# Ruminant Nutrition: Amino acids and metabolism

**454 Energy requirements for pregnant and nonpregnant Nellore cows.** Mateus P. Gionbelli*[1], Marcio S. Duarte[2], Sebastiao C. Valadares Filho[2], Edenio Detmann[2], Tathyane R. S. Gionbelli[1], Diego Zanetti[2], and Luiz H. P. Silva[2], *[1]University of Lavras, Lavras, Minas Gerais, Brazil, [2]University of Viçosa, Viçosa, Minas Gerais, Brazil.*

Forty-nine adult Nellore cows (32 pregnant and 17 nonpregnant) with average initial body weight of 451 ± 10 kg were used in a comparative slaughter study aiming to describe equations and relationships for prediction of net, metabolizable and dietary energy requirements for adult, pregnant and nonpregnant, *Bos indicus* cows. Feeding control was measured individually and cattle were fed either HIGH (ad libitum) or LOW (restricted feeding 1.2 times maintenance according to the NRC). The 32 pregnant cows were separated at random into 4 groups of 8 cows each (4 cows per each feeding level) and harvested at 136 ± 1, 189 ± 1, 239 ± 1 and 269 ± 1 d of pregnancy. The nonpregnant cows were harvested at different times of the experiment (85 to 216 d of feeding control) to keep them in experiment for a similar amount of time as the pregnant cows. The digestible energy and losses of energy as methane and urine were directly measured to establish the relations between GE, DE and ME. Energy content was analyzed in empty body and pregnant compounds. A set of relationships and equations based in the factorial method from ARC was used to estimate the nutrient requirements. The net energy requirement for pregnancy ($NE_p$) estimated in this study was about 3/4 of those estimated by NRC. When estimated by a logistic model, the daily requirements for pregnancy showed an exponential increase up to 250 d of gestation and then decreased. However, when an allometric model was used to estimate the daily requirements for pregnancy, the maximum daily requirements were at birth. There were no differences in the dynamics of energy ($P = 0.388$) in the cow's empty body weight pregnant free ($EBW_{np}$) suggesting that the pregnancy does not affect the requirements for accretion of body reserves in cows. The partial efficiencies for use of metabolizable energy for maintenance, weight gain and pregnancy ($k_m$, $k_g$ and $k_c$) were respectively 70, 53 and 12%. The efficiency of transformation of DE in ME was 0.80.

**Key Words:** *Bos indicus*, conceptus, metabolizable energy

**455 Rumen-protected methyl donors during the transition period: 1. Better postpartal performance in dairy cows supplemented with rumen-protected methionine (Smartamine M) than choline (ReaShure).** Z. Zhou*[1], M. Vailati Riboni[1], E. Trevisi[2], J. K. Drackley[1], D. N. Luchini[3], and J. J. Loor[1], *[1]University of Illinois at Urbana-Champaign, Urbana, IL, [2]Università Cattolica del Sacro Cuore, Piacenza, Italy, [3]Adisseo NA, Alpharetta, GA.*

