.com), Purina Feed; Donkin, Shawn (sdonkin@purdue.edu), Purdue University; Eun, Jong-Su (jseun@usu.edu), Utah State University; Erdman, Richard (erdman@umd.edu), University of Maryland; Fadel, James (jgfadel@ucdavis.edu), University of California, Davis; Firkins, Jeffrey (firkins.1@osu.edu), Ohio State University; Hackmann, Timothy (thackmann@ufl.edu), University of Florida; Harvatine, Kevin (kharvatine@psu.edu), Secretary, Pennsylvania State University; Kebreab, Ermias (ekebreab@ucdavis.edu), University of California- Davis; Rossow, Heidi (harossow@ucdavis.edu), University of California – Davis; Benfield, David (benfield.2@osu.edu), Administrative Assistant; Smith, Steve (sismith@nifa.usda.gov), NIFA-USDA (on conference call).

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The following includes a summary of minutes of the annual meeting (A), summary of station reports, including list of publications (B), and Impacts (C):

A. Minutes of the Annual Meeting (October 21-22, 2013):

Monday, October 21

The Administrative Assistant, Dr. David Benfield, talked about the following items:

- Successful renewal of the project as NC2040.
- Termination report for NC-1040 covering 2007 to 2013 due by Dec. 22nd 2013.

Dr. Steve Smith, USDA-NIFA participated in the meeting via a conference call. Dr. Smith provided the following information:

- Funding update
  - ✓ Overview of USDA funding and success rates of recent sections
  - ✓ Budget for FY2014 not clear
  - ✓ Discussion of methods to increase funding and especially funding high risk experiments.

The following station reports were presented: Cornell University (Yves Boisclair), Kansas State University (Barry Bradford), University of California Davis (James Fadel), University of California Davis (Ermias Kebreab), Washington State University (John McNamara), University of Maryland (Rich Erdman), South Dakota State University (David Casper), and Penn State University (Kevin Harvatine).

Lastly, the committee elected new officers and set the date for next years meeting: Chair and Secretary for the 2014 meeting: Kevin Harvatine (Penn State University) and Timothy Hackmann (University of Florida), respectively. The 2014 meeting will be held October 20 – 21 in Chicago, IL.

Tuesday, October 22

The following station reports were presented: UC Davis (Heidi Rossow), Utah State University (Jong-Su Eun), Purdue University (Shawn Donkin), University of Wisconsin (Lou Armentano), Ohio State University (Jeff Firkins), University of Florida (Tim Hackman), Iowa State University (Matt O'Neal).

A final discussion of the termination report was made. The meeting adjourned.

B. Summary of Station Reports:

Yves Boisclair (Cornell University, Obj., 2)

Biology of the adipokine Adiponectin in early lactating dairy cows was investigated. Variation in hormone responsiveness is a fundamental homeorhetic mechanism coordinating maternal metabolism during pregnancy and during the subsequent transition to lactation (transition period). For example, a growing insulin resistance (IR) during the transition period optimizes the use of endogenous reserves in liver, adipose tissue and skeletal muscle and in so doing, allows near peak milk production soon after parturition despite inadequate intake of all major classes of nutrients. This is also the time when IR becomes excessive in a proportion of cows, leading to inefficient use of endogenous reserves, losses of productivity and increased disease susceptibility. In other species, adiponectin is the single most potent known adipokine regulating insulin action but virtually nothing is known about adiponectin in transition dairy cows. The effect of the transition period on plasma adiponectin was examined in a randomly selected group of cows. Energy indicators in these cows varied in a manner typical of high

