APPENDIX D
SAES-422
Format for Multistate Research Activity
Accomplishments Report

Project Number: NC-2040
Project Title: Metabolic Relationships in Supply of Nutrients for Lactating Cows
Period covered: November 1, 2014 to October 31, 2015
Date of This Report: December 2015
Annual Meeting Dates: October 19th – 20th, 2015

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Participants submitting a written report, but not present:

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

A. **Minutes:** Brief Summary of Minutes of Annual Meeting
Monday, October 19th:
The Administrative Advisory, Dr. David Benfield, discussed the following items:
Successful renewal of this project NC2040.

Dr. Steve Smith, USDA-NIFA participated in the meeting via conference call. Dr. Smith provided the following information:
Funding update. No budget approved for 2016.
AFRI Budget for FY2016: either flat or slight increase.

RFA released for programs supported by both executive/legislative branches.
Funding success for AFRI foundational programs on Animal health/products. Success rate below 10% for most.
No major changes in Hatch funds or other programs.
Foundational Program FY 2016 RFA should be released in Nov 2015.
RFAs should be released on a more predictable and shorter (<1 yr) cycle
Exploratory program (introduced in 2015) is for high-risk, high-reward ideas.

Dr. Robin White (VT) gave a workshop on meta-analysis.
Accounting for other things that add variability to our models. Terms of the models used in meta-analysis. Meta-analysis of random and fixed effects (homogenous population). Fitting procedures, assumptions and uncertainties. Understanding the variability of the models. Grouping variables. Discrete explanatory variable vs continues explanatory variables.
Methods of weighing across the different studies in the meta-analysis, weighing by n, cow-period in Latin square designs, calculating the standard errors from data reported in the literature. Importance of weighing. Dealing with studies without standard errors. Propagating standard errors.

Station reports presented:
Lou Armentano, Barry Bradford, Shawn Donkin, Richard Erdman, Jeffrey Firkins, Matthias Hess, James G. Fadel, Jill Davidson, Brian Crooker.

Tuesday, October 20st
Election of new officers for NC2040 2015-6
Secretary: Heather White
Chair: Agustin Rius
Next year's meeting
Time: Oct 19th to 20th
Venue: St Louis, Missouri
Possible metabolomics workshop and visit to LandO'lakes research facilities in Gray Summit.

Station reports presented:
Kevin Harvatine, Timothy Hackmann, Hidie Ross, Mark Hanigan, Michael VandeHaar, Michigan State University, Agustin Rius, Yves Boisclair.
Meeting adjourned at noon.

B. Summary of Station Reports

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

Can a 95% Byproduct Diet Support High Milk Production? Twelve dairy cows were selected from the KSU Dairy herd. Cows were all post-peak (154 ± 20 DIM, mean ± SD) and produced on average 42.7 ± 5.5 kg of milk per day at the beginning of the study. Cows were housed in tie stalls and individually fed 2 different diets in a 2-period crossover design. One diet was a typical lactation TMR and the other was comprised entirely of byproduct feeds.

Each period lasted 20 days, of which the first 17 days allowed for diet adaptation and days 18-20 comprised the sampling period. Milk yield, milk composition, and feed intake were measured and averaged across each sampling period. Feed ingredient and TMR samples were collected, composited, and analyzed for nutrient composition. Blood samples were collected on day 20, immediately before feeding, and analyzed for plasma glucose and insulin concentration. Body weights and body condition scores were recorded at the beginning and end of each period. Average feed intake during the last 3 days of each period did not differ by treatment, although there was an effect of parity, with multiparous cows consuming more feed than primiparous cows. Milk production for first parity cows was unaffected by diet, but multiparous cows produced on average 3.6 lb less milk per day on the byproduct diet than on the control. Milk fat decreased with the byproduct diet, but milk protein concentration was unaffected. Parity interacted with diet for fat-corrected (FCM) and energy-corrected milk (ECM). Both ECM and FCM decreased more for the multiparous cows on the byproduct diet than for the primiparous cows. Milk urea nitrogen levels were higher for the control diet than for the byproduct diet. No treatment differences were detected for body condition score or body weight, although a period effect for body weight was observed, with cows on both diets weighing less after the second period. Plasma glucose concentrations tended to be increased by the byproduct diet, but plasma insulin concentrations did not differ between diets.

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

Estimating intake and diet effects on digestibility of nutrients. The typical high-producing dairy cow requires 4 to 6 times more nutrients than she does at maintenance. Nutrition models are used to formulate diets that are balanced to meet requirements. To determine the supply of absorbed nutrients, both dry matter intake (DMI) and the digestibility of nutrients are needed. Most nutrition models, such as NRC (2001) assume that digestibility is decreased at higher DMI but the database used to determine effects of DMI on digestibility included mostly animals that were producing less milk than that of modern commercial cows. The objectives of this study were to determine the effects of diet, animal characteristics, and DMI on the digestibility of dry matter, NDF and starch (DMD, NDFD and StarchD, respectively) using recent data with high producing cows. The database contained 1942 observations of 635 cows from 56 studies from 3 universities with most data collected in the last 15 years. Criteria for inclusion were that data on individual cows was available for BW, DMI, and total tract digestibility of nutrients on diets with known composition. The forage sources in these studies were corn, alfalfa, wheat straw and orchardgrass, and these were classified as corn, alfalfa, and grass. Diets contained 37 - 80% forage (%DM), 23 - 53% NDF (%DM), 22 - 93% forage NDF (%NDF), 11 – 41% starch (%DM), 0.6 - 8.3% FA (%DM), CP (%DM), 434 - 949 kg BW, and 3 - 35 kg DMI. The database was centered and analyzed by random regression using the HPMIXED procedure of SAS v. 9.4. The first step to analyze the data was to check for multi-collinearity by the VIF option and then remove the outliers based on extreme observations. The full model contain all the diet and animal characteristics described previously, all possible 2-way interactions between diet components and DMI, BW versus DMI, and quadratic terms for BW and DMI as a fixed effect; and cow, block, period, treatment and study as random effects. To determine the best fit model we used the backward procedure, where the variable with highest p value is removed in successive steps until the model contains only significant variables. To determine the overall effect of DMI on

digestibility, each variable was replaced by its mean value in the database; DMD, NDFD and StarchD decreased 0.27, 0.065 and 0.31%, respectively, for each 1 kg increase in DMI. Assuming maintenance intake is about 6 kg DMI for a cow, these translate to 1.5%, 0.4%, and 1.8% decreases in digestibility for DM, NDF, and starch per multiple of maintenance; these values are much lower than those for NRC 2001. Because forage source and DMI interacted to affect DMD, we simulated diets high in grass, alfalfa or by-products (low forage, high NDF) and DMD decreased 0.30, 0.28, 0.36%, respectively, for each 1 kg increase in DMI. The effect of intake on NDFD and StarchD was the same as the overall response for these diets. Our preliminary results confirm that digestibility is reduced as intake increases but at a lower rate than that used by NRC (2001).

Genomic and farm decision tools to enhance feed efficiency. The objective of the current study was to determine the repeatability of feed efficiency across diets differing in starch and forage fiber. Method: 64 Holstein cows, primiparous and multiparous, were housed on individual tie stalls on the MSU Dairy Research Teaching Center. Animals were milked twice daily and fed the treatment diet once a day. Diet treatments were formulated varying the amount of fiber vs starch ratio. Forage source included Corn silage, Alfalfa hay silage and wheat straw. Main starch sources were ground dry corn and high moisture corn. The two treatments evaluated were: A High Forage-Low Starch diet (HF) and a Low Forage-High Starch Diet (LF) Data recorded included daily Dry Mater Intake (DMI), Milk yield, Body Weight (BW), Body Condition Score (BCS) and milk components. Two Experimental groups were conducted with 32 cows each. Both followed a crossover switch back design with 2 treatment periods of 31 days and 28 days respectively. Statistical analysis was performed using a GLM procedure from SAS 9.4. After modeling cows to obtain the RFI value; cows were ranked using standard deviation of the RFI value as HRFI (>+0.5stdev), MRFI (±0.5stdev) or LRFI <-0.5stdev). A group rank was established for every cow for each diet treatment. In experiment one, the LF treatment had a significant effect over cow performance. A significant increase over Milk yield of 3.6kg, NEmilk of 2.4kg and Milk components yield of fat, protein and lactose was observed. Body Weight was also significantly increased with a difference of between treatments of 4.82kg. The HF Treatment had a significant effect decreasing the average DMI by 3.07kg. This reduction in intake as well as the difference in energetic density of the diet could explain the differences observed in production performance. A significant reduction of BCS was also reported on cows on the HF treatment interestingly the LF diet did not have a significant impact over bcs. In the analysis for the repeatability of feed efficiency, across nutritional treatments we compared the RFI group ranking and how it fluctuate when diet was change. 38% of the cows maintained their group ranking across both diet treatments. 36% change ranking by moving only one group in the ranking e.g. from HRFI to MRFI. Only 2 cows or 3% or all subject move in the ranking from the HRFI to the LRFI or vice versa. The repeatability of RFI across the two diets for 64 cows was r = 0.46. This is less than the repeatability observed for replacing starch with nonforage fiber. The high forage diet significantly reduced Intake and milk production compared to the high starch diet. This diet effect shows that our study was reasonable for testing our hypothesis. Residual Feed Intake was repeatable at r = 0.46 across starch and forage concentration; however, this repeatability was less that that observed when starch was replaced with non forage fiber.