The objective of this study was to evaluate the efficacy of supplemental rumen-protected methionine (MET) and choline (CHO) on performance and health of transition cows. Eighty-one multiparous Holstein cows were used in a randomized complete block design with 2 × 2 factorial arrangement of MET (Smartamine M, Adisseo NA) and CHO (ReaShure, Balchem Inc.) level (with or without). Treatments (20–21 cows each) were control (CON), no MET or CHO; CON+MET (SMA); CON+CHO (REA); and CON+MET+CHO (MIX). From −50 d to −21 d before expected calving, all cows received the same diet (1.24 Mcal/kg DM) with no MET or CHO. From −21 d to calving, cows received the same close up diet (1.54 Mcal/kg DM) and were assigned randomly to treatments (CON, SMA, REA, or MIX). From calving to 30 DIM, cows were on the same postpartal diet (1.69 Mcal/kg DM) and continued to receive the same treatments through 30 DIM. MET supplementation was adjusted daily at 0.08% DM of diet and REA was supplemented at 60 g/cow/d. No differences ($P = 0.34$ or greater) were detected for pre- or postpartal body weight and body condition score. However, MET supplementation (SMA, MIX) led to greater ($P < 0.05$) DMI compared with other treatments (CON, REA) in both close-up (14.40 vs. 13.13 kg/d, SEM 0.40) and first 30 d postpartum (19.30 vs. 17.15 kg/d, SEM 0.63). Milk yield in MET-supplemented cows (SMA, MIX) also was greater ($P < 0.05$) compared with other (CON, REA) treatments (44.32 vs. 40.32 kg/d, SEM 1.29). Milk fat % did not differ in response to MET ($P = 0.91$; 3.72% vs. 3.74%, SEM 0.11%) or CHO ($P = 0.46$; 3.78% vs. 3.68%, SEM 0.10%). However, milk protein % was greater in MET-supplemented ($P < 0.01$; 3.32% vs. 3.14%, SEM 0.05%) but not CHO-supplemented ($P = 0.23$; 3.27% vs. 3.19%, SEM 0.05%) cows. CHO led to greater ($P = 0.02$) blood glucose. No MET or CHO effects were detected for blood NEFA ($P = 0.56$ or greater) or BHBA ($P = 0.11$ or greater), but a MET × time effect ($P = 0.10$) was observed for NEFA due to lower concentrations on d 4. Results from the present study indicate peripartal supplementation of rumen-protected methionine has positive effects on cow performance.

**Key Words:** methionine, choline, transition cow

**456 Rumen-protected methyl donors during the transition period. 2. Biomarkers of inflammation and oxidative stress reveal better liver and immune function in cows supplemented with rumen-protected methionine (Smartamine M) than choline (ReaShure).** Z. Zhou*[1], M. Vailati Riboni[1], E. Trevisi[2], F. C. Cardoso[1], D. N. Luchini[3], and J. J. Loor[1], *[1]University of Illinois at Urbana-Champaign, Urbana, IL, [2]Università Cattolica del Sacro Cuore, Piacenza, Italy, [3]Adisseo NA, Alpharetta, GA.*

The objective of this study was to evaluate the efficacy of supplemental rumen-protected methionine (MET) and choline (CHO) on alleviating inflammation and oxidative stress through their use for synthesis of antioxidants or other methyl-group requiring compounds. Forty-eight multiparous Holstein cows were used in a randomized complete block design with 2 × 2 factorial arrangement of MET (Smartamine M, Adisseo NA) and CHO (ReaShure, Balchem Inc.) level (with or without). Treatments (12 cows each) were control (CON), no MET or CHO; CON+MET (SMA); CON+CHO (REA); and CON+MET+CHO (MIX). From −50 d to −21 d before expected calving, all cows received the same diet (1.24 Mcal/kg DM) with no MET or CHO. From −21 d to calving, cows received the same close-up diet (1.54 Mcal/kg DM) and were assigned randomly to each treatment. From calving to 30 d, cows were on the same postpartal diet (1.69 Mcal/kg DM) and continued to receive the same treatments until 30 d. MET supplementation was adjusted daily at 0.08% DM of diet and CHO was supplemented at 60 g/cow/d. Blood was collected at −10, 4, 8, 20, and 30 d for profiling of 16 biomarkers. Neutrophil and monocyte phagocytosis and oxidative burst were assessed at d 1, 3, 7, 14, and 28 d. MET (SMA, MIX) led to greater ($P = 0.04$; 36.55 vs. 35.53 g/L, SEM 0.35) albumin, lower ($P = 0.08$; 0.35 vs. 0.47 g/L, SEM 0.05) haptoglobin, and greater ($P = 0.07$, 93.09 vs. 84.54 U/ml, SEM 3.20) paraoxonase (antioxidant) compared with other treatments. This suggests a more pronounced inflammatory state in cows without supplemental MET. MET-supplemented cows had greater ($P < 0.01$; 60.43 vs. 54.88%, SEM 1.65) neutrophil phagocytosis capacity. Both SMA and REA had greater ($P < 0.01$) monocyte (SMA, 26.93 vs. 16.96%, SEM 2.71; REA, 25.96 vs. 16.96%, SEM 2.52) and