yielding dairy cows, with net energy balance of lactation and plasma non-esterified fatty acids (NEFA) averaging +6.2 Mcal/d and 123 μM in late pregnancy (LP, 4 weeks before parturition) *vs.* -9.1 Mcal/d and 397 μM during the first 3 wk of lactation ($P < 0.01$ for all, pregnancy *vs.* first 3 wk of lactation). By day 56 of lactation, net energy balance and plasma NEFA were +7.8 Mcal/d and 129 μM and no longer differed from LP values. Plasma adiponectin varied in quadratic fashion with highest levels in LP, a maximal reduction of 45% on the day after parturition and a progressive return to LP values by day 56 of lactation (Quadatic contrast, $P < 0.01$). Adiponectin circulates in multiple complexes in other species so we examined its molecular weight distribution 28 days before parturition and again on day 8 of lactation by gel filtration chromatography. Adiponectin eluted as a single peak consistent with the majority of adiponectin circulating in complexes of ~ 540 kDa. We next asked whether a change in adiponectin mRNA abundance contributes to the reduction of plasma levels in lactation. Adiponectin expression was measured in biopsies of adipose tissue obtained 40 days before parturition and on day 7 of lactation. In these cows, plasma adiponectin was decreased by 32% from pregnancy to lactation ($P < 0.001$). Adiponectin expression in adipose tissue did not vary, even though expression of leptin was reduced by 75% over this interval ($P < 0.05$). Adiponectin expression was negligible in other tissues (liver, skeletal muscle and mammary gland). Finally, we measure the expression of the 2 major adiponectin receptors (AdipoR1 and AdipoR2) in the major metabolic tissues. AdipoR1 expression in muscle was 6.6-fold higher than in liver and 2-fold higher than in adipose tissue and mammary gland ($P< 0.05$ or less). AdipoR2 expression was 2.5-5.5-fold higher in adipose tissue than in the other surveyed tissues ($P < 0.01$ or less). When transcripts of each receptor were compared within tissue, AdipoR1 abundance was approximately 2-fold higher than AdipoR2 in muscle and mammary gland ($P < 0.01$ or less). The reciprocal situation occurred in liver and adipose tissue whereby AdipoR2 was 3-4-fold more abundant than AdipoR1 ($P < 0.01$ or less). Early lactation caused a 2.5-fold increase in AdipoR1 expression in muscle ($P < 0.05$) but had no effect on its expression in liver or adipose tissue. In contrast, early lactation increased AdipoR2 expression 2.5-fold in liver ($P < 0.05$) but had no effect in other tissues. We conclude that the adiponectin system is dynamically regulated in transition dairy cows and could contribute to the changes of insulin resistance observed over this period.

Barry Bradford (Kansas State University, Obj. 1 and 2):
The effects of chromium propionate and supplemental amino acids on dairy cattle performance near peak of lactation was investigated. Forty-eight lactating Holstein cows (21 primiparous and 27 multiparous, 38 ± 15 days in milk) were used in a randomized complete block design with 4 treatments. Treatments were control, Cr propionate (CrPr; 8 mg/day Cr in the form of 20 g/day KemTRACE Chromium Propionate 0.04%, Kemin Industries, Des Moines, IA), rumen-protected lysine and methionine (RPLM; 10 g/day lysine and 5 g/day methionine, intestinally available), or both (CrPr+RPLM). The RPLM supplement was composed of 48.8 g/day of LysiPEARL and 15.3 g/day of MetiPEARL (Kemin Industries). Dry matter intake was significantly increased by CrPr ($P < 0.05$), but was not significantly affected by RPLM when fed for 5 wk near peak lactation. Although neither RPLM nor CrPr significantly altered yields of milk or milk components, CrPr tended to increase energy corrected milk (ECM; $P = 0.09$) by 6%. In addition, there was evidence of parity × CrPr interactions for both DMI ($P = 0.06$) and milk protein yield ($P = 0.04$), in both cases indicating positive responses to CrPr in primiparous cows but not in multiparous cows (Figures 1A and 1B). Feed efficiency was unaffected, because the increases in milk yield and DMI in response to Cr supplementation paralleled each other. Basal TNFα

transcript abundance in neutrophils was not affected (P > 0.10) by treatment or day. After LPS activation, TNFα transcript abundance increased compared with non-stimulated controls. Dietary CrPr increased (P = 0.02) TNFα abundance in LPS-activated neutrophils. There was a RPLM × parity interaction (P < 0.01), reflecting increased TNFα transcript abundance in LPS-stimulated neutrophils from primiparous cows (P < 0.01) and a tendency for decreased TNFα in multiparous cows (P = 0.07) in response to RPLM. Basal IL-1β transcript abundance in neutrophils was decreased (P = 0.05) by RPLM, and tended to be increased by CrPr (P = 0.08, Figure 2C). Basal IL-1β transcript abundance was increased between d 21 and d 35 (P = 0.01), and was greater (P = 0.04) in primiparous cows than multiparous cows (12.2 vs. 2.7 ± 7.77 arbitrary units). After LPS activation, IL-1β transcript abundance increased dramatically and a RPLM × parity interaction (P < 0.01) was observed, reflecting increased IL-1β transcript abundance in primiparous cows (P = 0.03) and decreased IL-1β in multiparous cows (P = 0.01) in response to RPLM.

