Physiology and Endocrinology: Nutrition, reproduction and metabolism

311 Dietary omega-3 supplementation alters gene expression in equine endometrial and embryonic tissues. Robert D. Jacobs*[1], Alan D. Ealy[1], Parker M. Pennington[2,3], Budhan Pukazhenthi[2], Lori K. Warren[4], Ashley L. Wagner[5], Tanja M. Hess[6], and Rebecca K. Splan[1,7], *[1]Virginia Polytechnic Institute and State University, Blacksburg, VA, [2]Smithsonian Conservation Biology Institute, Front Royal, VA, [3]George Mason University, Fairfax, VA, [4]University of Florida, Gainesville, FL, [5]Cooperative Research Farms, Richmond, VA, [6]Colorado State University, Fort Collins, CO, [7]Virginia Tech MARE Center, Middleburg, VA.*

Adverse maternal events around the time of conception influence embryonic development. Thus, aberrations in the uterine environment during early pregnancy, such as maternal metabolic or nutritional disruption, can alter gene expression in the developing embryo, leading to alteration in its developmental trajectory. Dietary supplementation of long-chain omega-3 polyunsaturated fatty acids (LCPUFA) improves metabolic and reproductive health across species. The objective of this study was to evaluate effects of peri-conceptual LCPUFA supplementation on endometrial and embryonic gene expression in overweight horses. Light horse mares (n = 13; mean age = 13.56 ± 0.11 yr; mean BCS = 7.07 ± 0.21) were supplemented with concentrate (n = 6) or an isocaloric, isonitrogenous diet containing 0.06 mg/kg BW marine-derived omega-3 LCPUFA (n = 7) 60 d before first sample collection. Four consecutive ovulatory cycles were monitored and uterine endometrial samples were obtained 12 d post-ovulation 1, 3 and 4. Mares were bred to one stallion on ovulatory cycles 2, 3 and 4, and embryos were flushed 12 d post ovulation. Candidate genes essential to inflammation, prostaglandin synthesis and embryonic development were evaluated by quantitative PCR. Data were log-transformed and analyzed using the GLM procedure in SAS (v9.3). When examining the data independent of breeding and pregnancy status, samples from LCPUFA supplemented mares contained reduced ($P = 0.04$) *IL6* mRNA abundance and tended to have increased transcript abundance for Uterocalin ($P = 0.09$), *SAA* ($P = 0.06$) and *IL10* ($P = 0.06$). Mares fed LCPUFA pregnant in cycle 3 contained greater *IL10* ($P < 0.001$) and *PLA2G3* mRNA ($P = 0.009$) and had a tendency for increased *SAA* abundance ($P = 0.08$). Supplemented mares bred but not pregnant in cycle 3 had a tendency for reduced expression of *PTGER2* ($P = 0.100$). In the conceptus, relative transcript abundance of *GATA4* and *GATA6*, markers of endoderm differentiation, along with *GATA3* and *ELF3*, markers of trophectoderm differentiation were greater ($P < 0.05$) in embryos from LCPUFA supplemented mares (n = 5), than controls (n = 5). These results indicate that LCPUFA supplementation during the peri-conceptual period may alter the post-ovulatory uterine environment and early embryonic development in the horse.

Key Words: fatty acid, pregnancy, fetal programming

312 Omega-6 fatty acid-rich sunflower oil supplements in diet affect uterine health, ovarian function and oocyte characteristic in heat-stressed dairy cows. Chainarong Navanukraw*[1,2], Aree Kraisoon[1], Vilaivan Khanthusaeng[1], Suthipong Uriyapongson[1], and Chuchart Kamollirt[3], *[1]Department of Animal Science, Khon Kaen University, Khon Kaen, Thailand, [2]Agricultural Biotechnology Research Center for Sustainable Economy, Khon Kaen University, Khon Kaen, Thailand, [3]Department of Anatomy, Faculty of Veterinary Medicine, Khon Kaen, Thailand.*