Effect of two fat supplements differing in saturation on milk production and energy partitioning. Effects of feeding diets containing fat supplements differing in saturation on milk

production and energy partitioning were evaluated. Holstein cows (n=32; 93±35 DIM) were randomly assigned to treatment sequence in a crossover design experiment. Treatments were diets containing a saturated fat supplement (2.5% DM palmitic acid-enriched triglyceride [BergaFat T-300], SAT) or an unsaturated fat supplement (2.5% DM soybean oil, UNSAT). Diets utilized corn silage and alfalfa silage as forage sources and contained 25% NDF, 18% forage NDF, 32% starch, 18% CP, and 4.6% FA. Treatment periods were 28 d in length with the final 5 d used for sample and data collection. The statistical model included the random effect of cow and fixed effects of treatment and period. Compared with UNSAT, SAT increased milk fat concentration (3.07% vs. 2.42%; P<0.01) and yield (1.35 vs. 1.11 kg/d; P<0.01), but reduced milk protein concentration (3.05% vs. 3.12%; P<0.01) and yield (1.40 vs. 1.44 kg/d; P<0.05). Treatment did not alter milk yield (46 kg/d; P=0.6), but SAT did increase FCM (41.9 vs. 38.1 kg/d; P<0.01) and ECM (42.6 vs. 39.8 kg/d; P<0.01) compared with UNSAT. DMI and energy intake did not differ between treatments and averaged 25 kg/d and 41.2 Mcal/d, respectively (both P>0.6). However, SAT increased the milk to feed ratio (ECM/DMI) compared with UNSAT (1.67 vs. 1.53; P<0.01). Compared with UNSAT, SAT reduced BW gain (5.2 vs. 12.8 kg/28 d; P<0.05) but did not alter BCS (P=0.8) or fat thickness over the rump (P=0.7) and rib (P=0.5). SAT decreased plasma concentration of insulin (1.18 vs. 1.34 μg/L, P<0.05), NEFA (122 vs. 137 μEq/L, P<0.01), and triglycerides (7.9 vs. 8.5 mg/dL, P=0.05) compared with UNSAT. There was no effect of treatment on plasma concentration of glucose (P=0.3). Treatments did not alter DM digestibility (64.9% vs. 64.2%; P=0.34) but UNSAT tended to reduce NDF digestibility (29.1% vs.26.4%; P=0.09). UNSAT treatment increased 16-carbon FA digestibility (52.4% vs. 68.5%; P<0.01). UNSAT increased FA digestibility in period 1 but had no effect in period 2; in contrast, UNSAT treatment decreased 18-carbon FA digestibility in period 2 but had no effect in period 1. On the concentration basis, UNSAT treatment reduced de novo synthesized and mixed source FAs, but increased preformed milk FAs (all P<0.01). However, on the yield basis, UNSAT reduced de novo synthesized milk FAs (P<0.01), increased mixed source milk FAs (P<0.01), but had no effect on preformed milk FAs (P=0.27). UNSAT increased t-10C18:1 and t -10, c-12 C18:2 FAs no matter in concentration basis or yield basis (all P<0.01). In conclusion, the two diets resulted in similar NEL intake but the SAT diet containing the palmitic acid-enriched triglyceride increased milk fat yield and partitioned more energy toward milk, while the UNSAT diet containing soybean oil reduced milk fat yield and partitioned more energy toward body gain.

3. J.W. Schroeder (NDSU) Objectives 1.

Supplementation of corn dried distiller's grains plus solubles to gestating beef cows fed low-quality forage. Objective: To investigate the effects of corn dried distiller's grains plus solubles (DDGS) supplementation to cows fed corn stover and silage during late gestation. Objectives include evaluating the impacts of feeding DDGS as a protein supplement during the last trimester on the use of cornstalks as a reliable source of forage; intake, feeding behavior, and maintenance of cow body condition; uterine blood flow to the fetus; colostrum quality; and growth and health of the offspring. Twenty-seven multiparous beef cows (674 ± 17 kg) were divided randomly into 2 pens equipped with Insentec feeders. For 10 wk., both groups were fed the basal diet for ad libitum intake while one group was supplemented (SUP; n = 12) with DDGS at 0.3% of BW (DM basis). Following parturition, all cows received the same diet for

an additional 8 wk. Ipsilateral and contralateral uterine blood flow (BF) and cross sectional area (CSA) of each uterine artery was measured by Doppler ultrasonography on d 180, 216, and 246 of pregnancy; Mammary gland BF relative to the pregnant uterine horn was measured on d 245 of gestation and d 44 of lactation. After parturition colostrum samples were collected. Milk production was assessed on d 44 of lactation. During gestation, SUP cows consumed more total feed than non-supplemented cows (CON). Supplemented cows gained BW ($P < 0.01$) while CON cows tended to lose BW ($P = 0.06$). A main effect of treatment ($P = 0.02$) and day ($P < 0.01$) was observed for total BF. Circulating concentrations of both progesterone (P4) and estradiol-17β (E2) were affected by an interaction of treatment by day ($P <0.01$); both increased with gestation and were greater in CON cows. Aldo-keto reductase (AKR) 1C activity was influenced by an interaction of treatment by day ($P \leq 0.01$). Calves born to SUP cows tended to be heavier than calves born to CON cows ($P = 0.06$). No effect of maternal diet was observed on total mammary BF ($P = 0.33$), but SUP cows tended to produce more milk on d 44 ($P = 0.07$). During lactation, DMI increased ($P < 0.01$) over time. Both groups gained ($P < 0.01$) BW with advancing lactation. In conclusion, distiller's grains altered feeding behavior and likely increased digestibility of corn stover. Blood flowing to the developing fetus was increased and birth weights were greater because of distiller's grains supplementation. Cows supplemented with distillers during gestation produced more milk and heavier calves than control cows.

4. Jong-Su Eun (Utah State). Objective 1:

Effects of supplementing slow-release urea in combination with steam-flaked corn or high-moisture corn on ruminal fermentation and lactational performance of dairy cows. The objective of this experiment was to determine if supplementing slow-release urea with either steam-flaked corn (SFC) or high-moisture corn (HMC) would improve ruminal fermentation and lactational performance of dairy cows. Eight multiparous Holstein cows (32 ± 8.2 days-in-milk) were used in a duplicated 4 × 4 Latin square with one square consisting of ruminally cannulated cows. A 2 × 2 factorial arrangement was used to test 4 dietary treatments: SFC without SRU (SFC–SRU), SFC with SRU (SFC+SRU), HMC without SRU (HMC–SRU), and HMC with SRU (HMC+SRU). The SRU was supplemented at 0.45% dietary dry matter (DM), replacing a mixture of soybean meal and canola meal in a 50:50 ratio. Diets were isonitrogenous and isocaloric averaging 17.4% crude protein (CP) and 1.66 Mcal/kg (NEL). Supplementing SRU with SFC but not with HMC increased DM intake (DMI), resulting in a tendency for an interaction between corn grain (CG) and SRU supplementation (CG × SRU; $P = 0.06$). Intake of CP followed the same pattern as DMI ($P = 0.04$). Total tract digestibility of NDF decreased with SRU supplementation in SFC diet, but increased in HMC diet, leading to a tendency for a CG × SRU ($P = 0.09$). Milk yield did not differ among treatments (39.0 ± 0.37 kg/d). Milk true protein concentration increased with SFC+SRU, whereas it decreased with HMC+SRU ($P = 0.01$). Dietary treatments did not affect ruminal concentration of volatile fatty acids and ammonia-N. Feeding HMC tended to increase feed efficiency for milk production ($P = 0.09$) and N utilization efficiency for milk N ($P = 0.10$), but supplementing SRU did not influence the efficiency parameters. Overall results from this study indicate that HMC can be a good source of readily fermentable carbohydrate to enhance ruminal fermentation and nutrition utilization of early lactating dairy cows without any negative impact if it would be fed at a relatively low dietary concentration.

Ruminal fermentation characteristics of lactation dairy diets with different forage-to-concentrate ratios without or with lipid extract algae in continuous cultures: The current in vitro experiment was performed to test the effects of supplementing lipid extracted algae (LEA) in lactation dairy diets on ruminal fermentation in a 2 (level of forage in diets) × 2 (without vs. with LEA) factorial design with 4 independent runs of continuous cultures (n = 4). Diets with LEA completely replaced mixture of soybean meal and canola meal (50:50 in a DM basis). Feeding LEA decreased culture pH, regardless of level of forage, but the decrease of culture pH was greater under high-forage diet compared with low-forage diet, resulting in an interaction between level of forage and LEA. Under high-forage diet, total VFA concentration increased with feeding LEA, but it was not affected in low-forage diet, leading to a tendency (P = 0.08) of level of forage and LEA interaction. Adding LEA decreased ammonia-N concentration both in high- and low-forage diet. Overall results in this experiment indicate that feeding LEA in lactation dairy diets did not interfere with in vitro ruminal fermentation. The decreased ammonia-N concentration due to feeding LEA may have resulted from less degradation of N fraction in LEA compared with mixture of soybean meal and canola meal.