Jim Fadel (UC Davis, Obj. 3):
The difference in AM vs. PM milk for different parities was investigated. The objective this study was to estimate and characterize the variances of milk constituents from samples taken twice daily from 24 cows over 22 days. The preliminary results show that parity affects percent protein and fat and AM vs. PM milking is different for percent solids and fat. Also, the between animal variance is greater than within animal variance. Details of this study will be presented next year.

Secondly, a structural equation model to analyze energy utilization in lactating dairy cows was developed. The objective of this study was to propose a multivariate framework for analyzing energy balance data from lactating cows and to investigate the changes in energetic parameters over the years. Data was composed of 1,094 energy balance records from 40 studies conducted from 1963 to 1995. These preliminary results show that maintenance requirements increase over the decades in all models. The k_L values increase similarly over the decades in all models tested. Our model shows consistently that $k_T > k_G$ but the other two models have inconsistent results where some cases $k_T < k_G$. Our k_T values are all higher than the other models.

Ermias Kebreab (University of California Davis; Obj. 3)
The objectives of this study were to: i) identify key explanatory variables in the prediction of methane emissions; ii) develop methane emission prediction equations using a large database of lactating and growing cattle; iii) conduct a cross validation of prediction equations; iv) examine the improvement in predictive ability of methane equations with an increase in model complexity and v) investigate the appropriateness of current models used in determining methane emissions from cattle enteric fermentation. A prediction model based on a large database of enteric methane emissions from North American lactating and growing cattle was developed and tested. Explanatory variables, which play a key role in predicting emissions, at the dietary and animal complexity levels were selected through a Bayesian model selection procedure, namely a Reversible Jump Markov Chain Monte Carlo (RJMCMC) sampler. Gross energy intake, dietary fiber and lipid proportions, animal body weight and milk fat proportion were identified as key explanatory variables for predicting emissions. Models here developed substantially outperformed models currently used in national greenhouse gas inventories. Additionally, estimates of repeatability of methane emissions were lower than the ones from the literature and

multicollinearity diagnostics suggested that prediction models are stable. In this context, various enteric methane prediction models are proposed which require different levels of information availability and can be readily implemented in national greenhouse gas inventories of different complexity levels.

John McNamara (Washington State University; Obj. 1, 2, & 3)
A systems model approach was used to assess the potential effect of changes in gene expression in adipose tissue of dairy cattle on production and reproductive efficiency. Specific changes in transcription of genes in adipose tissue may affect metabolic and reproductive efficiency. In order to help identify patterns of physiological control of metabolic flux and reproductive functions in the most efficient dairy cattle, an existing mechanistic metabolic model (Molly, UC Davis) was used and expanded to include initiation of cycling, IGFI and follicular growth, and degradation of estrogen and progesterone in the rumen. Our objective was to test the effects of changes in gene transcription of specific control proteins in adipose tissue in the model, based on observed data, on patterns of metabolism and potential effects on reproductive processes. The model explicitly describes the substrate sensitivity and maximum velocity for lipogenesis, esterification and lipolysis in adipose tissue which were what our candidate genes were determined for. Data on gene transcription and metabolic flux in the adipose tissue were collected from 1st and 2nd parity cows from 28 d prepartum to 56 DIM, from studies that measured nutrient intake, milk component output, changes in adipose tissue lipid, visceral and body protein and lipid, and metabolism rates in adipose tissue. We observed decreases in early lactation in transcription for genes coding for lipogenic pathways, as well as decreases in rates of lipogenesis; and observed increases in rates of lipolysis without increases in mRNA abundance. Explicit inputs into the model included nutrient intake, initial body fat and protein, milk production, fat, and protein output. The control steps in the model that were altered were substrate sensitivity and maximal velocity for lipogenesis, esterification and lipolysis to simulate the observed changes. Increasing rates of lipogenesis did not change milk production or feed intake, but decreased milk fat production and decreased the postpartum interval to first ovulation; while increases in rates of lipolysis did not change milk production but increased milk fat and increased the interval to first estrus. Increases in metabolic rate (by any organ) increased rates of estrogen and progesterone degradation. This model may be used to help interpret genomic and transcriptomic data leading to changes in productive and reproductive efficiency.