neutrophil (SMA, 59.19 vs. 43.32%, SEM 4.58; REA, 53.89 vs. 43.32%, SEM 4.25) oxidative burst activity compared with CON. Data suggest that supplementing rumen-protected methionine peripartal has positive effects on immune and liver function at least in part by reducing oxidative stress and inflammation.

**Key Words:** methionine, choline, inflammation

**457 Rumen-protected methyl donors during the transition period: 3. Hepatic one-carbon metabolism flux in response to supplemental Smartamine M or ReaShure.** Z. Zhou*[1], T. A. Garrow[1], M. Vailati Riboni[1], F. C. Cardoso[1], D. N. Luchini[2], and J. J. Loor[1], *[1]University of Illinois at Urbana-Champaign, Urbana, IL, [2]Adisseo NA, Alpharetta, GA.*

Nutrients such as methionine (MET), choline (CHO), folate, and betaine are vital for provision of methyl groups required by various cellular pathways in tissues such as liver. In the context of dietary methyl-donors, the peripartal dairy cows are characterized by negative MET and CHO balance, both of which can play a role in allowing cows to achieve optimal performance while maintaining good health. Although studies of 1-carbon metabolism and MET cycle in ruminant liver already were performed in sheep, similar data are not available for high-producing Holstein dairy cows. Objectives were to measure activity of enzymes governing S-adenosyl MET formation (methionine adenosyltransferase 1), the transsulfuration pathway (Cystathionine β synthase), and endogenous synthesis of MET via CHO (betaine homocysteine S-methyltransferase, BHMT). Forty multiparous Holstein cows were used in a randomized complete block design with 2 × 2 factorial arrangement of MET and CHO level (with or without). Treatments (10 cows each) were control (CON), no MET or CHO; (SMA), CON+Smartamine M, Adisseo NA; (REA) CON+ReaShure, Balchem Inc.; and (MIX) CON+SMA+REA. From −50 d to −21 d before expected calving, all cows received the same diet (1.24 Mcal/kg DM) with no MET or CHO. From −21 d to calving, cows received the same close-up diet (1.54 Mcal/kg DM) and were assigned randomly to each treatment. From calving to 30 d, cows were on the same postpartal diet (1.69 Mcal/kg DM) and continued to receive the same treatments through 30 d. MET supplementation was adjusted daily at a rate of 0.08% (DM basis) of diet and CHO was supplemented at 60 g/cow/d. Liver samples were harvested at −10, 10, 20 and 30 d relative to calving. Compared with −10 d, liver BHMT activity increased ($P < 0.01$) by 2-fold postpartum regardless of treatment and remained high through at least 30 d. CHO supplementation had no affect ($P > 0.05$). Although no main effect of MET was detected ($P > 0.05$), a significant MET × Time interaction ($P < 0.01$) occurred due to higher BHMT activity in MET vs. CON cows on d 20. Data underscore the high demand for endogenous MET synthesis and increased flux through the MET cycle during early lactation.

**Key Words:** methionine, choline, betaine homecysteine S-methyltransferase (BHMT)

**458 Supplemental Smartamine M in high-energy diets during the peripartal period improves production and hepatic biomarkers of oxidative status in Holstein cows.** Mario Vailati Riboni*[1], Johan S. Osorio[2], Erminio Trevisi[3], James K. Drackley[1], Daniel Luchini[4], and Juan J. Loor[1], *[1]University of Illinois at Urbana-Champaign, Urbana, IL, [2]Oregon State University, Corvallis, OR, [3]Università Cattolica del Sacro Cuore, Piacenza, Italy, [4]Adisseo S.A.S., Alpharetta, GA.*