5. Brian Crooker (University of Minnesota, Obj. 2)

Cows (n = 12/genotype) from unselected (stable milk yield since 1964, UH) and contemporary (CH) Holsteins that differed by more than 4,500 kg milk/305 d were fed the same diet ad lib and housed together for more than 4 months before being blocked (2/genotype) by DIM and randomly assigned within genotype to receive saline or 0.25 μg LPS (Escherichia coli 055:B5) per kg BW. Cows were synchronized to be at day 8 of their estrous cycle for the first challenge (C1) at 70-84 days in milk. Jugular catheters were implanted 24 h before C1. Blood samples were collected at -1, -0.5, 0, 1, 2, 3, 4, 6, 8, 12, and 24 h relative to treatment administration and plasma harvested. Body temperatures (BT) were determined at these times and at 5 and 7 h. Liver biopsies and blood for flow cytometry and hemogram assays were obtained at 0, 4, and 24 h. A second identical challenge (C2) and sampling was conducted 4 d later. Data were analyzed by repeated measures using PROC MIXED (SAS). Means differed when $P < 0.05$. Pre-challenge glucose and IGF-1 were greater ($P<0.01$) and BT was less ($P<0.01$) in UH than CH. Glucose response to LPS was greater ($P<0.01$) in UH than CH, but IGF-1 and BT response was similar in both genotypes. TNFα (Figure 3) and cortisol response to LPS was greater during C1 than C2 ($P<0.02$). TNFα response to LPS was greater ($P<0.05$) in UH than CH in C1 but similar in C2. Cortisol response to LPS increased in both genotypes but returned to baseline earlier in CH than UH ($P<0.05$). LPS decreased white blood cell count ($P<0.01$) but response did not differ between genotypes or challenge. Neutrophil oxidative burst was greater ($P<0.05$) and phagocytic capacity tended ($P=0.07$) to be greater in UH than CH. CD11b expression increased ($P<0.05$) in response to LPS at 4h, was less in CH than UH at 24h and did not differ between C1 and C2. L-selectin decreased in response to LPS at 4hr but did not differ between challenge or genotype. Results indicate genotype impacts bovine response to LPS and this impact differs among the response variables assessed.

Heifers (n = 4/genotype) from unselected (stable milk yield since 1964, UH) and contemporary (CH) Holsteins that differed in milk yield (6,200 and 11,100 kg milk/305 d) or from Red Angus cows (RA) were fed the same diet ad lib and housed together for 47 d before being challenged with 0.5 μg LPS/kg BW. Heifers were 20 months old and pregnant except for 2 CH heifers that were synchronized to be at day 8 of their cycle at the first LPS challenge

(C1). Progesterone exceeded 3.5 ng/mL at C1. Jugular catheters were implanted 24 h before LPS (Escherichia coli O111:B4). Blood samples were collected at -1, -0.5, 0, 1, 2, 3, 4, 6, 8, and 24 h relative to LPS administration and plasma harvested. Body temperatures (BT) were determined at these times and at 5 and 7 h. A second identical LPS challenge (C2) was administered 4 d later. Data were analyzed by repeated measures using PROC MIXED (SAS). Means differed when $P<0.05$. Cortisol, interleukin-6 (IL-6), xanthine oxidase (XO), and tumor necrosis factor α (TNFα) were greater ($P<0.01$) in C1 than C2, BT and IGF-1 were less ($P<0.01$) and glucose and nitric oxide (NOX) did not differ ($P>0.11$) between C1 and C2. There were genotype x challenge x time interactions ($P<0.05$) for BT, glucose and TNFα. During C1, BT increased earlier and peaked higher in RA than UH or CH, glucose increased less in RA than UH or CH, and TNFα increased more in CH than UH and RA. Glucose, TNFα, and BT did not differ ($P>0.10$) among genotypes during C2. There was an interaction of genotype and challenge for IL-6 as response in UH was greater than in CH or RA in C1 but there was no effect of genotype on IL-6 in C2 (Figure 4). There was a trend for NOX ($P=0.10$) to be less in RA than in UH or CH and a trend ($P=0.10$) for a genotype x challenge interaction for XO as XO was greater in UH than in CH and RA in C1 but not in C2. Results indicate the reduced response during a repeated challenge decreases the ability to detect impact of genotype on LPS. Regardless, results indicate genotype impacts bovine response to LPS and that the impact of genotype differs among the response variables assessed in this study.

Using direct comparison of 45,878 SNPs between a group of Holstein cattle unselected since 1964 and contemporary Holsteins that on average take 30 d longer for successful conception than the 1964 Holsteins, we conducted selection signature analyses to identify genome regions associated with dairy fertility. Several genes known to affect reproduction were located in or near genome regions with strong selection signals. These genes include the fibroblast growth factor 1 gene (FGF1) on Chr07; the follicle stimulating hormone receptor gene (FSHR) and the luteinizing hormone choriogonadotropin receptor gene (LHCGR) on Chr11; the KIT ligand gene (KITLG or KITL), the fibroblast growth factor 6 and 23 genes (FGF6 and FGF23) and the cyclin D2 gene (CCND2) on Chr05; the placental growth factor gene (PGF or PLGF) and the estrogen-related receptor β gene (ESRRB) 2Mb downstream of PGF on Chr10; and the prolactin receptor gene (PRLR) on Chr20. The selection signal for the region containing FGF1 was among the strongest selection signals we observed. According to the literature on these genes, FGF1 is involved in broad mitogenic and cell survival activities including embryonic development, PGF plays a key role in embryogenesis, ESRRB plays an essential role in placenta development, FSHR is necessary for follicular development and is expressed on the granulosa cells that are closely associated with the developing female gamete in the ovary of mammals, and LHCGR is necessary for follicular maturation and ovulation. Mouse knockout models showed that FSHR, KITLG, CCNG2, and PRLR were involved in female fertility proteins. These known gene functions related to reproduction and the fact that these genes were in or near chromosome regions with strong selection signals indicate that these genes could be involved in the vast difference in fertility between contemporary Holsteins and the 1964 Holsteins.

Objectives were to determine the effects recombinant bovine somatotropin (rbST) treatment during the peripartum period on hepatic mRNA expression of genes related to inflammation and immune response. Holstein cows were assigned randomly to receive no treatment (control; n = 10), 87.5 mg (rbST87.5; n = 12), or 125 mg (rbST125; n = 10) of rbST

every 7 d from −21 to 21 d relative to calving. Liver biopsies were collected −21, −7, and 7 d relative to calving. Twenty-four genes were assessed by direct molecular counts using NanoString technology. Continuous data were analyzed by ANOVA. Gene expression on d −21 was used as a covariate for analyses of mRNA expression on d −7. No differences in mRNA expression were observed among treatments on d −21 for all the genes except SOCS3, which had lower ($P \leq 0.05$) mRNA expression in control cows compared with rbST87.5 cows. On d −7, expression of mRNA for ANGPTL4 and SCARB1 was higher ($P \leq 0.05$) in rbST87.5 and rbST125 cows than control. Cows in the rbST87.5 treatment had ($P \leq 0.05$) higher mRNA expression for HP, ICAM1, SOCS2 and XBP1 on d −7 than control cows. Control cows had ($P \leq 0.05$) higher mRNA expression for HIF1A than rbST125 cows on d −7. On d 7, control cows had ($P \leq 0.05$) higher mRNA expression for CXCL1, IL1RN, MYD88, NFKBIA, and SOCS3 compared with rbST87.5 and rbST125 cows. Control cows had ($P \leq 0.05$) higher mRNA expression for ICAM1 and XBP1 than rbST125 cows and had higher mRNA expression for HIF1A than rbST87.5 cows. On the other hand, expression of mRNA for NR3C1 and SOCS2 was ($P \leq 0.05$) lower in control cows than rbST125 and rbST87.5 cows, respectively. Treatment did not affect hepatic expression of the genes CEBPD, JUN, M-CSF1, NFKB1, PPARGC1A, STAT5B, TLR2, TNF, TNFRSF1 and TNFRSF5. The gene G-CSF was not detected. Weekly treatment of periparturient cows with rbST regulates liver mRNA expression of genes related to inflammation and immune response during the prepartum and postpartum periods. Increased postpartum mRNA expression of inflammatory and immune responses genes in control cows might be a consequence of increased incidence of postpartum diseases.

6. Ermias Kebreab (University of California, Davis, Obj. 3) James Fadel (University of California, Davis, Obj. 3)

Several studies have identified dietary manipulation strategies for the mitigation of emissions, but studies examining the costs of reducing methane by manipulating diets are scarce. The objective of this study was to develop an optimization framework for the joint minimization of dietary costs and methane emissions based on the identification of a set of feasible solutions for various levels of tradeoff between emissions and costs. Such a set of solutions was created by the specification of a systematic grid of goal programming weights, enabling the decision maker to choose the solution that achieves the desired tradeoff level. Moreover, the model enables the calculation of emission-mitigation costs imputing a trading value for methane emissions. Emission imputed costs can be used in emission-unit trading schemes, such as cap-and-trade policy designs. An application of the model using data from lactating cows from dairies in the California Central Valley is presented to illustrate the use of model generated results in the identification of optimal diets when reducing emissions.

A genetic algorithm was implemented to select models to predict fecal, urinary, and total manure N excretions, and milk N secretions from 3 classes of animals: lactating dairy cows, heifers and dry cows, and steers. Two tiers of model classes were developed for each category of animals based on model input requirements. A total of 6 models for heifers and dry cows and steers and an additional 2 models for lactating dairy cattle were developed. Evaluation of the models using K-fold cross validation based on all data and using the most recent 6 yr of data showed better prediction for total manure N and fecal N compared with urinary N excretion, which was the most variable response in the database. Compared with extant models from the literature, the models developed in this study resulted in a significant improvement in

prediction error for fecal and urinary N excretions from lactating cows. For total manure production by lactating cows, extant and new models were comparable in their prediction ability. Both proposed and extant models performed better than the prediction methods used by the US Environmental Protection Agency for the national inventory of greenhouse gases. Therefore, the proposed models are recommended for use in estimation of manure N from various classes of animals.