The objective was to examine effects of plant oil supplement on uterine health, ovarian function, and oocyte characteristic in heat-stressed dairy cows. Prepartum cows (n = 36) were randomly allocated to one of 3 dietary treatments; control, 4% refined palm oil (PO), and 4% refined sunflower oil (SO). ANOVA using the general linear model procedure was performed to determine treatments effect. All variables were analyzed with a nested ANOVA with treatment, animal (treatment), and time included in the model. Differences between specific means were evaluated by least significance difference. Placental expulsion and uterine involution in cows fed oil supplements occurred earlier than control cows (3.4 h and 33.2 d for cows fed SO, 4.9 h and 42.0 d for cows fed PO, and 21.0 h and 50.4 d for control cows; $P < 0.05$). Numbers of class 1 (2–5 mm) and class 3 (≥10 mm) follicles of cows fed oil supplements were greater than those of control cows ($P < 0.05$). In the second experiment, lactating cows (n = 42) were hormonally synchronized and were randomly offered diets supplemented either with 4% PO or 4% SO for 42 d. Follicle and oocyte numbers and corpus luteum (CL) volume were determined on d 5, 9, and 13 of the estrous cycle and morphologic quality assessed. Neither follicle nor oocyte characteristics were affected by the dietary treatment, however volume of CL and progesterone concentrations on d 13 in cows fed SO were greater ($P < 0.05$) than those fed PO diet (8,290 vs. 7,875 mm^3 and 5.0 vs. 3.9 ng/mL). The data support the beneficial effects of SO supplement in diet on reproductive function in dairy cows

Key Words: plant oil, ovarian function, dairy cow

313 Effects of excess dietary MP from corn gluten meal or soybean meal on ovarian function of beef cows consuming low quality forage. Taylor C. Geppert*[1], George A. Perry[2], and Patrick J. Gunn[1], *[1]Department of Animal Science, Iowa State University, Ames, IA, [2]Department of Animal Sciences, South Dakota State University, Brookings, SD.*

The objective of this study was to determine the effects of feeding excess MP from feedstuffs differing in rumen degradability on ovarian function of beef cows. Non-pregnant, nonlactating beef cows (n = 18) were stratified by age, BCS, and BW to 1 of 2 isocaloric, isonitrogenous diets: (1) corn gluten meal (CGM) or (2) soybean meal (SBM), supplemented at 150% of MP requirements with ad libitum access to corn stalks for 58d. After a 20d supplement adaptation period, cows were synchronized for ovulation using the 5-d CO-Synch + CIDR protocol. Ten d after synchronization completion, 100 µg of GnRH was administered to reset follicular growth. Starting at GnRH administration and daily thereafter until spontaneous ovulation, transrectal ultrasonography was performed to diagram ovarian follicular growth, and blood samples were collected for hormone and metabolite analyses. Seven d after visual detection of estrus, corpus luteum (CL) size was determined and supplements were terminated. Data were analyzed using the MIXED procedures of SAS. Body weight and BCS, ovulatory follicular wavelength, antral follicle count, size of ovulatory follicle at dominance and duration of dominance were not different ($P = 0.13$) between treatments. Cows supplemented with CGM had greater ($P < 0.01$) post-dominance dominant follicle growth, larger ($P = 0.03$) dominant follicles at spontaneous luteolysis, shorter proestrus (36 vs. 68 h; $P < 0.01$) and larger (15.4 vs. 13.5 mm; $P = 0.03$) ovulatory follicles than SBM cows. No differences ($P = 0.11$) in estradiol, ratio of estradiol to ovulatory follicle volume, or plasma urea nitrogen concentrations were observed between treatments. Although CL volume and the ratio of progesterone to CL volume were not affected

by treatment ($P = 0.24$), CGM treated cows tended to have lesser (4.7 vs. 5.7 ng/mL; $P = 0.07$) concentrations of circulating progesterone 7d post-estrus than SBM. In summary, these data illustrate that excess MP when supplemented to cows consuming a low quality forage may differentially affect ovarian function depending on rumen degradability of the protein source.