Feeding higher-energy prepartum is a common practice in the dairy industry. However, recent data underscore how it could reduce performance, deepen negative energy balance, and augment inflammation and oxidative stress in fresh cows. We tested the effectiveness of rumen-protected methionine in preventing the negative effect of feeding high-energy prepartum. Twenty-one multiparous Holstein cows were fed a controlled-energy diet (CON, n = 7; 1.24 Mcal/kg DM; high-straw) during the whole dry period (~50 d), or switched to a higher-energy (OVE, n = 7; 1.54 Mcal/kg DM), or OVE plus Smartamine M® (OVE+SM, n = 7; Adisseo NA) during the last 21 d before calving. Afterward cows received the same lactation diet (1.75 Mcal/kg DM). Smartamine M was top-dressed on the OVE diet (0.07% of DM) from −21 through 30 d in milk (DIM). Liver samples were obtained via percutaneous biopsy at −10, 7 and 21 DIM. Expression of genes associated with energy and lipid metabolism, hepatokines, methionine cycle, antioxidant capacity and inflammation was measured. Although prepartal dry matter intake (DMI) was not affected ($P = 0.21$) by diet, it was lower ($P < 0.005$) in OVE than CON and OVE+SM. Milk yield and ECM were lower ($P < 0.10$) in OVE than CON and OVE+SM. Milk protein and fat percentages were lower ($P < 0.05$) in CON and OVE compared with OVE+SM. Feeding OVE compared with CON led to lower ($P < 0.05$) *PCK1* and *PPARA*. At 7 DIM, *GSR* was greater ($P < 0.05$) in OVE than CON-fed cows, suggesting a greater antioxidant demand. Feeding OVE+SM resulted in similar expression of *GSR* compared with CON. Expression of *SAHH*, *STAT5B* and *MTR* was greater ($P < 0.05$) prepartum in OVE+SM compared with both CON and OVE, and at 7 DIM ($P < 0.05$) for CON and OVE+SM compared with OVE. *FGF21* was lower ($P < 0.05$) prepartum in OVE than CON and OVE+SM, and increased ($P < 0.05$) postpartum only in OVE. Expression of *DMT3A* was greater ($P < 0.05$) in OVE and OVE+SM, while *MTTP* was lower in OVE+SM than CON or OVE. Data suggest, supplemental Smartamine M was able to compensate the negative effect of prepartal energy-overfeeding by increasing production and alleviating the demand for intracellular antioxidants.

**Key Words:** methionine, transition period, nutrigenomics

**459 Effect of strategic ration balancing with use of Prolak, MetaboLys, and Smartamine M on the efficiency of milk protein production and environmental impact in primiparous cows.** Yanting Chen*[1], Joe Harrison[1], Pius Ndegwa[1], Deb Wilks[2], Lynn VanWieringen[1], and John Azzone[3], *[1]Washington State University, Puyallup, WA, [2]EPL Feeds, Lynden, WA, [3]H J Baker, Fayetteville, PA.*

The objective of this study was to evaluate the effect of reduced CP on milk production and environmental impact in a commercial dairy herd. Primiparous cows were completely randomized to 2 groups with 84 cows each, and each group had similar DIM (181 ± 7.14 vs. 195 ± 7.85) before initiation of the study. The control diet was the current general herd ration. The reformulated diet was supplemented with Prolak, MetaboLys and Smartamine M, reduced the dietary CP (17.7 ± 0.65 vs. 16.7 ± 0.7% DM), and increased methionine (1.8 vs. 2.2% MP) and lysine (6.6 vs. 6.9% MP) concentrations. Diets were fed in a 6-wk switch back design trial with 2 periods. DMI was recorded daily (1 pen per treatment), and milk yield and composition of individual cows were measured weekly. Urine and feces samples were mixed together and used in a closed chamber incubation to determine $NH_3$ emission. Feeding the reformulated diet numerically decreased DMI 0.4 kg (19.3 ± 0.49 vs. 18.9 ± 0.58 kg), however milk yield ($P = 0.91$), protein yield ($P = 0.74$), % protein ($P = 0.49$), fat yield ($P = 0.19$), % fat ($P = 0.09$), and lactose yield ($P = 0.28$) did not differ. Lactose % and MUN ($P < 0.001$) decreased when cows were fed the reformulated diet. Cows