South Dakota State University (David Casper)

High forage TMR rations were evaluated in mid- to late-lactation dairy cows. Twenty mid- to late-lactation lactating Holstein dairy cows were randomly assigned within blocks to 1 of 2 rations based on ration forage concentration. Forages were 60% 2012 1st cutting alfalfa haylage and 40% 2012 corn silage blended on a DM basis and then fed at either 60% (Low Forage:LF) or 80% (High Forage:HF) of the ration DM. Milk production was reduced ($P<0.01$) by feeding the HF diet compared to cows fed the LF (28.1 and 24.1 kg/d for LF and HF, respectively), while milk fat (3.98 and 3.96%), milk protein (3.17 and 3.11%), milk lactose (4.81 and 4.77%), and milk solids-not-fat (8.87 and 8.77%) percentages were similar ($P>0.10$) for cows fed both rations. The forage quality and digestibility in this study was not adequate to support the milk production of mid- to late-lactation dairy cows. The digestibility of DM (DMD=75.7%) and NDF (NDFd=55.7%) for the alfalfa haylage was above average but, the corn silage quality was average (DMD=72.9, NDFd=52.3%, and starch=32.1%) in this study. In this study, the forage

quality and digestibility when fed at high rates did not support similar milk production in mid- to late-lactation dairy cows.

Kevin Harvatine (Penn State University)
The effects of feeding time on the circadian pattern of feed intake, milk production, and plasma hormones and metabolites was investigated in dairy cows. The object of this study was to determine if the timing of feeding can entrain circadian rhythms (24 h repeating cycles) of dairy cows. Nine Holstein cows were arranged in a replicated 3 x 3 Latin Square design with 14 d periods in an automated feed observation system that recorded feed weight every 10 s. Treatments were feeding 1 x/d at 0830 h (AM) or 2030 h (PM) and feeding 2 x/d in equal amounts at 0830 and 2030 h (AMPM). All treatments were fed at 110% of daily intake. Cows were milked 2 x/d at 0500 h and 1700 h. Daily measurements were analyzed by a mixed model that included the random effect of cow and period and the preplanned contrasts were AM vs PM and AM vs AM/PM. Time course data were analyzed with mixed model procedures using repeated measures and the planned contrast were tested at each time point. Secondly, a cosine function was fit to time course data by nonlinear regression. There was no effect of treatment on DMI, digestible DMI, and total tract digestibility of DM, OM, and NDF. Over 16 and 24% of DMI was consumed in the first hour after feeding for the AM and PM, and 12 and 19% for AMPM in the morning and evening, respectively. The rate of intake at other times of day did not differ between treatments including a low level of intake overnight (2400 to 0500 h; 2.2%/h ± 0.74 mean ± SD) and a moderate level of intake in the afternoon (1200 to 1700 h; 4.8%/h ± 1.1, Mean ± SD). Milk yield and composition did not differ among treatments, but milk yield at the 0500 h milking was 1.3 kg higher than the 1700 h milking ($P < 0.01$). There was no main effect of treatment on fecal NDF or plasma insulin, glucose, and NEFA, but there was an effect of time ($P < 0.001$). Plasma BUN was increased 0.52 and 0.89 mg/dL by PM and AMPM compared to AM at X h, respectively ($P < 0.05$). A cosine function with a 24 h period fit fecal NDF concentration ($P < 0.001$). Briefly, the amplitude of fecal NDF was increased 31% by PM and decreased 28% by AMPM compared to AM (mean = 56.45, 56.10, and 56.55%, mean amplitude = 2.34, 3.07 and 1.68% for AM, PM, and AMPM, respectively). In conclusion, feeding time entrains the circadian rhythms of key physiological variables, but has little impact on daily total intake or milk production.

Heidi Rossow (UC Davis, Obj. 1, 2, and 3):
Correlation of feed efficiency with mitochondrial efficiency was investigated. Significant differences in mitochondrial oxygen consumption are observed in steers with high and low RFI. Data suggest that mitochondrial function is a maternally inherited trait, however important proteins such as outer mitochondrial membrane, intermembrane space, inner membrane and matrix proteins are encoded in the cell nuclei and therefore, could be inherited by both the sire and the dams (Lymbery et al., 2001). It is still unknown if there's any correlation between lineage of sires and mitochondrial oxygen consumption. The objective of this experiment is to analyse mitochondrial efficiency for two sires with high and low RFI. Two popular Angus bulls were selected based on the HD 50K MVP genetic test (Pfizer Animal Genetics) and were used as sires at the Sierra Foothill Research and Extension Center. Eight offspring (10-11mo) from each sire were selected based on body weight and shipped to the UC Davis feedlot. Following a diet adaptation period of 14 d, steers were housed in individual pens to allow individual measurements of feed intake for 70-105 d and fed a typical feedlot finishing diet with 63% rolled corn and 17% dry distilled grains (DDG) four times a day. Slaughter criteria