7. Yves Bosclair (Cornell University)

Mammals meet the increased nutritional demands of lactation through increased feed intake and a collection of adaptations known as adaptive metabolism (e.g., mobilization of endogenous reserves, glucose sparing mechanisms, etc,). In the modern dairy cow, adaptive metabolism predominates over feed intake at the onset of lactation and develops concurrently with a reduction in plasma leptin. We sought to address the role of leptin in adaptive metabolism by reversing its reduction in early lactation. Starting on day 8 of lactation, cows received a constant intravenous infusion of either saline (control) or human leptin (hLeptin) for 96 consecutive hours (5 cows per treatment). In the hLeptin group, human leptin rose to a steady state concentration of 14.0 ± 1.9 ng/ml within 2.3 h of infusion, consistent with a calculated half-life of 28.0 ± 5.0 min in the vascular compartment. The hleptin infusion did not impact voluntary feed intake, milk production or estimated energy balance. The hleptin infusion was also devoid of effects on plasma glucose, free fatty acids and insulin but caused significant reductions of 73 and 28% in liver glycogen and triglyceride content, respectively. Finally we examine the effects of hLeptin on secretion of growth hormone and the thyroid hormones. hLeptin infusion increased the average plasma growth hormone concentration but had no effect on plasma IGF-I. Cows infused with human leptin experienced significant increases of ~ 45% in the plasma concentrations of both thyroxine and triiodothyronine. We conclude that leptin is a major determinant of plasma thyroid hormone levels in early lactating dairy cows.

Ruminants remain productive during the energy insufficiency of late pregnancy or early lactation by evoking metabolic adaptations sparing available energy and nutrients (e.g., higher metabolic efficiency and induction of insulin resistance). A deficit in central leptin signaling triggers these adaptations in rodents but whether it does in ruminants remains unclear. To address this issue, five mature ewes were implanted with intracerebroventricular (ICV) cannula in the third ventricle. They were used in 2 experiments with an ovine leptin antagonist (OLA) when well-conditioned (average body condition score of 3.7 on a 5 point scale). The first experiment tested the ability of OLA to antagonize leptin under in vivo conditions. Ewes received continuous ICV infusion of artificial cerebrospinal fluid (aCSF), ovine leptin (4 µg/h) or the combination of ovine leptin (4 µg/h) and its mutant version OLA (40 µg/h) for 48 h. Dry matter intake (DMI) was measured every day and blood samples were collected on the last day of infusion. ICV infusion of leptin reduced significantly DMI by 24%, and this effect was completely abolished by OLA co-infusion. A second experiment tested whether a reduction in endogenous leptin signaling in the brain triggers metabolic adaptations. This involved continuous ICV infusions of aCSF or OLA alone (40 µg/h) for 4 consecutive days. The infusion of OLA did not alter voluntary DMI over the treatment period or on any individual day. OLA did not affect plasma variables indicative of insulin action (glucose, non-esterified fatty acids, insulin and the disposition of plasma glucose during an insulin tolerance test) or

plasma cortisol, but reduced plasma triiodothyronine and thyroxine. Overall, these data show that a reduction of central leptin signaling has little impact on insulin action in well-conditioned mature sheep but show that reduced central leptin signaling plays a role in controlling thyroid hormone production.

8. Jeff Firkins (Ohio)

Nitrates have been successfully fed to dairy cows to decrease methane emissions in several experiments. In the nitrate assimilatory pathway, bacteria reduce nitrate to nitrite to ammonia. Because the second step can be rate-limiting, nitrite accumulation poses health risks such as methemoglobinemia, which would hinder adoption of nitrate feeding to compete with methanogens for H2 while assimilating the N from nitrate into microbial protein. The yeast Saccharomyces cerevisiae has the potential to anaerobically respire nitrite through its cytochrome c oxidase; it also can stimulate populations of bacteria that express nitrate and nitrite reductases. For this project, 4 dual flow continuous culture fermenters were used in 5 periods with 7 d of adaptation and 3 d of sampling. Fermenters were fed 40 g DM (50:50 ratio of concentrate:alfalfa pellet). Treatments were arranged in a 2 x 2 factorial with NO3 (1.5% of DM) or urea as an isonitrogenous control and without or with Yea-Sacc ® (Alltech Inc., Nicholasville, KY) fed at a recommended 0.010 g/d. Gas production was measured over 3 d by closed circuit respirometry; 1 fermenter's gas production was omitted for all periods (unrelated to treatment). Objectives were to test the hypothesis that the combination of live yeast culture and nitrate would mitigate methane production in continuous culture compared to the control (a statistical interaction). However, there were no interactions ($P > 0.10$). The main effect of nitrate decreased ($P < 0.05$) CH4 emission compared to urea control (29.6 vs 21.0 mmol/d). There was no difference ($P > 0.10$) for H2 emission for nitrate or yeast (averaging 0.149 mmol/d; SEM = 0.051), but the main effect of nitrate was decreased ($P < 0.01$) for aqueous H2 concentration compared with urea (1.23 vs 1.88 μM). Total VFA production (averaging 148 mmol/d; SEM = 15) and acetate:propionate (averaging 3.37, SEM = 0.12) did not differ ($P > 0.10$) among treatments. Nitrate decreased methanogenesis without affecting H2 variables. No interactions were detected, but live yeast might offer a useful protection against incompletely adapted rumen microbial populations.

Nutritional appreciation of the gut microbiome has extended past the single species concept based on characterizations of pure cultures of prokaryotes. Although previous studies based on rRNA gene sequence analyses have provided intriguing results for the field of ruminant nutrition, opportunity exists for more robust association of phylogenetic distributions to help decrease the predictive variation in digestibility and nutrient supply to the animal. We need to assess the functional importance of key members of the rumen microbiome and the redundancy of important taxa, how they are related among different animals or different dietary conditions, and how (or how long it takes) to strategically manipulate certain taxa. Historically, efficiency of protein usage in the rumen has been associated with types and/or counts of protozoa; yet, very large ranges exist among species in the biomass per cell. The hyper-ammonia producing bacteria catabolize amino acids for energy and metabolic intermediates in addition to synthesis of cellular protein, but these bacteria are not well integrated into any model because of their low abundance. The non-growth metabolism (maintenance and energy spilling) of a mixed community might help explain the variation remaining in prediction of microbial growth efficiency. The fibrolytic consortium is more

diverse than the sum of characterized fibrolytic species, and fiber surface area (not bacterial abundance) limits fiber digestibility. A better characterization of bacterial colonization should help understand and therefore improve the consistency of fiber digestibility under different dietary conditions. The hindgut needs more attention in some dietary conditions that shift site of fiber digestibility. Although metagenomic approaches have greatly aided our understanding of bacteria involved in various steps of biohydrogenation, can abundance of certain bacteria be better related to activity to explain the large differences among studies? Can the discovery of novel glycosyl hydrolases help extend phylogenetic analyses to the functional level? Can metagenomics analysis of the complex microbiome help explain varying degrees of VFA interconversion and recovery of hydrogen in hydrogen balance models? Can metagenomics analysis of the rumen from cows fed monensin extend inferences past culture-based conclusions based on Gram staining and acetate:propionate ratio to assess more complicated microbial interactions? Answers to these types of questions might better explain how the diversity/structure of rumen microbiome varies with varying concentrations of metabolites and biomarkers. Therefore, semi-quantitative sequence distributions need more quantitative verification of abundance of taxa that are functionally important or at least empirically associated (indicators) with important nutritional responses.

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

Most rumen bacteria cannot be cultured, making their niche in the rumen difficult to identify. Fluorescent substrates could potentially identify substrates preferences and thus the niche of these uncultured bacteria, but specificity and kinetics of their uptake have not been thoroughly evaluated. Our objective was to determine if cultured strains of rumen bacteria would transport a fluorescent analog of glucose (2-NBDG) with the same specificity and kinetics as glucose.

Streptococcus bovis JB1 and other pure cultures of bacteria were harvested in the mid-to-late log phase, washed, and dosed with 2-NBDG or radiolabeled sugar (0 to 100 μM). Transport was halted by adding −5°C stop buffer and filtering through a membrane. The membrane was taken for fluorometry or liquid scintillation counting. For S. bovis JB1, we could detect 2-NDBG transport quantitatively and within 2 s. We found Vmax of 2-NBDG transport was 2.9-fold lower than that for [14C]-glucose, whereas Km was 9.9-fold higher. The mannose phosphotransferase system (PTS) was found responsible for transport, based on experiments with wild-type and mutant strains. When examining twelve species of glucose-utilizing rumen bacteria, only the five which possessed a mannose PTS were shown to transport 2-NBDG (Fig. 3). Those five uniformly transported [14C]-mannose and [14C]-deoxyglucose (other glucose derivatives at the C-2 position) at high velocities. Species that did not transport 2-NBDG at detectable velocities did not possess a mannose PTS, though they collectively possessed several other glucose transporters. Preliminary cell sorting shows many mixed rumen bacteria take of 2-NBDG and could be separated for downstream analysis (e.g., for sequencing and taxonomic assignment). These results suggest that 2-NBDG could identify uncultured, glucose-utilizing bacteria, but only those with a mannose PTS.

10. Richard Erdman (University of Maryland, Obj. 1 and 2)

Although many studies have focused on the influence of dietary cation-anion difference (DCAD) on animal performance, few of them have examined the effect of DCAD on the rumen ionic environment.