fed the reformulated diet had higher Met ($P = 0.09$) and less Tyr ($P = 0.06$) in blood plasma than control cows. Feeding the reformulated diet numerically increased feed efficiency (1.62 vs. 1.66 ± 0.01) and milk true protein efficiency (28.4 vs. 30.8 ± 0.01%). Cows fed the reformulated diet consumed 7.2% less N, produced 0.6% more milk total N, excreted 9.9% less predicted urinary N and 18.0% less calculated fecal N than control cows. The difference in income over feed cost for the reformulated diet was $0.15 and $0.18 based on the milk price of Washington State in 2013 and 2014, respectively. The emitted $NH_3$ flux rates of manure were numerically similar (122.6 ± 10.26 vs. 124.1 ± 11.99 $mg \cdot h^{-1} \cdot m^{-2}$) between diets. These results illustrated that feeding low CP with balanced AA diet could improve the efficiency of nitrogen utilization and reduce the environmental impact without compromising the profitability of milk production in primiparous cows.

**Key Words:** bypass lysine, methionine, milk production

**460 Sodium salicylate alters ruminal digestion in vitro and in situ.** Abigail J. Carpenter*, Claudio F. Vargas-Rodriguez, Jacob A. B. Jantz, and Barry J. Bradford, *Kansas State University, Manhattan, KS.*

Although sodium salicylate (SS) administration after calving increases 305-d milk production, it is associated with hypoglycemia in some circumstances. We hypothesized that this may be in part due to decreased glucogenic substrate supply from fermentation. Six heifers were drenched once daily for 3 d with either 62.5 g of SS in water (SS) or an equal volume of water (CON). A series of batch cultures were performed the day before the start of treatment and 1, 13, and 35 d following. Strained fluid from each heifer was combined in a 2:1 ratio with McDougall's buffer, and 150 mL of the inoculum was added to each flask (n = 4/heifer) with 2.5 g substrate. Gas production was measured with the ANKOM$^{RF}$ Gas Production System. Following each rumen fluid collection, Dacron bags containing approximately 1 g of substrate DM were inserted into the rumen of each heifer in duplicate at 2, 8, 16, 24, and 48 h time points to estimate rate of DMD. The 48-h time point was used to estimate rumen-undigested substrate. Measurements from the pre-treatment batch culture and in situ experiments were used as covariates for statistical analysis but were removed from the model if they were not significant ($P > 0.05$). Overall, there was no effect of treatment on batch culture final pH across time ($P = 0.70$), although dry matter disappearance (DMD) was decreased across time due to treatment ($P < 0.01$; treatment × day: $P = 0.01$). One day following treatment, SS had no effect on DMD ($P = 0.70$); however, DMD was decreased ($P < 0.01$) in batch culture 13 d after SS treatment (45.0 vs. 38.9 ± 0.9% of DM for CON vs. SS, respectively), and it remained lesser for the SS treatment 35 d after the end of treatment ($P < 0.01$, 44.0% vs. 40.3 ± 0.9% of DM for CON vs. SS, respectively). No differences were observed due to treatment for volume, rate, or lag in gas production ($P \geq 0.60$). For the in situ experiment, no differences were detected in any 48-h time points ($P \geq 0.17$). Treatment with SS tended to decrease DMD rate ($P = 0.10$; treatment × day: $P = 0.50$). Given the sustained effect of SS drenches on in vitro DMD, it is likely that SS modifies the rumen microbiota to impair ruminal digestion.