Key Words: follicle, ovary, RUP

314 Effects of supplementing excess amounts of MP from a moderately abundant RUP source on ovarian function of beef cows consuming low quality forage. Taylor C. Geppert*[1], George A. Perry[2], and Patrick J. Gunn[1], *[1]Department of Animal Science, Iowa State University, Ames, IA, [2]Department of Animal Sciences, South Dakota State University, Brookings, SD.*

The objective of this experiment was to determine the effects of excess MP supplementation from a moderately abundant RUP source on ovarian function in beef cows consuming low quality forage. Non-pregnant, nonlactating mature beef cows (n = 16) were allocated by age, BW and BCS to 1 of 2 isocaloric supplements designed to maintain BW for 60 d. All cows were offered ad-libitum access to corn stalks and individually offered a corn gluten meal-based supplement at (1) 125% (MP125) or (2) 150% (MP150) of NRC MP requirements. After a 20-d supplement adaptation period, cows were synchronized for ovulation using the 5-d CO-Synch + CIDR protocol. Ten days after synchronization, 100 μL of GnRH was administered to reset follicular growth. Starting at GnRH administration and daily thereafter, transrectal ultrasonography was performed to diagram follicular waves, and blood samples were collected for hormone and metabolite analyses. Seven days after observation of estrus, corpus luteum (CL) size was determined via ultrasound and supplementation was terminated. Data were analyzed using the MIXED procedures of SAS. There were no differences ($P \geq 0.21$) between BW and BCS. Preovulatory ovarian follicle characteristics including size at dominance, duration of dominance, post-dominance growth, size at spontaneous luteolysis, post-luteolysis growth, length of proestrus, and wavelength were not different between treatments ($P \geq 0.11$). However, ovulatory follicles were larger ($P = 0.04$) and average antral follicle count was greater ($P = 0.01$) in the MP150 than MP125 treatment. Estradiol concentrations and the ratio of estradiol to ovulatory follicle volume was not affected ($P \geq 0.51$) by treatment. Although CL volume 7 d post-estrus was greater ($P < 0.01$) in MP150 than MP125, circulating progesterone 7 d post-estrus and the ratio of progesterone to CL volume was not different ($P \geq 0.21$). In conclusion, supplementation of CP at 150% of NRC MP requirements from a moderately undegradable protein source may enhance growth of the ovulatory follicle and subsequent CL compared with MP supplementation at 125% of NRC requirements.

Key Words: crude protein, follicle, ovulation

315 Effect of top-dressing rumen-protected methionine in lactating Holstein cows II: Fertility and embryo development. Mateus Z. Toledo*[1], Giovanni M. Baez[1], Eduardo Trevisol[1], Nelson E. Lobos[1], Alvaro Garcia-Guerra[1], Jerry N. Guenther[1], Daniel Luchini[2], Randy D. Shaver[1], and Milo C. Wiltbank[1], *[1]University of Wisconsin-Madison, Madison, WI, [2]Adisseo, Alpharetta, GA.*

Experimental objectives were to evaluate the effects of supplementation with rumen-protected methionine (RPM) from 31 ± 2 to 127 ± 2 DIM (61 d after timed AI; TAI) on fertility and embryo development of dairy cows. Holstein cows (n = 309) were housed in a free-stall barn, milked twice daily, fed a basal diet formulated to 16.7% CP to deliver 2521 g of metabolizable protein (MP) with 6.93 lysine as % of MP and randomly assigned to once daily top-dressing with either (1) RPM, 21.2 g of Smartamine M mixed with 38.8 g of dry distillers grains (2.34 methionine as % of MP) or (2) Control (CON), 60 g of dry distillers grain (1.87 methionine as % of MP). All cows were synchronized using a Double Ovsynch protocol (GnRH-7d-PGF-3d-GnRH-7d-GnRH-7d-PGF-56h-GnRH-16h-TAI) to receive first TAI at 66 ± 3 DIM. Pregnancy diagnosis was done at 28 d (pregnancy-specific protein B) and at 32 and 61 d (ultrasound) after TAI. Embryo size and amniotic vesicle volume were determined by ultrasound on d 33 of pregnancy. Binomial data were analyzed by parity using Chi-squared or Fisher's exact test. Continuous outcomes were analyzed by *t*-test or Wilcoxon sum-rank test. Only synchronized cows (92.1%, determined by progesterone concentrations) were used in the final analysis (n = 285). There was no effect of treatment on pregnancies per AI at 28 (65.5 vs. 66.7%; CON vs. RPM), 32 (58.6 vs. 61.4%), or 61 (54.4 vs. 58.3%) d after TAI. However, pregnancy loss was greater in multiparous cows for CON compared with RPM cows from 28 to 61 d (19.6 vs. 6.1%; $P = 0.04$) or from 32 to 61 d (8.9 vs. 0.0%, $P = 0.03$). However, there was no effect of treatment on pregnancy loss in primiparous cows (28 to 61 d, $P = 0.50$; 32 to 61 d, $P = 0.50$). Consistent with data on pregnancy loss, RPM increased embryonic size in multiparous cows (RPM vs. CON; amniotic vesicle volume, 592 vs. 472 mm^3, $P = 0.03$; embryo abdominal diameter, 11.0 vs. 10.5 mm, $P = 0.01$; crown-rump length, 5.8 vs. 5.4 mm, $P = 0.13$), but had little effect on embryonic size in primiparous cows. Thus, top-dressing RPM increased embryo size and pregnancy maintenance in multiparous cows.