were 11mm of backfat using ultrasound (SONOVET 2000). Statistical analysis was performed in R Project for Statistical Computing (version 2.15.1) and the data analysed using ANOVA. Respiratory control ratio (RCR) is the ratio of oxygen consumption in State 3 and State 4 respiration and provides an indication of mitochondrial coupling and efficiency. State 3 (maximum ATP stimulated respiration) and State 4 (leak-dependent oxygen consumption) did not differ between the 2 group of animals (P=0.87) and (P=0.99), respectively. Also, no differences in RCR (±SD) were found with averages of 2.98 (0.45) and 3.03 (0.39) for low and high RFI steers, respectively (P=0.85).These results differ from Keisler et al. (2006) in which low RFI steers had greater RCR values. Therefore even though there were differences in RFI between the two groups, their liver mitochondria did not present differences in maximum oxygen consumption, proton leak dependent respiration or uncoupling.

Jong-Su Eun (Utah State University, Obj. 1):
The first experiment presented was "Improving nutrient utilization, feed efficiency, and lactational performance of dairy cows by feeding protein supplements in high forage lactation diets." The objective of this study was to assess the effects of protein supplementation in high forage dairy diets on nutrient utilization, feed efficiency, and lactational performance of lactating cows. Twelve multiparous dairy cows were used in a triple 4 × 4 Latin square design with one square consisting of ruminally cannulated cows. Treatments included: 1) control, 2) slow release urea (**SRU**), 3) yeast derived microbial protein (**YMP**), and 4) combination of SRU and YMP (**S+Y**). Cows fed protein supplements had lower intakes of DM and NDF, and tended to have lower CP intake (P = 0.06) compared to those fed the control. Milk yield was similar across all diets, but the YMP tended (P = 0.08) to increase milk true protein yield compared with other treatments. Milk urea-N concentration increased in the S+Y compared with the control. Feed efficiencies expressed as milk yield/DMI and ECM yield/DMI were improved in all diets with protein supplementation, with the YMP being the highest. In addition, milk N-to-N intake ratio tended to increase (P = 0.10) in the YMP. Total VFA concentration tended to decrease (P = 0.10) when protein supplements were added because of reduced feed intake. Overall results of this study indicate that feeding protein supplements in high forage dairy diets improved feed and N utilization efficiencies for milk production, which can contribute to increasing dairy profitability.

Shawn Donkin (Purdue University):
Regulation of bovine hepatic gene expression of gluconeogenic enzymes by nutrients and hormones including differential regulation of bovine PEPCKC promoter by propionate and hormones were investigated. In nonruminants, the activity of PEPCK-C is regulated by a variety of dietary and hormonal signals at transcriptional level. Glucagon and glucocorticoids stimulate hepatic gluconeogenesis by inducing the gene expression of PEPCK-C. Insulin dominantly counteracts the effects of these hormones and results in deinduction of PEPCK-C expression. In our in vivo study, direct effect of propionate on expression of PEPCK-C cannot be separated from the commensurate increases in circulating insulin in lactating cows. In vitro promoter experiment allows us to study the direct effect of propionate as well as its interaction with various hormones on the PEPCK-C expression at transcription level. A Series of bovine PEPCKC promoter-pGL3-Basic luciferase constructs with 5' end deletions of the promoter region were generated using PCR. Constructs contained regions corresponding to -1238, -815,-409, -251, and -85 through +221 relative to the transcription start site (TSS) of bovine PEPCK-C gene. 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. These studies provide direct evidence of control of PEPCK expression by propionate and provide insight to the region of DNA that responds to the presence of propionate.

Louis Armentano (University of Wisconsin, Obj. 1)

Dietary polyunsaturated fatty acids are a risk factor for milk fat depression and recent experiments examining the effect of "Exogenous fatty acid composition" on cow performance in low fat diets was discussed. Twenty four primiparous and thirty six multiparous cows were enrolled in the study. A 6x6 Latin squares experiment with 21 d periods was used. There were two control diets, a corn based control (CC) and a low oil control (LOC). LOC was formulated to have similar NDF and CP to the CC control while having lower fatty acid content. The LOC was formulated mostly by replacing corn grain, corn silage and corn distillers grains in the CC standard diet with corn starch, alfalfa silage and low-fat corn proteins. The LOC diet is the control for the oil treatment diets. Dietary oil supplement treatments were formulated by replacing corn starch in the LOC diet with different fat sources to make the oil supplemented diets equal in total fatty acid. Treatments included 1.7% of DM of a 50:50 blend of corn oil and high linoleic safflower oil (corn oil or CO), high oleic sunflower oil (oleic oil or OO), palm oil (PO), and 1.8% of DM of calcium salts of palm fatty acids (Megalac or ML). Milk production was lower for the LOC diet when compared to all other diets. Milk fat yield and concentration were lower for CO vs. the rest of the oil treatments so this diet was able to induce milk fat depression even at fairly low levels of total dietary fat. Numerically, the rank of fat yields for treatment diets was as anticipated. PO achieved a level of fat similar to CC whereas OO was intermediary and CO resulted in less fat than the LOC which was not supplemented with fat. Future work will investigate the effect of soybean fatty acid profile in dairy cows using high oleic soybeans (Plenish, Dupont).