**Key Words:** salicylate, fermentation, rumen modification

**461 Effect of rumen acidosis and short-term feed restriction on short-chain fatty acid concentrations and permeability of the bovine gastrointestinal tract.** Rae-Leigh A. Pederzolli*[1], Steve Hendrick[2], John Campbell[1], Katie M. Wood[1], and Gregory B. Penner[1], *[1]University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [2]Coaldale Veterinary Clinic, Lethbridge, Alberta, Canada.*

The objective of this study was to identify whether ruminal acidosis (RA) or feed restriction (FR) differentially affect permeability of the gastrointestinal tract (GIT). Twenty-one Holstein steers were randomly assigned to 1 of 3 treatments: control (CON); ruminal acidosis (ACID), and feed restriction (REST). Steers were fed a common diet with a 50:50 F:C ratio once daily at 0800 h for a 5-d baseline period followed by a challenge period. Rumen acidosis was induced by restricting feed to 25% DMI for 1 d and then offering pelleted barley (30% DMI:BW) the next day. Steers on the REST treatment were restricted to 25% DMI for 5 d. Steers were killed and tissues were collected from the rumen, omasum, duodenum, jejunum, ileum, cecum, and proximal and distal colon for measurement of $^{14}$C-mannitol and $^{3}$H-inulin flux in Ussing chambers as markers for gut permeability. Data were analyzed as a randomized complete block design using Proc Mixed. Rumen pH was recorded throughout the study. During baseline, there were no differences for DMI or rumen pH (7.1 kg/d and 6.53, respectively; $P > 0.1$), but DMI and pH during the challenge were 1.7 kg/d and 6.94, and 7.9 kg/d and 5.86 for REST and ACID calves, respectively. The proportion of acetate in the proximal colon from CON (56.8%) was greater (treatment × region, $P = 0.01$) than for FR (31.0%) and ACID (31.0%). Butyrate concentration was less in the proximal colon of CON (16.0%; interaction $P < 0.01$) than ACID (31.0%). There were no region × treatment interactions or treatment effects ($P > 0.10$) for inulin or mannitol flux. However, inulin flux (nmol/($cm^2$ × h)) was greater ($P < 0.01$) in rumen (21.1), omasum (26.9), and duodenum (21.3) than ileum (4.2), cecum (7.5), and distal colon (2.4). Mannitol flux (nmol/($cm^2$ × h)) was greatest ($P < 0.01$) in rumen (36.9), duodenum (59.5), jejunum (48.2), and proximal (41.4) and distal colon (33.0) relative to ileum (13.0). These data indicate that feed restriction and ruminal acidosis do not appear to differentially affect permeability of the GIT. The duodenum and rumen are likely regions with greatest permeability.

**Key Words:** acidosis, feed restriction, permeability

**462 Effect of gluconeogenic precursors on blood metabolites and milk yield in Chilean transition Holstein cattle.** Pedro Melendez*[1], Katherine Severino[2], Maria P. Marin[2], Patrick Pithua[1], and Pablo Pinedo[4,5], *[1]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, MO, [2]College of Veterinary Medicine, University Santo Tomas, Viña del Mar, Chile, [3]3Department of Animal Sciences, College of Veterinary Medicine, University of Chile, Santiago, Chile, [4]Texas A&M AgriLife Research, Amarillo, TX, [5]5Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University System, College Station, TX.*

The objective of this study was to determine the effect of feeding a gluconeogenic precursor containing calcium propionate, propylene glycol, and glycerol on serum concentrations of β-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), and milk yield in transition Holstein cows under Chilean conditions. The study was conducted in a commercial dairy farm located in central Chile. Cows were housed in a freestall system with headlocks, milked 3 times per day and fed a total mixed ration containing alfalfa hay, corn silage, and concentrate to meet or exceed the requirements proposed by the National Research Council (2001). At 21 d before expected parturition 40 multiparous cows were assigned at random to either a control or a treated group to achieve at least 15 cows per group at 30 DIM. The cows in the control group received the default periparturient TMR diet. Cows in the treatment group received 300 g of a supplement containing 70 g of calcium