Key Words: methionine, dairy cattle, reproduction

316 Intravenous glucose infusion in early postpartum dairy cows: Effects on plasma metabolites, milk production, and interval to first ovulation. Stephen Butler*[1], Shane Leane[1], Francis Curran[1], Stephen Moore[1], Mark Crowe[2], and Matthew Lucy[3], *[1]Teagasc Moorepark-Animal & Grassland Research and Innovation Centre, Fermoy, Ireland, [2]School of Veterinary Medicine, University College Dublin, Dublin, Ireland, [3]Department of Animal Sciences, University of Missouri, Columbia, MO.*

Glucose supply is less than required during early lactation. The glucose deficit affects the homeorhetic mechanisms that coordinate milk production and also impinges upon reproduction. The effect of glucose infusion during early lactation was tested. Postpartum dairy cows (n = 22) that calved in either the autumn (n = 11) or spring (n = 11) were treated with either glucose (GLUC; n = 11) or saline (SAL; n = 11). The GLUC cows received 750 g of glucose per day via continuous intravenous infusion of 40% dextrose solution (0.52 g glucose/min). The SAL cows received an equal volume of 0.9% saline. Infusions began during the second week postpartum (7.9 ± 1.9 d) and continued for 14 d. Blood was collected 4 times daily. Data were analyzed by using PROC MIXED of SAS. Compared with SAL, the GLUC cows had greater blood glucose concentrations (69.7 ± 1.5 vs. 61.7 ± 1.5 mg/dL; $P < 0.01$). There was a tendency ($P < 0.11$) for a treatment by season interaction for plasma glucose (spring: 71.6 ± 2.3 vs. 60.0 ± 2.5 ng/mL, $P < 0.01$; autumn: 67.7 ± 2.0 vs. 63.4 ± 1.8 mg/mL, $P > 0.10$; GLUC vs. SAL) and there was a treatment by season interaction ($P < 0.01$) for plasma insulin (spring: 4.4 ± 0.4 vs. 3.1 ± 0.4 ng/mL, $P < 0.05$; autumn: 3.4 ± 0.5 vs. 3.1 ± 0.5 ng/mL, $P > 0.10$; GLUC vs. SAL). GLUC cows had lesser plasma nonesterified fatty acids (NEFA; 448 ± 39 vs. 580 ± 39 μEq/L; $P < 0.03$) and lesser plasma β hydroxybutyrate (BHBA; 0.63 ± 0.10 vs. 1.15 ± 0.10 mM; $P < 0.01$). Insulin sensitivity (RQUICKI) was less in spring cows compared with autumn cows (0.50 ± 0.01 vs 0.53

± 0.01; $P < 0.05$). There was no effect of treatment on milk produced (27.8 ± 1.1 vs. 27.8 ± 1.1 kg/d; GLUC vs. SAL) or as-fed consumption of total mixed ration (44.1 ± 1.6 vs. 45.1 ± 1.7 kg/d; GLUC vs. SAL). The number of cows with first ovulation during the infusion was not affected by treatment [5/11 (45%) for GLUC and SAL] and interval to first ovulation was similar (13.7 ± 0.9 d postpartum). There was a tendency ($P < 0.10$) for more autumn cows [7/11 (64%)] compared with spring cows [3/11(27%)] to ovulate during infusion. Glucose infusion affected plasma hormone and metabolite concentrations but did not affect milk production or interval to first ovulation.

