Physiology and Endocrinology: Growth and Metabolism

395 ASAS Early Career Award Presentation: Placental programming: How the maternal environment can impact placental growth and function. K. A. Vonnahme*, C. O. Lemley, L. E. Camacho, L. A. Lekatz, D. A. Redmer, L. P. Reynolds, and J. S. Canton, *Center for Nutrition and Pregnancy, Department of Animal Sciences, NDSU, Fargo.*

As placental growth and vascularity precedes exponential fetal growth, not only is proper establishment of the placenta important, but a continual plasticity of placental function throughout gestation. Inadequate maternal environment has been documented to alter fetal organogenesis and growth, thus leading to improper postnatal growth and performance in many livestock species. The timing and duration of maternal nutritional restriction appears to influence the capillary vascularity, angiogenic profile, and vascular function of the placenta in cattle and sheep. Moreover, upon realimentation, it appears as if the placenta may try to "overcompensate" allowing for enhanced blood flow and nutrient delivery. In environments where fetal growth and/or fetal organogenesis are compromised, potential therapeutics may augment placental nutrient transport capacity and improve offspring performance. Supplementation of specific nutrients, including selenium and protein, as well as hormone supplements, such as indolamines during times of nutrient restriction may assist placental function. Current use of Doppler ultrasonography has allowed for repeated measurements of uterine and umbilical blood flow including assessment of uteroplacental hemodynamics in cattle and sheep. Moreover, these variables can be monitored in conjugation with placental capacity and fetal growth at specific time points of gestation. Elucidating the consequences of inadequate maternal intake on the continual plasticity of placental function will allow us to determine the proper timing and duration for intervention.

396 Blood metabolites and hormones as potential markers of body reserves dynamic and energetic balance in ruminants. E. González-García*[1], N. Debus[1], P. Hassoun[1], S. Camous[2], M.-R. Aurel[3], F. Bocquier[1], and F. Barillet[4], *[1]INRA UMR[868], Systèmes d'Èlevage Méditerranées et Tropicaux (SELMET), Montpellier, France, [2]INRA UMR[1198], Biologie du Développement et Reproduction (BDR), Domaine de Vilvert, Jouy-en-Josas Cedex, France, [3]INRA UE[0321], Domaine Expérimental de La Fage, Roquefort-Sur-Soulzon, France, [4]INRA UR[0631], Station d'Amélioration Génétique des Animaux (SAGA), Chemin de Borde Rouge, Auzeville, BP [52627], Castanet-Tolosan Cedex, France.*

Under strict controlled conditions, and throughout a whole lactation period, we evaluated the consistence of some plasma metabolites and hormones as potential markers of body reserves status (mobilization or accretion) in ruminants. Forty-eight confined primiparous (PRIM; n = 48) and multiparous (MULT; n = 48) dairy Lacaune ewes were monitored from late pregnancy to late lactation in a 2 × 2 × 2 factorial design. Parity (PRIM or MULT), litter size (singletons –SING– or twins –TWIN) and energetic balance (milked once –ONE– or twice –TWO) were the fixed effects. NEFA, glucose (GLUC) and triglycerides (TRIG) plasma metabolites, and leptin (LEPT), insulin (INSU) and tri-iodothyroidine (T3) hormones were monitored. Animals received a 70:30 hay:concentrate TMR diet. Blood samples (10 mL) were individually collected (≈biweekly) early morning by jugular venipuncture in EDTA or heparin tubes. Plasma was harvested from blood samples that were centrifuged immediately after collection. After centrifugation, samples were transferred to 5-mL tubes and frozen at –20°C until analyses. Energy restriction (i.e., TWO higher energy expenditure than ONE) resulted in higher concentrations of NEFA (PRIM, $P < 0.001$; MULT, $P < 0.05$); in contrast, concentrations of INSU (PRIM, $P < 0.05$; MULT, $P < 0.001$) decreased until 1 mo after ONE; in MULT ewes, LEPT consistently decreased in TWO when compared with ONE (11.15 ± 0.571 vs. 14.31 ± 0.676, $P < 0.05$). However, LEPT was affected ($P < 0.05$) by litter size × number of milking interaction in PRIM, SING × ONE and SING × TWO ewes showing the highest (12.33 ± 1.04) and lowest (7.36 ± 0.989 ng.mL^{-1}) values, respectively. Ewes with TWIN had higher NEFA and lower INSU or LEPT than SING from before lambing until weaning. T3 results showed contradictory variability. Either for parity or number of milking, no differences at all were found for TRIG. For GLUC, differences (ONE > TWO) appeared just 1 wk after passing to ONE. Even when differences in BW or BCS were not found, NEFA, LEPT and INSU showed the higher sensitivity as potential markers of body reserves mobilization under the conditions of this experiment.