Jeff Firkins (Ohio State University, Obj. 1) and Timothy Hartmann (University of Florida)

Experiments were conducted to quantitatively evaluate chemical and physical properties of protein and energy sources which determine the availability of nutrients critical to milk protein secretion in lactating dairy cows.

First, methods were evaluated to detect changes in reserve carbohydrate for mixed rumen microbes. Thin-layer chromatography and other analyses revealed that rumen microbes accumulated large amounts of glycogen. The aim of this study was to identify a method that would most accurately quantify accumulation and utilization of this reserve carbohydrate. Anthrone detects all carbohydrate, including that not used for reserve carbohydrate. In contrast, amyloglucosidase might not hydrolyze all reserve carbohydrate and might be less appropriate for quantitative recoveries of carbohydrate and energy. For whole microbial cells, the anthrone reaction detected more ($P<0.001$) carbohydrate than did hydrolysis with amyloglucosidase, even after exhaustive extraction by bead beating (45 min) or KOH digestion (3 h). Less carbohydrate was detected after isolating reserve polysaccharide by ethanol precipitation. Compared to amyloglucosidase hydrolysis, anthrone detected a larger ($P=0.017$) increase in cell carbohydrate when glucose (20 mM) was dosed in cultures. Additionally, anthrone detected a larger ($P=0.049$) decrease in cell carbohydrate after glucose was exhausted. Thus, anthrone detected more reserve carbohydrate that accumulates during energy excess and is utilized for energy during starvation. Assuming a constant detection of non-reserve material before vs after glucose dosage, recoveries for energy (97.5%), carbon (100.2%), and cell components (99.8%) were high, indicating

carbohydrate was completely detected. For the amyloglucosidase hydrolysis method, recoveries of energy (88.9%), carbon (91.6%), and cell components (92.8%) were lower. Iodine did not stain glycogen remaining after cells were incompletely extracted intentionally. The anthrone method appeared to accurately quantify changes in reserve carbohydrate and shows merit for quantitative studies, whereas the amyloglucosidase method detected smaller changes and was less consistent with expected carbon and energy recovery.

Secondly, the response of mixed rumen microbes to excess carbohydrate was investigated. The aim of this study was to determine if a mixed microbial community from the bovine rumen would respond to excess carbohydrate by accumulating reserve carbohydrate, energy spilling (dissipating excess ATP energy as heat), or both. Mixed microbes from the rumen were washed with N-free buffer and dosed with glucose. Total heat production was measured by calorimetry. Energy spilling was calculated as heat production not accounted by (i) endogenous metabolism (heat production before dosing glucose) and (ii) synthesis of reserve carbohydrate (heat from synthesis itself and reactions yielding ATP for it). For cells dosed with 5 mM glucose, synthesis of reserve carbohydrate and endogenous metabolism accounted for nearly all heat production (93.7%); no spilling was detected ($P = 0.226$). For cells dosed with 20 mM glucose, energy spilling was not detected immediately after dosing, but it became significant ($P < 0.05$) by approximately 30 min after dosing with glucose. Energy spilling accounted for as much as 38.7% of heat production in one incubation. Nearly all energy (97.9%) and carbon (99.9%) in glucose were recovered in reserve carbohydrate, fermentation acids, CO_2, CH_4, and heat. This full recovery indicates that products were measured completely and that spilling was not a methodological artifact. These results should aid future research aiming to mechanistically account for variation in energetic efficiency of mixed microbial communities.