Key Words: glucose, dairy, reproduction

317 Intravenous glucose infusion during pregnancy in dairy cows: Effects on plasma hormones, metabolites, milk production, and conceptus growth. Matthew Lucy*[1], Shane Leane[2], Francis Curran[2], Stephen Moore[2], Mark Crowe[3], and Stephen Butler[2], *[1]Department of Animal Sciences, University of Missouri, Columbia, MO, [2]Teagasc Moorepark-Animal & Grassland Research and Innovation Centre, Fermoy, Ireland, [3]School of Veterinary Medicine, University College Dublin, Dublin, Ireland.*

Glucose supply in postpartum dairy cows may impinge upon early pregnancy by affecting maternal and placental endocrinology as well as substrate availability to the developing conceptus. The objective was to test the effect of glucose infusion from d 32 to 45 of pregnancy on plasma hormones and metabolites, milk production and conceptus growth. Dairy cows (n = 10) were assigned to glucose (GLUC; n = 5) or saline (SAL; n = 5)]. The GLUC cows received 750 g of glucose per d via continuous intravenous infusion of 40% dextrose (0.52 g glucose/min). The SAL cows were infused with an equal volume of 0.9% saline. Infusions began on d 32 of pregnancy (98 ± 15 d postpartum) and ended on d 45 of pregnancy. Blood was collected twice daily during the infusion. Fetal and amniotic vesicle length and width were measured on d 31 (1 d before infusion) and d 33, 35, 37, 39, 41, 43 and 45. Data were analyzed by using PROC MIXED of SAS. Compared with SAL, the GLUC cows had greater blood glucose (81.7 ± 2.1 vs. 74.2 ± 2.1 mg/dL; $P < 0.05$). Plasma progesterone (P4; 9.5 ± 1.3 vs. 9.5 ± 1.3 ng/mL), insulin (7.7 ± 1.7 vs. 7.0 ± 1.7 ng/mL), IGF1 (159 ± 27 vs. 143 ± 27 ng/mL), β hydroxybutyrate (0.93 ± 0.08 vs. 1.02 ± 0.08 m*M*), nonesterified fatty acids (280 ± 10 vs. 264 ± 10 μEq/L) and pregnancy-associated glycoproteins (PAG; 64 ± 21 vs. 73.2 ± 21% of assay control) were not affected ($P > 0.10$; GLUC vs. SAL). There was no effect of treatment on milk yield (26.8 ± 2.4 vs. 24.6 ± 2.2 kg/d; GLUC vs. SAL) or as-fed consumption of total mixed ration (63 ± 4 vs. 67 ± 3 kg/d; GLUC vs. SAL). There was an effect of day of pregnancy ($P < 0.001$) but no effect of treatment on length, width, or volume of the fetus or amniotic vesicle. All hormone and metabolic data were provided as independent variables to explain variation in conceptus measurements using backward elimination (GLMSELECT of SAS). The only significant effects remaining were day ($P < 0.001$) and plasma progesterone (P4; $P < 0.001$; positive association for P4 with all conceptus measurements). Glucose infusion from d 32 to 45 of pregnancy increased plasma glucose but did not affect growth of the conceptus. Greater plasma P4 was associated with larger conceptuses.

Key Words: glucose, pregnancy, dairy

318 Rumen-protected methyl donors during late pregnancy: 1. Maternal Smartamine M and its association with neonatal Holstein calf blood immunometabolic biomarkers. Carolina Bespalhok Jacometo*[1], Zheng Zhou[2], Erminio Trevisi[3], Daniel Luchini[4], Marcio Nunes Corrêa[1], and Juan J. Loor[2], *[1]Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil, [2]University of Illinois, Urbana, IL, [3]Università Cattolica del Sacro Cuore, Piacenza, Italy, [4]Adisseo NA, Alpharetta, GA.*