propionate, 95 g of propylene glycol and 330 g of glycerol top dressed on the TMR during morning feedings when cows were head-locked. This feeding protocol was continued into the postpartum period until 30 d in milk. Seventeen and 18 cows completed the protocol in the control and the treatment group, respectively. Blood samples were collected at calving for NEFA quantitation and later at 7, 14 and 21 d postpartum for BHBA determination. The average daily milk yield up to 60-d of lactation was significantly higher (2 kg per day) in the treated than the control group ($P < 0.05$). NEFA (mEq/L) at calving were higher in the control (0.75 ± 0.1) than the treated group (0.55 ± 0.1; $P < 0.05$). The concentration of serum BHBA at 14 d postpartum was lower in control (0.60 ± 0.11 mmol/L) than treated cows (0.98 ± 0.11 mmol/L; $P < 0.05$). In conclusion, supplementing the default diet with gluconeogenic precursors during the pre- and postpartum period improved milk yield during the first 60 d of lactation and maintained a moderated metabolic energy status in Chilean dairy cattle.

**Key Words:** transition cow, metabolite, dairy cattle

**463 Expression of G-protein coupled fatty acid receptors during the transition period.** Alea Agrawal*, Johan S. Osorio, and Juan J. Loor, *University of Illinois at Urbana-Champaign, Urbana, IL.*

G-protein coupled receptors (GPR) have been widely studied within human medicine as drug targets for metabolic disorders. They play central roles in many cell signaling processes, and also have application within dairy nutrition as targets for specific dietary components; for example, omega-3 fatty acids. To combat metabolic disorders prevalent in dairy cows during the transition period, which often co-occur with negative energy balance and lipid mobilization, it may be helpful to identify locations, activities, and roles of free fatty acid receptors (FFAR) and other members of the GPR family. To provide insight on tissue-specific differences in gene expression, and therefore, potential downstream pathways of fatty acid-sensing GPR in bovine, quantitative RT-PCR (qPCR) of subcutaneous adipose, liver, and polymorphonuclear leukocyte (PMN) samples during the transition period (−15, +10, and +30 d) were used for expression profiling of medium- (MCFA) and long-chain fatty acid (LCFA) receptors *GPR120* and *GPR40*, MCFA receptor *GPR84*, and niacin receptor *GPR109A*. Results were analyzed in SAS to examine differences in expression over time ($P < 0.05$). In adipose tissue, *GPR120* expression was highest at −15 d, decreased at +10 d, and further decreased at +30 d. *GPR40* expression was highest at +10 d relative to other time points, and *GPR109A* expression was lower prepartum than both postpartum time points. *GPR84* was undetectable. In liver tissue, *GPR84* and *GPR109A* were nearly undetectable and did not differ ($P > 0.05$) across time. *GPR40* had lower expression at +30 d than −15 or +10 d. *GPR120* was undetectable. In PMN, *GPR120* expression was increased between +10 and +30 d but neither were significantly different from −15 d. *GPR84* expression was higher at +10 d relative to other time points. Neither *GPR40* nor *GPR109A* were altered in PMN. The data suggest that there is likely not a direct role for the selected GPR in the liver during the transition period, but they do play variable roles in adipose and PMN. Dietary supplementation or exclusion of LCFA and/or niacin may provide a method of regulating GPR function during the prepartal or early postpartal periods.

**Key Words:** transition cow, fatty acid, G-protein coupled receptor (GPR)

**464 Effects of prepartal dietary intake and calving on blood neutrophil transcriptome in Holstein cows.** Alea Agrawal*, Muhammad J. Khan, Daniel E. Graugnard, Sandra L. Rodriguez-Zas, and Juan J. Loor, *University of Illinois at Urbana-Champaign, Urbana, IL.*