Donald Beitz (Iowa State University):

The hypothesis that daily feeding of Bovamine (a commercial probiotic containing 3×10^{12} cfu of bacteria *Propionibacterium freudenreichii* and *Lactobacillus acidophilus*) to lactating dairy cows would improve milk production performance was tested. One hundred and four multiparous (parity 1 to 5) Holstein cows at differing stages of lactation (42 to 121 DIM) were enrolled in the study that was carried out in 3 blocks. On d 0, cows were assigned randomly to one of two treatment groups; TMR plus lactose (CON; 1 g of lactose per hd per day) and TMR plus Bovamine (BOV; 1 g Bovamine per hd per day). Each gram of Bovamine contains 3×10^{12} cfu of bacteria (*Propionibacterium freudenreichii* and *Lactobacillus acidophilus*) and lactose as a carrier. Treatments continued for 12 wk. Lactose and Bovamine were offered to cows once daily by topdressing the TMR with 115 g of ground corn mixed well with 1 g of lactose or Bovamine, respectively. Feed refusals were collected, weighed, and recorded daily to determine daily intake. Dry matter intake decreased 3.59% in cows in the Bovamine group compared to cows in the Control group ($P < 0.0001$). Both daily milk production and daily ECM production however, remained unchanged ($P = 0.3006$ and $P = 0.7376$, respectively) in response to treatment. The decrease in DMI without a change in milk production caused milk production efficiency and ECM production efficiency to increase ($P < 0.0001$ and $P = 0.0296$, respectively) by 6.1% and 5.3%, respectively. Milk fat, lactose, total solids, and MUN were not altered in the Bovamine group ($P > 0.1305$). Milk protein was increased ($P = 0.0103$) in the Bovamine group compared with the Control group. Somatic cell counts were not different ($P = 0.1712$) between and Control and Bovamine treatments. Total VFA concentration and individual VFA concentration were not altered by treatment ($P > 0.1842$). Additionally, treatment did not change

the acetate-to-propionate ratio ($P = 0.1842$) or the percentage of individual VFA ($P < 0.4223$). In conclusion, whereas milk production was not affected, feeding Bovamine decreased DMI resulting in an improvement in milk production efficiency and ECM production efficiency 6.1% and 5.3%, respectively. This effect was not associated with a change in ruminal VFA concentration as we hypothesized. Variability of VFA data, however, is also greater than previously reported and may be related to sampling technique and variability of sampling time relative to feeding. Future studies should focus on minimizing sampling variability to determine if a difference in VFA concentration can be detected.

Brian Crooker (University of Minnesota)

Impact of increased milk yield on hepatic gene expression in the periparturient Holstein was investigated. The o**bjectives** of this effort were to determine if expression of genes involved in the regulation of hepatic metabolism differed between the University of Minnesota's (UMN) unselected and contemporary multiparous Holsteins. These unselected Holsteins have been bred to semen from 1964 breed average Holsteins since 1964 and producing considerably less milk (about 4,000 kg/lactation) than contemporary Holsteins. Periparturient unselected UMN and contemporary Holsteins (n = 5 and 6, respectively) were managed identically and fed ad libitum quantities of the same diets. Hepatic biopsies were collected at -14, 3, 14, and 35 days of lactation and gene expression determined by digital multiplexed gene expression analysis using a nanoString nCounter. Expression of 45 individual genes of interest was normalized to the positive control and the geometric mean of 5 internal control genes. Expression of total and the liver specific growth hormone receptor (GHRtot and GHR-1a, respectively), insulin-like growth factor binding protein 3 (IGFBP3) and the acid liable subunit (IGF-ALS) of the IGFBP3 complex were greater ($P < 0.01$) and fibroblast growth factor 21 (FGF-21) and IGFBP2 less ($P < 0.01$) in unselected Holsteins. Genotype did not affect ($P > 0.3$) IGF1-receptor (IGF1-R), insulin receptor (INS-R) or β-Klotho. There were day by genotype interactions ($P < 0.05$) for IGFBP2, IGF-ALS, and β-Klotho. Not surprisingly, hepatic gene expression profiles in this effort and relationships among the genes (for example, the insulin like growth factor binding proteins IGFBP2 and IGFBP3 and the IGF acid liable subunit, IGF-ALS) were generally consistent with previous reports for periparturient Holstein cows. Our GHR-1a and total GHR (GHRtot) expression data support previous reports that most of the postpartum decrease in GHR expression is due to reduced GHR-1a expression. The more important knowledge gained from these results is the additional documentation that expression of genes involved with the regulation of metabolic function differs between the two genotypes (unselected vs. contemporary Holstein). These differences are due to genotype and not to any treatment imposed to alter metabolism. Of particular interest is the greater and prolonged (at least through 35 d PP) increase in hepatic expression of FGF21 in contemporary Holsteins. Others have suggested that hepatic FGF21 participates in the regulation of lipid reserves during lactation. Our hepatic FGF21 expression profile data indicate agreement with these results and indicate our animal model could help ascertain the role of FGF21 in the high producing periparturient cow.