The aim was to evaluate the effect of supplementing pregnant cows with rumen-protected methionine (MET) on growth and blood biomarkers of hepatic and energy metabolism, inflammation and oxidative stress. Forty Holstein calves born to cows receiving during the last ~4 wk of pregnancy MET (Smartamine M, Adisseo NA.; ~2.9:1 Lys:Met; n = 20) or control (CON, ~3.35:1 Lys:Met, n = 20) were used. Immediately after birth calves were separated from the dam, fed first colostrum (3.8 L with minimum IgG concentration of 50 g/L), housed individually and fed a common milk replacer (25% CP, 17% fat) twice daily. Calves were bled at birth (before colostrum), 24 h after first colostrum, at 14, 28 and 50 (~1 wk post-weaning) d of age. Data were analyzed as repeated measures using the MIXED procedure of SAS. No maternal diet effect ($P > 0.05$) was observed in calf growth (body weight and withers height) from birth through weaning. MET calves had lower glucose at birth (4.05 vs. 4.73 mmol/L, $P > 0.01$), but there was no overall maternal diet effect ($P = 0.18$). Regardless of maternal diet, glucose, AST and GGT increased markedly ($P < 0.01$) from birth to 24 h after colostrum intake, then decreased ($P < 0.01$) at 14 d and remained unchanged until 50 d. NEFA and creatinine concentrations had a sharp decrease after birth ($P < 0.01$) while BHBA concentrations increased ($P < 0.01$) over time. Paraoxonase, albumin and ceruloplasmin concentration increased ($P < 0.01$) over time. MET calves had lower albumin (30.1 vs. 30.9 g/L, $P = 0.09$) and ceruloplasmin tended to be lower (1.58 vs. 1.85 μmol/L, $P = 0.11$). IL1-B and IL-6 had a marked decrease ($P < 0.01$) from birth to 24 h after colostrum intake. Tocopherol (1.31 vs. 2.19 μg/mL), myeloperoxidase (466 vs. 544 U/L) and ROMt (12.4 vs. 15.5 mg H_2O_2/100 mL) were lower ($P < 0.05$) in MET calves at 14 d of age. Retinol increased over time ($P < 0.01$). Overall, data suggest that maternal supplementation with MET during the last ~4 wk of gestation affected some biomarkers of metabolism and oxidative stress, hence, seemed to elicit a beneficial effect on the neonatal calf.

Key Words: dairy cattle, fetal programming, nutrition

319 Rumen-protected methyl donors during late pregnancy: 2. Maternal Smartamine M and its association with hepatic gene expression in neonatal Holstein calves. Carolina Bespahok Jacometo*[1], Zheng Zhou[2], Daniel Luchini[3], Marcio Nunes Corrêa[1], and Juan J. Loor[2], *[1]Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil, [2]University of Illinois, Urbana, IL, [3]Adisseo NA, Alpharetta, GA.*

The aim was to evaluate the effect of supplementing pregnant cows with rumen-protected methionine (MET) on neonatal calf liver expression of genes related to energy/lipid metabolism, insulin signaling, growth hormone signaling and inflammation. Forty Holstein calves born to cows receiving during the last ~4 wk of pregnancy MET (Smartamine M, Adisseo NA; ~2.9:1 Lys:Met; n = 20) or control (CON, ~3.35:1 Lys:Met, n = 20) were used. Immediately after birth calves were separated from the dam, fed first colostrum within 6 h (3.8 L with minimum IgG concentration of 50 g/L), housed individually and fed a common milk replacer (25% CP, 17% fat) twice daily. Liver biopsies were harvested (n = 8/group) at 4, 14, 28 and 50 (~1 wk post-weaning) d of age. Data were analyzed as repeated measures using the MIXED procedure of SAS. No maternal diet effect ($P > 0.05$) was observed on calf growth (body weight and withers height) from birth through weaning. Expression of genes related to lipoprotein metabolism (*APOB, MTTP*) and growth hormone signaling (*IGF1, GHR1A*) were not ($P > 0.05$) affected by

maternal diet, but increased in expression over time ($P < 0.05$). *PCK1* and *FBP1* expression was greater ($P = 0.05$ and 0.02) in MET calves and increased ($P < 0.001$) over time in both groups. *PC* expression, however, was lower ($P = 0.007$) in MET calves and decreased ($P < 0.001$) over time in both groups. Lower ($P = 0.001$) *ACOX1* expression was observed in MET, while *CPT1A* was greater ($P < 0.001$). The insulin-signaling related genes *AKT2* and *SLC2A2* had greater ($P < 0.01$) expression in MET calves. Except for *FOXO1* and *SLC2A2*, all other genes evaluated in this pathway (*INSR*, *IRS1*, *AKT2*, *SREBF1*) increased ($P < 0.05$) expression over time regardless of maternal diet. MET calves had higher *NFKB* ($P = 0.009$) and *SOD2* ($P < 0.001$) expression, and also a trend ($P = 0.08$) for higher *SOD1*. Overall, the data suggest that maternal supplementation with MET during the last ~4 wk of gestation elicited changes in calf hepatic gene expression and, as such, might have led to functional differences in improving neonatal energy metabolism.