Previous work by Iwaniuk et al. (JDS . 98:1950, 2015). Showed that substitution of Na for K in diets that were DCAD constant increased milk fat percent. This suggested that strong ion source and not just DCAD alone might influence rumen fermentation. The objective of this study was to examine the effects of DCAD, cation source (Na vs. K), and anion source (Cl vs. bicarbonate or carbonate) on the rumen ionic environment and fermentation. The study used five rumen fistulated dairy cows, and treatments were applied using a 5 x 5 Latin square design with 2 wk experimental periods. Treatments consisted of: 1) the basal total mixed ration (TMR); 2) the basal TMR plus 340 mEq/kg (DM basis) sodium and chloride using NaCl; 3) the basal TMR plus 340 mEq/kg potassium and chloride using KCl; 4) the basal TMR plus 340 mEq/kg sodium using NaHCO3; and 5) the basal TMR plus 340 mEq/kg potassium using K2CO3. On last day of each experimental period, rumen samples were collected and pooled from five different locations at 0, 1.5, 3, 4.5, 6, 9, and 12 h post-feeding for measurement of rumen pH, strong ion and VFA concentrations.

Treatment had no effect on feed intake or milk production (Table 1) but we really did not expect to see a difference in performance as the cows used in the study were in mid to late lactation. As expected increased DCAD (bicarbonate and carbonate salt so Na and K, respectively) increased rumen pH (Table 2) but there was no effect of DCAD on total VFA concentrations. Dietary supplementation of individual strong ions increased their corresponding rumen ion concentration (Table 2). Rumen Na was decreased by 24 mEq/L when K was substituted for Na in the diet but added dietary Na had no effect on rumen K. Rumen Cl was increased by ~10 mEq/L in diets supplemented with Cl. There was a time post-feeding effect on rumen ion concentrations where Na decreased and K and Cl increased from 0 to 1.5 h post-feeding. This is consistent with literature data that shows a positive correlation between rumen K and Cl and that as Na decreases, K and Cl increase. The study demonstrated that rumen ion concentrations can be manipulated by dietary ion concentration but not DCAD. Potential implications of changes in rumen ion concentrations include responses to dietary ionophores that influence rumen fermentation by transporting cations into affected bacterial cells and disrupting homeostasis.

11. Shawn Donkin (Purdue University, Obj. 2)

Rationale: Chemical treatment may improve the nutritional value of corn stalk residues and their potential use as an alternative forage source for lactating dairy cows. Objective: The objectives of this study were to determine the effect of prestorage hydration and treatment with 6.6% Ca(OH)2 on feeding value of corn stalks as an alternative forage source on milk production, milk composition, and DMI. Experimental design and data collection: Mid-lactation multiparous Holstein cows (n = 30) were stratified by parity and milk production and randomly assigned to one of three diets. Corn stalks were chopped, hydrated, and treated with 6.6% Ca(OH)2 (DM basis) and stored in Ag-bag silos. Treated corn stover was fed in a TMR at 0, 15, and 30% of the diet DM. Treated corn stover replaced either alfalfa haylage (15% stover diet) or replaced alfalfa haylage and an additional portion of corn silage (30% stover

diet). Cows were individually fed in tie stalls for 10 weeks. Results: Milk production was not altered by treatment (P = 0.80). Compared with 0% stover diet, DMI was reduced when the 15% stover diet was fed (25.9 vs. 22.7 ± 0.88 kg/d, P < 0.05) and tended to be reduced (25.9 vs. 23.1 ± 0.88 kg/d, P = 0.08) when cows were fed the 30% stover diet. Milk production per unit DMI (kg/kg) tended to increase for cows fed 15% stover diet compared with the 0% stover diet (1.41 vs. 1.62 ± 0.07, P = 0.08) but was not different between cows fed the 0% and 30% stover diets (1.41 vs. 1.50 ± 0.07, P = 0.62). Milk composition, energy corrected milk production, and energy corrected milk produced per unit of DMI (kg/kg) was not different (P > 0.05) among treatments for the 10-week feeding period. Cows fed the 15% and 30% diets had stable DMI and daily milk production over the 10-week treatment period but DMI for cows fed 0% stover increased slightly (time x treatment effect, P < 0.05). Conclusions: These data indicate that corn stover processed through prestorage hydration with Ca(OH)2 results in an alternative forage source for lactating dairy cows that when fed to mid-lactation cows tends to improve the efficiency of conversion of feed to milk without altering milk production or milk composition.

Rationale: In nonruminants, the activity of PCK1 (the cytosolic form of the enzyme) 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 PCK1. Insulin dominantly counteracts the effects of these hormones and results in a depression in PCK. In our in vivo data indicate a lack of response in PCK1 to feed restriction, glucagon injection and other perturbation but an effect of propionate infusion and diets that elevate ruminal propionate production to elevate PCK1 expression. The source of the induction of PCK1 expression in ruminants is not known. In vitro promoter experiments using the bovine PCK1 promoter allows us to study the direct effect of propionate at transcription level. In examining the bovine PCK1 promoter transcription factor binding sequences were identified within the bovine PCK1 proximal promoter for cAMP response element (CRE) at -94 through -87 and for Hepatic Nuclear Factor 4α (HNF4α) at +68 through +72 and -1078 through -1074 respectively. We hypothesized that propionate, butyrate, cAMP, and dexamethasone activate common cis-regulatory elements within the bovine PCK1 promoter to stimulate its transcription. Objectives: To identify the location of the candidate cis-elements within the bovine PCK1 promoter, specifically CRE and HNF4α. To determine the essentiality of the consensus CRE and putative HNF4α as well as how they interact to mediate PCK1 transcription in response to propionate, butyrate, cAMP and dexamethasone. Methods and Materials To test control of transcription, the wild-type bovine PCK1 promoter from -1238 through +221 bp (PCK1 (WT)) was ligated to a luciferase reporter gene. The essentiality of CRE and the two putative HNF4α binding elements was determined using site-directed deletions of the core transcription factor binding regions within the PCK1 promoter DNA. H4IIE cells were transfected with the promoter-reporter constructs and exposed to 2.5 mM propionate, 2.5 mM butyrate, 1 mM cAMP, 5 μM dexamethasone or their designated combinations for 23 h. Results: Exposure to cAMP, dexamethasone, cAMP+ dexamethasone, propionate, cAMP+ propionate, cAMP+ dexamethasone + propionate, and butyrate induced expression of the PCK1 (WT) relative to the no addition controls by 2.0, 2.3, 3.9, 7.6, 9.2, 18.0 and 15.4± 0.9 X respectively. A similar pattern was observed for each single mutant and the double mutant lacking CRE at -94 through -87 bp and HNF4α at −1078 through −1074 bp. Responses to all treatments were completely abolished for the double mutant lacking CRE at -94 through -87 bp and HNF4α at +68 through +72 bp. Conclusions and Implications:

Propionate increases the transcriptional activity of the bovine PCK1 promoter. The data indicate that propionate, butyrate, cAMP and dexamethasone share common cis-acting elements to induce bovine PCK1 transcription. The CRE at -94 through -87 bp and HNF4α binding element at +68 through +72 bp act synergistically to mediate the full responsiveness of the bovine PCK1 promoter to propionate, butyrate, cAMP, and dexamethasone as well as the synergistic effect of cAMP, dexamethasone and propionate.

12. Lou Armentano and Heather White (University of Wisconsin)

Supplementing fat to lactating cows may reduce total tract NDF digestibility (ttNDFd). The objective was to analyze the effects of different types of fat across studies. The observed values for ttNDFd and DMI, as well as the numerical difference between control and fat supplemented diets for these two variables, were calculated within study and analyzed in SAS 9.4 using Proc Mixed. Studies were weighted based on the inverse of the standard error of ttNDFd. The models were analyzed by fat type or contained the fixed effect of fat type. Study was always included as a random effect. Other dietary parameters were included in the models if they were found to be significant. Fatty acid content of treatment diets never exceeded 10% of DM (mean= 6.2, SD=1.4). Supplementation of fats containing 12 and 14 carbon chain fatty acids (C12/C14) decreased ttNDFd in all models (average=9.6 percentage units). Oil significantly decreased ttNDFd by 1.9 percentage units at 3% supplementation in the regression model. No other type of fat decreased ttNDFd using the regression models, though saturated fats significantly increased ttNDFd by 1.7 percentage units at 3% supplementation and 2.4 percentage units in the individual regressions. In the models utilizing the difference from control oil decreased ttNDFd 2.4 percentage units at 3% supplementation and calcium salts of palm oil significantly increased ttNDFd by 2.7 percentage units at 3% supplementation and 2.1 percentage units utilizing the least squared means. In order to attempt to explain changes in ttNDFd, DMI was also analyzed and calcium salts of palm oils and other oils, and C12/C14 showed significant decreases for DMI. A regression analysis across all types of fat, except C12/C14, using Proc Glm in SAS 9.4 of the difference in ttNDFd versus the difference in DMI within study shows a positive relationship, though the r2 is low (0.06) and there is no relationship within the calcium salts or oil categories specifically (P>0.10). This suggests that the increase in ttNDFd for calcium salts of palm oil is not explained through the decrease in DMI. There is a positive relationship between the changes for ttNDFd and DMI in the saturated fat category (P=0.003) which would be consistent with greater digestibility allowing the cow to maintain her intake. Overall, these results indicate that the addition of a fat supplement, in which the fatty acids are 16C or greater in length, has minimal effects on ttNDFd.