Richard Erdman (University of Maryland)

Provided overview of the issue of an incorrect TDN discount that occurs in the NRC 2001. Secondly, described research relating to the effect of DCAD on milk fat synthesis. Increased DCAD increased digestion of DM and NDF and increased milk fat.

C. Publications:

List of peer-reviewed journal articles published by NC-1040 committee members during 2013 reporting year (includes papers in press, accepted, or submitted)

Acharya, I. P., D. J. Schingoethe, K. F. Kalscheur, and D. P. Casper. 2013. Canola meal or high protein dried distillers grains for lactating cows. J. Dairy Sci. 96:(Submitted).

Aguilar, M., M. D. Hanigan, H. A. Tucker, B. L. Jones, S. K. Garbade, M. L. McGilliard, C. C. Stallings, K. F. Knowlton, and R. E. James. 2012. Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. J. Dairy Sci. 95 :7261–7268

Appuhamy J.A.D.R, A. B. Strathe, S. Jayasundara, C. Wagner-Riddle, J. Dijkstra, J. France, and E. Kebreab 2013. Anti-methanogenic effects of monensin in dairy and beef cattle: a meta-analysis. J. Dairy Sci. 96 (8): 5161-5173.

Appuhamy, J. A. D. R. N., W. A. D. Nayananjalie, J. Escobar, and M. D. Hanigan. (in press). Effects of acetate, glucose and essential amino acids on cell signaling in bovine mammary epithelial cells. J. Dairy Sci.

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Dias, R. S., S. López, R. M. Patiño, T. S. Silva, J. C. Silva Filho, D. M. S. S. Vitti, M. R. S. R. Peçanha, E. Kebreab and J. France. 2013. Calcium and phosphorus utilization in growing sheep supplemented with dicalcium phosphate. J. Agric Sci. 151:424-433.

Doane, P. H., K. L. Perfield, and J.P. McNamara. 2013. Effect of monensin and a plant botanical mix on milk production and metabolic indices of lactating dairy cattle. J. Dairy Sci, under review.

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List of abstracts published by NC-1040 committee members during 2012 reporting year

Acetoze, G., K. L. Weber, A. L. Van Eenennaam, J.J. Ramsey, H. A. Rossow. (2013)Liver Mitochondrial Efficiency of two Lineages of Angus Bulls with High and Low Residual Feed Intake (RFI). American Society of Animal Science July 8-12.

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D. Impacts

- Improvement to quantitative predictions of metabolism of protozoa and their interaction with other microbes in mechanistic models. Overall effects are improved fiber digestibility, maintenance of milk production with lower protein content of diets and reduced risk of milk fat depression.

- Increased productivity (and immune function) of primaparous cows near peak lactation through dietary chromium propionate.

- Development of guidelines to feed and manage cows to increase mitochondrial (and feed) efficiency.

- Identification of the optimal times to offer fresh feed.

- Predictable effects of dietary fatty acids on ruminal fermentation, mammary *de novo* lipogenesis, and total lipid secretion by the mammary gland.

- Demonstration that protein synthesis is regulated in the mammary gland, fostering efforts by experimenters and modelers to include this phenomenon in metabolic models.

- Promotion of gluconeogenesis and overall health in dairy cows through understanding of transcriptional stimulation of the PEPCK gene by propionate and PC gene by specific fatty acids.

- Resolution of molecular mechanisms (gene transcription or post-translational modification of receptors and enzymes) regulating the flow of lipids in adipose tissue during lactation.

- Identification and characterization of adiponectin as regulators of metabolism at the onset of lactation.

- Literature reviews on greenhouse gases that producers and policy makers can use for understanding the impact of animal production on climate change and development of carbon credits.

- Development of improved equations to estimate enteric methane emissions from different classes of cattle allowing better estimates in national inventories and proper evaluation of mitigation options.

- Derivation of more precise estimation of maintenance and efficiencies of lactating dairy cattle for use by producers and experimentalists.

- Development of a cell signaling model underpinning mammary amino acid metabolism that can be used to improve existing whole animal models of amino acid and protein metabolism.

- Characterized the impact of slow release urea and yeast protein on rumen fermentation and nitrogen utilization efficiency in dairy cows.

- Ability of probiotics to modify ruminal volatile fatty acid profile.

- Increasing DCAD as a mechanism to increase milk fat.

Members have been very successful in the past year in receiving private and federal resources to leverage the work of the committee. The committee was also influential in organization of a Discovery conference on feed efficiency and modeling workshops. Committee members also remain active in speaking at regional and international conferences.