Key Words: fetal programming, nutrition, nutrigenomics

320 Rumen-protected methyl donors during late pregnancy: 3. Maternal Smartamine M and its association with neonatal Holstein calf neutrophil gene network expression. Carolina Bespalhok Jacometo*[1], Zheng Zhou[2], Erminio Trevisi[3], Daniel Luchini[4], Marcio Nunes Corrêa[1], and Juan J. Loor[2], *[1]Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil, [2]University of Illinois, Urbana, IL, [3]Università Cattolica del Sacro Cuore, Piacenza, Italy, [4]Adisseo NA, Alpharetta, GA.*

The aim was to evaluate the effect of supplementing pregnant cows with rumen-protected methionine (MET) on calf neutrophil expression of genes related to cell adhesion and chemotaxis, oxidative stress and inflammation. Forty Holstein calves born to cows receiving during the last ~4 wk of pregnancy MET (Smartamine M, Adisseo NA; ~2.9:1 Lys:Met; n = 20) or control (CON, ~3.35:1 Lys:Met, n = 20) were used. Immediately after birth calves were separated from the dam, fed first colostrum within 6 h (3.8 L with minimum IgG concentration of 50 g/L), housed individually and fed a common milk replacer (25% CP, 17% fat) twice daily. Blood neutrophils were isolated at birth (before receiving colostrum), 24 h after first colostrum and at 14, 28 and 50 (~1 wk post-weaning) d of age. Data were analyzed as repeated measures using the MIXED procedure of SAS. Neutrophil phagocytosis was not affected ($P > 0.05$) by maternal MET supplementation, but increased ($P < 0.01$) over time in both groups. Regardless of maternal diet *SELL*, *CADM1*, *LCP1* and *CYBA* expression increased ($P < 0.05$) from birth to 24 h after colostrum intake, then decreased ($P < 0.05$) until 28 d. *ZBP1* increased ($P < 0.01$) from birth to 28 d. *SELL* expression was overall greater ($P = 0.04$) in MET than CON calves. Expression of genes related to oxidative stress (*MPO*, *NOS2*, *SOD1*, *SOD2, NFE2L2*) was not affected ($P > 0.05$) by maternal diet. Similarly, blood biomarkers related to oxidative stress (ROMt, myeloperoxidase, retinol, tocopherol) were not affected ($P > 0.05$) by diet. *TLR2* had lower ($P = 0.04$) expression in MET calves, but other inflammatory mediators (*TLR4*, *MYD88*, *IRAK1*, *TRAF6*, *NFKB*, *TNF*, *IL1B, SLAMF7*) and blood IL-1B and IL-6 concentrations were not affected ($P > 0.05$). A marked decrease ($P < 0.01$) in both cytokines from birth to 24 h after colostrum intake was observed regardless of diet. Overall, the data suggest that maternal supplementation with MET during the last ~4 wk of gestation had a minor effect on calf neutrophil gene network expression and blood biomarkers of oxidative stress and inflammation.

Key Words: fetal programming, nutrition, nutrigenomics

321 Chromium supplementation alleviates heat stress in growing pigs. Fan Liu*[1], Jeremy J Cottrell[1], Danni Wijesiriwardana[1], Fletcher W. Kelly[1], Pietro Celi[2,1], Brian J. Leury[1], and Frank R. Dunshea[1], *[1]Facultry of Veterinary and Agricultural Sciences, the University of Melbourne, Parkville, VIC, Australia, [2]Faculty of Veterinary Science, the University of Sydney, Camden, NSW, Australia.*