Prior research feeding free oils has shown greater milk fat yield when feeding high oleic vs. iso-fat high linoleic diets (He, et al., 2012. JDS 95:1447-1461), but less work has been done with oilseeds of different fatty acid (FA) profiles. We hypothesized that Plenish High Oleic Soybeans ® (DuPont-Pioneer) would increase milk fat yield relative to conventional soybeans. Trial 1 used 63 cows (28 primiparous and 35 multiparous). Cows were housed in a common pen with 32 electronic feed gates and fed conventional or high oleic whole raw beans for 3 weeks following a covariate adjustment period. Preliminary results from this trial were reported previously to this regional research group. The second trial used 20 cows (10 primiparous, 10 multiparous) in a tie stall barn in two 5x5 Latin Squares within parity. These

pairs of squares used complementary sequences of treatments designed to remove one-period carryover effects. Raw Plenish or conventional beans, either ground or whole, formed 4 iso-fat diets in a 2x2 factorial, plus an additional lower fat diet without soybeans was fed. Diets were 55% forage, balanced for amino acids, and contained 2.9-3.9% ether extract from soybeans (15.9-19.1% soybeans, DM basis). The conventional soybeans contained approximately 50% linoleic acid and 25% oleic acid compared with the Plenish beans which contained approximately 5% linoleic acid and 80% oleic acid.

In trial 1, there was a parity by diet interaction; no diet effects were seen for production by primiparous cows, but multiparous cows fed the Plenish bean diet had increased milk fat yield. In trial 2 there was a particle size by bean interaction so that the Ground Plenish treatment had higher milk fat concentration and yield compared with the Ground conventional treatment whereas there was no significant difference between the whole beans. In trial 1 the milk fatty acid proportions show that the multiparous cows fed conventional soybeans had a higher proportion of milk fat synthesis. However, the yield data indicate that this is due to decreased incorporation of 18C FA while there is no difference in shorter chain FA yield. There was a significantly greater yield of trans-10 18:1 in both the multiparous and primiparous cows on the conventional bean diets though there were no differences in trans-10, cis-12 CLA. In trial 2 here were not significant differences in the proportion of FA, but Plenish cows yielded more of each due to their increased milk fat yield relative to control. There were significant differences in the proportion and yield of trans-10 18:1 with Plenish cows producing less and larger particle size producing less both in yield and proportionally though there were no significant differences in trans-10, cis-12 CLA. While the physical form of whole fat soybeans affect the response to added beans, beans with low levels of linoleic are less detrimental to milk fat even with more rapid oil availability.

The objective of this study was to characterize PNPLA3 protein abundance in transition cows subjected to fatty liver induction. Multiparous cows were blocked by expected calving date and randomly assigned to a control (n=3) ad libitum intake group, or a fatty liver induction (n=6) group that was overfed during the dry period, and feed restricted (80% of dry matter intake) at +14 days relative to calving (DRTC) until onset of clinical ketosis. Liver biopsies were taken in the prepartum (-28, -14 DRTC), lipid accumulation (+1, +14, +28 DRTC), and recovery (+42, +56 DRTC) periods and at the time of clinical ketosis. Liver PNPLA3 protein abundance was determined through Western blot analysis and normalized to total protein. Data were analyzed using PROC MIXED of SAS 9.3. Abundance of PNPLA3 was analyzed with main effects of treatment, period, and treatment x period, and random effect of cow. Protein abundance was also analyzed by PROC MIXED and PROC CORR based on total lipid accumulation diagnosed as high (>15%, dry matter) or low (<15%, dry matter). All fatty liver induction cows became clinically ketotic and developed fatty liver. Abundance of PNPLA3 was greater ($P = 0.02$) during the recovery period compared with the accumulation period (1.12 vs. 0.79 + 0.09, arbitrary units). Cows with high liver lipids had decreased ($P < 0.01$) PNPLA3 abundance compared to cows with low liver lipids (0.69 vs. 1.0 + 0.08, arbitrary units). There was a negative correlation ($P < 0.01$; $r = -0.427$) between liver lipid concentration and PNPLA3 abundance. These data indicate that regulation of PNPLA3 may influence liver lipid accumulation and potential recovery from fatty liver disease.

The objective of this experiment was to examine the regulation of genes controlling methyl group transfer in response to increasing concentrations of choline chloride (CC), dL-

met (dLM), and added fatty acids (FA). Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h prior to treatment with CC (33, 100, 200, 450 μM) and dLM (16, 30, 100, 300 μM), with or without a 1 mM FA cocktail in a factorial design. Concentrations mimicked expected physiological concentrations. After 24 h, media was collected for quantification of reactive oxygen species (ROS) by fluorometric assay and cells were collected for quantification of gene expression. Data were analyzed using PROC MIXED of SAS 9.4 with linear and quadratic contrasts in a model with fixed effect of treatment and random effects of calf. Interactions were not significant and therefore only main effects are discussed. Met can be generated from betaine via BHMT, or from homocysteine via MTR which also serves as the final step in regeneration of met after methyl-group donation. Increasing dLM concentration did not alter BHMT expression but did decrease (P = 0.003) MTR expression. Increasing concentration of CC did not alter BHMT but did increase (P = 0.02) MTR expression, suggesting that CC plays a key role in regeneration of met after methyl group donation. FA increased (P = 0.05) BHMT expression but decreased (P = 0.0001) MTR expression, which favors regeneration of met that is coupled with downstream glutathione production, which may aid the cell with oxidative stress associated with FA metabolism. Both CC and dLM decreased (P = 0.02) expression of MAT1A, the enzyme that generates SAM from met. PEMT expression was not affected by CC or dLM suggesting that dLM was not used to generate phosphatidylcholine. ROS tended to decrease (P = 0.08) with increasing CC treatment but was not changed with dLM treatment. After 24 h media was collected for VLDL quantification by ELISA. The ELISA (NeoBiolab, Cambridge, MA) is a competitive immunoassay that utilizes antibodies for apolipoprotein B100 and C, which in combination uniquely binds to VLDL particles. The assay was validated using cell culture and postpartum bovine serum samples with purified LDL and HDL spikes. Recovery of samples with spiked standards, compared with independently analyzed samples and standards, was 102 ± 1%. Increasing concentrations of CC linearly increased (P = 0.02) VLDL export from hepatocytes. Treatment with dLM did not alter (P = 0.7) VLDL export. These data suggest that CC may play a critical role in donating methyl groups and in decreasing ROS within the hepatocyte. Furthermore, these data support the role of CC, but not dLM, supplementation in increasing VLDL export from hepatocytes.

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

Use of Chloride concentration to identify ration sorting by dairy cattle HA Rossow Currently differences in proportions of particle sizes between the TMR fed and the residual TMR indicates if dairy cattle are sorting their feed. However, results using the Penn State Particle Sorter (PSPS) can be variable depending on the dry matter of the ration and how vigorously and consistently PSPS is shaken for each sample. Comparing differences in chloride concentration (CC) between the TMR fed and residual TMR is an easier and more accurate method to assess TMR sorting by dairy cattle. Therefore the objective of this study is to examine if CC in the TMR fed compared to the residual TMR could be used to assess ration sorting by dairy cattle. Ten samples each of the TMR dropped and the residual TMR were collected from pens at 5 dairies in Tulare Co., CA, including cows close to calving and cows 30-200 DIM. All samples were then sorted using the PSPS. CC was measured by soaking 30g of each sample in 200ml of de-ionized water for 2 hr and then measuring CC using an Oakton waterproof SaltTestr meter (Oakton Instruments, Vernon Hills IL) with a range of 0 to 1%

chloride. Statistics to compare % and CC of PSPS of the TMR dropped to the residual TMR were performed using Proc GLM (SAS Institute, 2013) for each dairy and pen to assess cows sorting of the TMR. CC increased with decreasing particle size with means (standard deviations) of CC of 0.195 % (0.048), 0.254 % (0.041), 0.264 % (0.055) and 0.277 % (0.080) for top, middle, screen and bottom trays of the PSPS, respectively. To assess mixer wagon function, CC of samples taken from the dropped wagon load must fall within a 10% CV in order to be considered a uniform load. Sorting is indicated when the dropped mixer wagon load CC CV is below 10% and residual CC CV is above 10%. Sorting can only be evaluated if the dropped wagon load was uniform. Otherwise the contribution of a non uniform mixer wagon load to sorting is unknown. Figure 1 shows that with the CC method, cows sorted in 4 pens (.). Using guidelines for indicating sorting with the PSPS (Figure 2), sorting is indicated in 8 pens ($P < 0.0001$) . Using the same guideline as CC (above 10% CV), the PSPS did not identify any non uniform mixer wagon loads. The PSPS did not take into account the uniformity of the dropped mixer wagon load TMR. Therefore more information about ration uniformity and cow TMR sorting can be obtained using the easier CC method but more research needs to be do done to determine the impact of non uniform mixer wagon loads and sorting as determined using CC on milk production.