In the dairy industry, cow health and farmer profits depend on the balance between diet (i.e., nutrient composition, daily intake) and metabolism. This is especially true during the transition period, where dramatic physiological changes foster vulnerability to immunosuppression, negative energy balance, and clinical and subclinical disorders. Using an Agilent microarray platform, the present study examined changes in the transcriptome of bovine PMNL, a representative cell of the immune system, due to time relative to parturition, prepartal dietary intake, or the combination. Sixteen Holstein cows were fed a high-straw, control diet (S; NEL = 1.34 Mcal/kg) or overfed a moderate-energy diet (M; NEL = 1.62 Mcal/kg) during the dry period. Blood for PMNL isolation and metabolite analysis was collected at −14 and +7 d relative to parturition. At an ANOVA false discovery rate (FDR) < 0.05, time (7 vs. −14 d) significantly influenced expression of 1758 genes, energy intake (M vs. S) influenced 3062 genes, and the interaction had an effect on 1673 genes. Dynamic Impact Approach (DIA) bioinformatics analysis classified effects on KEGG pathways, including: activated carbohydrate metabolism due to time and interactions, and activated amino acid (AA) biosynthesis and ribosome activity with dietary treatment. In contrast, DIA analysis revealed inhibition of riboflavin and fatty acid (FA) metabolism, unsaturated FA synthesis, and calcium reabsorption due to energy intake. These analyses suggest that processes critical for energy metabolism and immune function (e.g., calcium reabsorption, FA and vitamin metabolism, and AA synthesis) were affected by energy overfeeding with mixed results, but overall, strong effects from either main effect were mitigated by the interactions. Ingenuity Pathway Analysis (IPA) of genes significantly affected at an FDR <0.10 also revealed 50 upstream regulators for each main effect and interaction comparison. The widespread, transcriptome-level changes captured here confirm the importance of dietary energy adjustments around calving on the immune system.

**Key Words:** transition cow, PMNL, intake

**465 A cow mammary epithelial cell-free system based on crude lysosomes and cytosol proteins: Leucine activating mTOR at Ser2448.** Wen-ting Dai[1,2], Nan Zheng[1,3], and Jia-qi Wang[*1,3], *[1]Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products, Beijing, China, [2]Jilin University, Changchun, China, [3]Ministry of Agriculture-Milk and Dairy Product Inspection Center, Beijing, China.*

Essential amino acids, especially leucine, initiated the signaling of the mammalian target of rapamycin complex-1 (mTORC1) and protein synthesis in cow mammary epithelial cells (CMEC). CMEC-H is an immortalized cow mammary epithelial cell line. We established a cell-free system, which was based on crude lysosomes and cytosol proteins from CMEC-H. By trypan blue dye exclusion assay, we found passing through gauge-26 needle was a good cell lysis method of CMEC-H. Crude lysosomes were extracted by lysosome isolation kit and cytosol proteins (supernatant-100) were obtained by ultracentrifugation at 1 × $10^5$ *g* about 1 h Supernatants 100, which contained all the cytosolic soluble proteins, were identified by SDS-PAGE. Raptor, mTOR and mLST8, the essential components of mTORC1, played a vital role in regulating protein synthesis; Lamp2 represented lysosome. All these proteins were testified by western blots. The constructed cell-free model stimulated by 1× essential amino acids was regarded as positive control,

the cell-free model stimulated by no amino acids was regarded as negative control, and the cell-free model stimulated by 1× leucine was seen as the treatment group. The cell-free models stimulated by 1× leucine readded 20 nmol rapamycin and stimulated by 1× essential amino acids readded 20 nmol rapamycin were both regarded as the corresponding inhibition groups. The results showed that the model can partially duplicate aspects of amino acids signaling mTOR program in vitro; the addition of 1× leucine solely and 1× essential amino acids led mTOR to be activated, phosphorylated and then moved towards the crude lysosome fractions in the cell-free system. However, combined with 1× leucine or 1× essential amino acids, 20 nmol rapamycin was not able to completely prevent mTOR from being phosphorylated. This study may provide some directions for further construction of cell-free system duplicating amino acid signaling mTOR. Furthermore, the method may be applied to cell-free models of other cell lines.

**Key Words:** cell-free system, crude lysosomes, leucine