Reduced insulin sensitivity is a characteristic of heat stress (HS) in pigs. Therefore the aim of the experiment was to investigate the effect of chromium (Cr) supplementation in ameliorating HS due to its properties augmenting insulin sensitivity. Thirty-six gilts (Large White × Landrace, 29 ± 4 kg) were randomly assigned to 2 diets containing 0 (control) or 3200 ppb Cr picolinate (400 ppb Cr). After 14 d supplementation pigs were allocated to 8 d thermoneutral (20°C; TN) or cyclic HS (8h/d 35°C) (n = 9/group). Production performance was recorded in the thermal exposure period. Respiration rate (RR) and rectal temperature (RT) were measured at 0900, 1300 and 1600h daily, and blood gas was measured on d 7. Area under the curve (AUC) of glucose and NEFA was studied in an intravenous glucose tolerance test (n = 6/group) on d 8. Data were analyzed by ANOVA in Genstat. In TN pigs fed Cr diet had higher ADFI (2.0 vs. 2.3 kg, $P < 0.05$), but ADG was not improved (0.63 vs. 0.69 kg, $P = 0.26$). Heat stress decreased ADFI by 35% and ADG by 84% (both $P < 0.001$) and no effect of Cr was observed. Heat stress increased RT (38.8 vs. 40.0°C, $P < 0.001$) and RR (34 vs. 155 breaths/min, $P < 0.001$). The increased RR led to reductions in blood CO_2, bicarbonate and base excess in HS (all $P < 0.05$). Collectively the results indicate that heat treatment resulted in a "heat stressed" state. Besides, HS increased glucose and decreased NEFA AUC (both $P = 0.05$), suggesting reduced insulin sensitivity. Compared with control diet, Cr pigs had lower RT (40.2 vs. 39.9, $P < 0.05$) and RR (173 vs. 136, $P < 0.01$) under HS, indicating an amelioration in the level of HS experienced in Cr pigs. Chromium did not alter the glucose AUC in HS, although Cr reduced the glucose "basal to peak" increment in TN (4.15 vs. 2.55 m*M*, $P < 0.05$). Besides, Cr tended to increase NEFA recovery (20 to 90 min) rate and AUC in HS (both $P = 0.09$), indicating that Cr facilitated lipid mobilization in HS. In summary, pig growth performance was not improved by Cr during HS, possibly because of the severe reduction in ADFI. However, dietary Cr mitigated the physiological responses to HS, including lipid mobilization. Therefore, an inclusion of 400 ppb Cr may reduce HS in growing pigs.

Key Words: chromium, pig, insulin

322 Metabolome-based relationships of four biofluids from dairy cows. H. Z. Sun*[1,2], L. L. Guan[3], and J. X. Liu[1,2], *[1]Institute of Dairy Science, College of Animal Sciences, Hangzhou, China, [2]MoE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, China, [3]Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Canada.*

To date, most studies have focused on the improvement of dairy nutritional management strategies to enhance milk production and quality, whereas the physiological and metabolic mechanisms involved have not been well examined. This study was conducted to investigate the relationships of 4 biofluids from lactating dairy cow to identify mechanisms and potential biomarkers as well as key pathways involved in potential regulation of lactation. Eight multiparous Holstein dairy cows with similar lactation stage (164 ± 19.6 d, mean ± SD) and milk yield (30.4 ± 2.29 kg, mean ± SD) were offered a diet with 16.7% (DM basis) of crude protein and 1.57 Mcal/kg of net energy for lactation.

Rumen fluid, serum, milk, and urine were collected after 65-d feeding to identify the metabolome using gas chromatography–time of flight/mass spectrometry. A total of 165, 195, 218 and 156 metabolites were identified from rumen fluid, milk, serum, and urine, respectively, with 29 metabolites detected in all 4 biofluids. The TIC chromatograms showed a clear discrimination among 4 biofluids; and principal component analysis of the relative concentration of mutual metabolites revealed 4 separated metabolite profile clusters of 4 biofluids. The clusters derived from the rumen fluid, milk, and serum partly overlapped with each other, whereas the cluster from the urine was separated from the other 3 biofluids. The dendrogram of hierarchical clustering analysis revealed different subclusters containing varying numbers of metabolites within each biofluid; and the subclusters from rumen fluid and serum were grouped together and highly correlated with each other, but were separated from milk. Based on metabolomic profiles, urine is the most different biofluid, compared with other 3 biofluids. When the mutual metabolites were used for pathway analysis, the impact values of the pathway were 0.29, 0.28, and 0.11 for glycine, serine and threonine metabolism, glycerolipid metabolism, and tyrosine metabolism, respectively. These 3 pathways may play important roles in improvement of lactation performance. Our results suggest that all the 4 biofluids represent the comprehensive metabolism of dairy cow that can be further used for metabolic pathway analysis.

Key Words: biofluid, metabolomics, dairy cow