Use of Ultrasound for the assessment of muscle area and depth in preweaned Holstein calves J.H. Davis, C. Sparlin, P.V. Rossitto, J.D. Champagne, S.S. Aly, C.M. Barker, H.A. Rossow The prewean period is one of the most important stages of dairy calf development. A tool is needed to more accurately assess calf growth, specifically muscle development. The objective was to determine if ultrasound can be used to predict *longissimus dorsi* (ribeye) linear depth and *external carpi radialis* (front) and *semitendinosus* (hind) area in postmortem preweaned Holstein calves. Postmortem preweaned bull and heifer calves (n=191, age 17.3 ± 20.67 d, body weight 37.9 ± 19.07 kg) were obtained from two California calf ranches between April and July 2013. Ultrasound images of the ribeye, front, and hind muscles were collected using an Aloka 500V equipped with a 5-cm 7.5 MHz linear transducer. Ultrasound ribeye linear depth and front and hind areas were calculated using the Ultrasound Image Capture System®. The ribeye was dissected and measured for linear depth. The front and hind muscles were dissected and the cross-sectional planes were traced onto transparency paper. The transparency paper was photocopied and individual paper muscle tracings were cut out and weighed. The weights of the paper muscle tracings were then converted to areas using the known area of a standard 8.5 x 11 inch paper. Means were calculated using PROC GLM in SAS (version 9.2). Mean dissected values for the ribeye, front, and hind muscles (1.65 ± 0.44 cm, 6.23± 1.83 cm2, 9.05 ±2.37 cm2) were greater than the respective mean ultrasound values (1.46 ± 0.37 cm, 5.41± 1.49 cm2, 8.60 ±2.40 cm2) indicating ultrasound underestimated the true linear depth and area values consistently. The relationship between the dissected and ultrasound measurements was tested using Pearson's correlation coefficient (PROC CORR, SAS). Overall, there was a strong, positive relationship between both the dissected and ultrasound measurements for the ribeye (r=0.55, P<0.01), front (r=0.65, P<0.01), and hind muscle (r=0.80, P<0.01). The weight, age, and gender of the calf and the operator of the ultrasound may explain some of the variability not accounted for by the correlation coefficient. The *semitendinosus* muscle displayed the highest correlation coefficient and may be used in future studies to assess calf muscle growth and guide implementation of dynamic feeding changes on both dairy farms and calf ranches.

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

Volatile Fatty Acid (VFA) Production: A dual continuous flow fermentor study was conducted in 2014 at Ohio in collaboration with Firkins and Kohn of Maryland with the objective of determining the effect of varying VFA concentrations and pH on hydrogen and methane production and VFA production rates. Treatments were: control, acetate infusion (20 mmol/d), propionate infusion (7 mmol/d), and low pH (0.5 units lower than control). Methane and hydrogen production were reduced for the low pH treatment ($P < 0.05$). Net production of propionate was increased for the low pH treatment. However, contrary to our hypothesis, thermodynamic state did not affect rates of interconversion among VFA. The primary reason for increased propionate appears to be a shift in microbial metabolism. Outcome: As VFA are a primary energy source and affect hormonal signals, it is important to predict their production if we are to predict animal performance. We ruled out thermodynamic feedback as a contributor to net VFA production rates through interchange of VFA. This knowledge indicates the shift results from altered metabolic pathway use.

Molly model: The objective of this work was to develop an improved representation of rumen digesta outflow in the model. The work was a collaboration between VA, DairyNZ, and AgResearch and included 3 changes: 1) a medium-size particle pool was added to the rumen which was assumed to ferment and pass from the rumen; 2) particulate passage was made a function of particle size, particle concentrations in the rumen, and liquid passage rate; and 3) fermentation rate was made a function of particle surface area in the medium and small particle pools. Prediction accuracy of digestive functions was not substantially improved, but the model now reproduces observed patterns in rumen function and diet digestibility as affected by feed intake and dietary particle size including decreased diet digestibility and methane yield per unit of dry matter as intake increases. Predictions of acetate, propionate, and butyrate concentrations were improved and the previously observed slope bias completely removed. Prediction errors for ruminal ammonia concentrations were also substantially reduced. Outcomes: The revised model performed better in predicting patterns of rumen metabolism within the day and across days. A key improvement was the correction of an inappropriate pattern of diet digestibility and methane yield as feed intake increased. This model is better able to predict the effects of increasing production and different feed management on animal performance and the carbon footprint of the product produced.

The objective of the work was to use digestion coefficients from the Feng et al. (2015) model (VT model) to calculate phosphorus (P) bioavailability of common feeds used in dairy production. Compared to the new model, the NRC (2001) underestimates the bioavailabilities of alfalfa hay, alfalfa silage, corn silage, grass hay, mixed legume silage, and high moisture corn; and overestimates the bioavailabilities of corn grain. Two dairy diets were formulated using nutrient values from the NRC (2001): a standard diet which includes minimal byproducts and a high byproduct diet. Total bioavailable P was calculated for each diet using values from the NRC (2001) and the VT model. Comparison of P balance for each diet was made for a representative cow with an absorbed P requirement of 59.4 g/d. The standard diet supplied 56.7 g and 53.5 g of bioavailable P per day using bioavailabilities from the NRC (2001) and VT models, respectively, resulting in a P balance of -2.7 and -5.9 ± 0.26 g/d. The byproduct diet provided 75.7 and 78.5 g/d of bioavailable P yielding P balances of 16.3 and 19.1 ± 0.37 g per day respectively using the two sets of bioavailabilities. Outcomes: A model of digestion and

absorption that considers the differential digestibility of 3 phosphorus forms was used to assign bioavailability values to a range of ingredients. This change in knowledge can be used with greater confidence in balancing low P rations minimizing phosphorus excretion leading to improved surface water quality.

15. Alex Hristov (Pennsylvania State University, Obj. 1) Kevin Harvatine (Pennsylvania State University, Obj. 2

Effects of rumen-protected methionine, lysine and histidine on lactation performance of dairy cows. The objective of this study was to evaluate the effects of rumen-protected (RP) Met, Lys and His supplementation to a metabolizable protein (MP)-deficient diet on performance of dairy cows. The experiment was a 9-wk randomized complete block design with 36 Holstein cows (DIM, 132 ± 30 d; BW, 611 ± 81 kg) and is currently being repeated with another group of 36 cows. After a 2-wk covariate period, cows were blocked by DIM, milk yield, and parity, and randomly assigned to 1 of the following 6 treatments: control [AMP; +245 g/d of MP over NRC (2001) requirements]; MP-deficient diet (DMP; -118 g/d of MP); DMP supplemented with RPMet (30 g/d of Mepron; Evonik Industries AG; DMPM); DMP supplemented with RPLys (130 g/d of AjiPro-L; Ajinomoto Co., Inc.; DMPL); DMP supplemented with RPHis (120 g/d of an experimental product; DMPH); and DMP supplemented with RPMet, RPLys and RPHis (DMPMLH). The AMP and DMP diets consisted of (DM basis): 42% corn and 21% alfalfa silages and 37% concentrates and contained 16.5 and 14.5% CP, respectively. DMI tended to be decreased ($P = 0.07$) by DMP compared with AMP (28.0 vs. 29.4 kg/d). Milk and energy-corrected milk yields were decreased ($P < 0.03$) by DMP (40.5 and 36.1 kg/d) vs. AMP (44.1 and 42.1 kg/d). Milk protein content was increased ($P \leq 0.03$) by DMPH and DMPL (3.17 and 3.20%) compared with DMP and AMP (3.01%), and tended ($P = 0.06$) or was numerically higher ($P \leq 0.15$) for DMPMLH and DMPM (3.15 and 3.12%) vs. DMP and AMP. Milk fat content was decreased by DMP vs. AMP (3.33 and 3.90%; $P = 0.04$) and was increased by DMPH and DMPMLH (3.93 and 4.01%; $P \leq 0.03$) compared with DMP. Yields of milk protein and milk fat were decreased ($P = 0.01$) by DMP vs. AMP (by 10 and 20%, respectively). Cows fed AMP had higher MUN (11.7 mg/dL; $P < 0.01$) compared with cows fed the DMP diets (on average 8.09 mg/dL). Overall, feeding an MP-deficient diet decreased DMI and yields of milk, protein, and fat. Addition of RPAA to the DMP diet generally increased milk protein content but did not affect protein yield. Supplementation of RPHis alone or in combinations with RPMet and RPLys also increased milk fat content.

Effect of 3-nitrooxypropanol on ruminal fermentation, methane and hydrogen emissions, and methane isotopic composition in dairy cows. The objective of this crossover experiment was to investigate the effect of a methane inhibitor, 3-nitrooxypropanol (3NOP), on rumen fermentation and enteric CH4 emission in lactating dairy cows. Six ruminally-cannulated late-lactation (235 DIM; SD = 20 d) Holstein cows were assigned to 2 treatments: control and 3NOP (60 mg/kg DMI). Each experimental period consisted of 10 d for adaptation and 4 d for sample collection. Compared with the control, 3NOP decreased ($P < 0.001$) CH4 emission by 31% (487 vs. 335 g/d, respectively) and increased ($P < 0.001$) that of H2 from 0.005 to 1.33 g/d. CH4 emissions per kg of DMI or milk yield were also decreased ($P < 0.001$) 34 and 37%, respectively, by 3NOP. The isotopic composition of CH4 was similar between treatments: control, $\delta 13CCH4 = -20.91 \pm 0.32‰$, $\delta DCH4 = -266.92 \pm 0.14‰$, and

Δ13CH3D = -1.96 ± 1.78‰; and 3NOP, δ13CCH4 = -24.91 ± 1.72‰, δDCH4 = -266.94 ± 0.27‰, and Δ13CH3D = -1.72 ± 2.97‰. Concentrations of total VFA and propionate in ruminal fluid were not affected by treatment. Acetate concentration tended to be lower (P = 0.08) and acetate:propionate ratio was lower (P < 0.001) for 3NOP compared with the control. Butyrate and iso-valerate concentrations tended to be or were increased (P ≤ 0.08) by 3NOP. Methanogenic archaea (Methanobrevibacter, Methanosphaera, and Methanomicrobium) were not affected (P ≥ 0.46) by 3NOP. Prevotella spp., the predominant bacterial genus in ruminal contents (22 to 23% of the total isolates), was also not affected (P = 0.54) by 3NOP. Compared with the control, Ruminococcus and Clostridium spp. were decreased (P ≤ 0.03) and Butyrivibrio spp. was increased by 3NOP: 8.2 vs. 6.5%, 6.2 vs. 4.1%, and 3.6 vs. 4.8%, respectively. This experiment demonstrated that a substantial inhibition of enteric CH4 emission in dairy cows resulted in increased H2 emission and decreased acetate concentration, but had no effect on rumen archaea. The isotopic composition of CH4 was similar between the two treatments, supporting the conclusion that there was little to no change in the metabolic strategy of the rumen archaeal population

Comparison between the GreenFeed system and the sulfur hexafluoride tracer technique for measuring enteric methane emissions from dairy cows. The objective of this study was to compare 2 commonly used techniques for measuring CH4 emissions from ruminant animals, the GreenFeed (GF) system and the sulfur hexafluoride (SF6) technique. The study was part of a larger experiment, in which a CH4 inhibitor, 3-nitrooxypropanol (3NOP), fed at 4 application rates (0, 40, 60, and 80 mg/kg feed DM) decreased enteric CH4 emission by 25 to 32% in a 12-wk experiment with 48 lactating Holstein cows. The larger experiment used a randomized block design and was conducted in 2 phases (Feb-May, phase 1 and Jun-Aug, phase 2), with 24 cows in each phase. Methane emissions using GF were measured during experimental wks 2, 6, 9, and 12. During each GF measurement, 8 spot samples of gas emissions were collected from each cow, staggered over a 3-d period (a total of 0.67 h/cow). Emission data using the SF6 technique were collected for 3, 24 h periods (a total of 77 h/cow) during wks 2, 6 or 9, and 12. An outlier analysis removed 1 observation from the GF dataset (1,271 observations) and 6 observations from the SF6 dataset (451 observations). Methane yield data (g/kg DMI) were averaged per cow for the statistical analysis. The mean CH4 yield, SD, lower and upper 95% CL, CV, and min and max values for the GF dataset were (g CH4/kg DMI or as indicated): 12.8, 3.63, 12.8 and 13.9, 27.2% (18.1 and 21.2%; control and 3NOP cows, respectively), and 6.7 and 26.4. For the SF6 dataset these values were: 14.7, 5.60, 14.7 and 17.0, 35.3% (30.4 and 29.9%, control and 3NOP cows), and 7.2 and 36.5. Data were analyzed within experimental phase, sampling wk, and treatment to compare CH4 yield between GF and SF6. The difference between the 2 methods (SF6 – GF) within treatment was 1.9 to 4.1 g CH4/kg DMI (P < 0.001 to 0.06) for phase 1 and 1.1 to 2.4 g/kg DMI (P = 0.06 to 0.38) for phase 2. In the conditions of this experiment, the SF6 technique produced larger variability in CH4 yield than the GF method. The difference between the 2 methods was not consistent over time, perhaps influenced by barn ventilation and background CH4 and SF6 concentrations.

A novel method to determine rumen biohydrogenation kinetics of alpha-linolenic acid (18:3 n-3). Biohydrogenation (BH) of unsaturated fatty acids (FA) has been extensively studied in vitro. but BH rates and intermediates formed in vitro may not parallel BH pathways in vivo. The objective was to develop an in vivo method to determine the rate of alpha-linolenic acid (18:3 n-3) BH and identify intermediates formed. Eleven rumen cannulated high-producing

Holstein cows [40 ± 6 kg milk/d (Mean ± SD)] were fed at a rate of 6%/h of expected total DMI a diet balanced to 29% NDF and 5.9% EE (1.5% soybean oil). A single bolus consisting of 200 g of flaxseed oil (53% 18:3) and 15 g of tridecanoic acid (13:0) was mixed with rumen contents and rumen digesta was collected at -1, 0.1, 0.5, 1, 2, 3, 4, 6 and 8 h relative to the bolus. Samples were immediately placed in dry ice, stored at -20oC, freeze-dried, methylated and analyzed by gas chromatography. Data were first analyzed using PROC Mixed with repeated measures for time point comparison. Secondly, the disappearance of 13:0 and 18:3 was fit to a single exponential decay model using the nonlinear procedure of JMP Pro. The bolus increased total fat in the rumen from 4.3 to 6.0% and enriched 13:0 from 0.04 to 2.2% of FA and 18:3 from 2.0 to 11.3% of FA. The fractional rate of disappearance of 13:0 was 0.4%/min (r2 = 0.98) and of 18:3 was 2.5%/min (r2 = 0.99), with 18:3 reaching pre-bolus concentration within 4 h. Assuming that 13:0 disappeared only by passage, 18:3 disappeared by passage and biohydrogenation, and the rate of passage of 13:0 and 18:3 are the same, the extent of bolused 18:3 BH was 85%. The concentration of cis-9, trans-11, cis-15 18:3 peaked at 1.2% of FA at 1 h (8-fold increase), trans-11, cis-15 18:2 peaked at 3.9% of FA at 2 h (13-fold increase), and trans-11 18:1 peaked at 6.6% FA at 3 h (43% increase). In conclusion, the in vivo method resulted in the expected extent of biohydrogenation and biohydrogenation intermediates, but the rate of ruminal biohydrogenation of 18:3 was much higher than that commonly observed in vitro. The method developed provides an in vivo assay of ruminal biohydrogenation for use in future experiments

16. Agustin Rius (The University of Tennessee, Obj. 1)

Heat stress (HS) alters metabolism of amino acids and reduces synthesis of caseins in bovine mammary glands. The mammalian target of rapamycin (mTOR) cascade regulates the initiation of the translation of protein synthesis and is mediated by protein factors that are activated or inhibited upon phosphorylation. It has been reported that essential amino acids increased protein synthesis by activating the mTOR cascade. Our objective was to determine the effect of HS in phosphorylating mTOR protein factors in immortalized bovine mammary cells line (MAC-T). It was hypothesized that the phosphorylation activity of mTOR signaling factors would be altered in MAC-T cells exposed to HS. Cells were cultured in 15 mL of Dulbecco's Modified Eagle Medium with 10% fetal bovine serum at 37°C and 5% CO2. Cells were subjected to one of two treatments: 1) 37°C (control) and 2) 41.5°C (HS) for 12 hours. The treatments were repeated 5 times in 5 different days. Cell proteins were harvested and separated by gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Western blotting was conducted to identify total and site-specific phosphorylated forms of protein kinase B (Akt; Thr308/Ser473), P70 S6 kinase (S6K1; Thr389), ribosomal protein S6 (rpS6; Ser235/236), and eukaryotic elongation factor 2 (eEF2; Thr56). Relative densities for phosphorylated and total forms of Akt, S6K1, rpS6 and eEF2 were quantified and expressed as phosphorylated to total ratio. Analysis of variance was conducted using a mixed model. Compared with control, cells exposed to HS decreased phosphorylation to total ratio of Akt (0.41 vs. 0.29; P<0.001), S6K1 (1.65 vs. 0.97; P=0.042), and rpS6 (1.45 vs. 1.07; P<0.001). However, preliminary results indicated that HS did not affect the ratio of eEF2. These results indicate that HS impaired the translation of protein by altering the phosphorylation activity of mTOR signaling factors in MAC-T cells.

Insulin increases protein synthesis by activating the signaling pathway that regulates protein translation in mammary tissue. Lactating cows exposed to heat stress (HS) have increased basal levels of insulin but exhibit reduction in milk protein synthesis. The activity of mammalian target of rapamycin (mTOR) signaling cascade is mediated upon phosphorylation and dephosphorylation of protein kinase B (Akt), P70 S6 kinase (S6K1), ribosomal protein S6 (rpS6), and eukaryotic elongation factor 2 (eEF2). The objective of this study was to determine the effects of insulin and HS on phosphorylating activity in Akt, S6K1, rpS6, and eEF2 factors in immortalized bovine mammary cell line (MAC-T). Cells were cultured in 15 mL of Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1 µg/mL of insulin at 37°C and 5% CO2 before the treatments were imposed. The experimental design consisted of a 2 x 2 factorial arrangement of treatments with two temperature environments, 37°C thermoneutral or 41°C HS, and two insulin concentrations, 0 µg/mL and 1 µg/mL, for 12 hours. Cell lysates were separated by gel electrophoresis and transferred onto a polyvinylidene fluoride membrane. Western blotting was conducted to identify total and site-specific phosphorylated forms of Akt (Thr308/Ser473), S6K1 (Thr389), rpS6 (Ser235/236) and eEF2 (Thr56). The relative densities for phosphorylated and total forms of Akt, S6K1, rpS6 and eEF2 were quantified and expressed as phosphorylated to total ratio. Preliminary results indicate a significant HS by insulin interaction for rpS6 ($P<0.05$). There was an increase in phosphorylated to total ratio in response to insulin when cells were exposed to HS. However, there was a reduction of this ratio in response to insulin when cells were exposed to thermoneutral conditions. The remaining protein factors were not affected by treatments. These results would indicate that the response of mTOR signaling cascade to insulin was altered in MAC-T cells exposed to HS.

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D. Impacts

Members have been successful in the past year in receiving private and federal funds 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.