Key words: metabolites, hormones, markers of body reserve status

397 Metabolic gene expression in bovine ruminal tissue in response to age and pre and postweaning plane of nutrition. A. Naeem*, J. Stamey, J. K. Drackley, and J. J. Loor, *University of Illinois, Urbana.*

We evaluated expression of 22 genes encoding enzymes involved in ketogenesis, cholesterogenesis, TCA cycle flux, long-chain fatty acid (LCFA) oxidation, and transcriptional regulation in ruminal tissue of male Holstein calves fed a conventional milk replacer (20% CP, 20% fat; 1.25% of birth BW as solids) and starter (19.6% CP, DM basis; control) or enhanced milk replacer (28.5% CP, 15% fat; 2% of BW; ENH) and enhanced starter (25.5% CP, DM basis). All calves were weaned on d 42. Groups of calves in control and ENH were harvested after 43 d (wk 5) and 71 d (wk 10) of feeding. There was marked upregulation of HMGCS2, the rate-limiting enzyme of hepatic mitochondrial ketogenesis in non-ruminants, between wk 5 and 10 regardless of diet. This response paralleled an increase in plasma BHBA concentration (0.09 vs. 0.24 mmol/L) between wk 5 and 10. Expression of other ketogenic (BDH1, HMGCL), cholesterogenic (HMGCS1), and TCA cycle-related enzymes (LDHA, GOT2, PCCA) also increased by wk 10 regardless of diet. A higher expression of CPT1A and ACADVL at wk 5 in calves fed ENH vs. control suggested greater LCFA oxidation potentially driven by the greater intake of LCFA from milk replacer. This suggestion is supported by the greater concentration of plasma NEFA (128 vs. 95 μEq/L) at wk 5 due to ENH vs. control. Expression of peroxisome proliferator-activated receptor-δ increased ~8-fold between wk 5 and 10 regardless of diet, suggesting a role for this nuclear receptor in postweaning ruminal tissue development. In conclusion, several metabolic enzymes were upregulated at wk 10 regardless of diet suggesting a coordinated response to support ruminal tissue development. The mRNA of HMGCS2 accounted for ca. 50% of total genes measured (e.g., HMGCS1 was 0.2%), suggesting this enzyme is key for regulating ketogenesis in ruminal epithelium as in liver. Enhanced nutrition during the first 5 wk of life had minor effects on the selected genes.

Key words: ketogenesis, nuclear receptor, dairy calf

398 Functional genomics of liver in crossbred beef cows in two forage allowances during gestation and lactation period. J. Laporta*[1], G. Greif[2], P. Zorrilla[2], H. Naya[2], G. J. M. Rosa[3], and M. Carriquiry[1], *[1]Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay, [2]Instituto Pasteur, Montevideo, Uruguay, [3]University of Wisconsin, Madison.*

Beef cows in rangeland conditions are subjected to climate variations that affect pasture quality and availability along the year as cow physiological stage changes from pregnancy (autumn-winter) to calving (spring) and lactation (spring-summer). A large-scale microarray experiment was conducted using 8 Angus-Hereford crossbred cows in high and low forage allowances (10 vs. 6 kg of DM/100 kg of LW/d) to study the molecular basis of such physiological processes. Liver biopsies were collected at −170, −15, +15 and +60 d relative to parturition and total RNA was extracted. RNA integrity and quality were evaluated using the Agilent 2100 Bioanalyzer (RIN 6.4 ± 0.4). A single-channel microarray analysis was performed using Agilent 4x44K Bovine (v2) Gene Expression array. After data cleaning and normalization, a 2-way ANOVA test was performed using Agilent GeneSpring (v11.5) Software. Significance levels were adjusted for multiple comparisons using a false discovery rate of 0.2. Out of 2,484 differentially expressed genes, 2,353 changed across time (169 ≥ 2.5 fold change), and 146 with forage allowance, but there was no significant interaction between the 2 factors. Differentially expressed genes were hierarchically clustered to study expression profiles and a Gene Set Enrichment Analysis was performed. More than 45 significant ($P \leq 0.01$) gene sets across time (only for −170 vs. −15 d) with positive (metabolism of RNA, mRNA splicing, proteasome, protein export, TGF signaling pathway) and negative (fatty acid, pyruvate, steroid, glucose, glycolysis and gluconeogenesis, lipid and lipoprotein metabolism; cholesterol biosynthesis; PPAR signaling pathway, among others) enrichment scores (ES) were identified. No genes sets were enriched for peripartum and lactation period. Only 3 genes sets were positively enriched ($P \leq 0.01$) when high vs. low forage were compared: glycolysis, gluconeogenesis, and glucose metabolism. These results contribute to identify pathways that are up or downregulated as physiological stage of cows change as well as due to the different levels of nutrition in grazing conditions.

Key words: microarrays, liver, grazing

399 Alterations in the somatotropic axis during a dual stress and *M. haemolytica* challenge in beef steers. S. M. Falkenberg*[1], J. A. Carroll[2], M. A. Ballou[5], J. L. Sartin[3], J. O. Buntyn[1], T. Elsasser[4], S. Kahl[4], and T. B. Schmidt[1], *[1]Mississippi State University, Mississippi State, [2]Livestock Issues Research Unit, USDA-ARS, Lubbock, TX, [3]Auburn University College of Veterinary Medicine, Auburn, AL, [4]Bovine Functional Genomics Lab, USDA-ARS, Beltsville, MD, [5]Texas Tech University, Lubbock.*

The objective of this trial was to characterize the potential impact of individual and multiple stressors before, simultaneously, and post *M. haemolytica* (MH) challenge on the somatotropic axis. Forty-eight beef steers (207.7 ± 22.1 kg BW) vaccinated against MH were randomly assigned to 1 of 8 treatments (trt). Treatments consisted of steers that received MH (given at 0 h for all 8 trts) and corticotrophin releasing hormone + arginine vasopressin in tandem as the stressor (Stress) administered before, simultaneously, or after the challenge. The 8 trt were: 1) MH only; 2) Stress at −7 h + MH; 3) Stress at 0 h + MH; 4) MH followed by Stress at 7 h; 5) Stress at −7 h and 0 h + MH; 6) Stress at −7 h and 7 h + MH; 7) Stress at 0 h and 7 h + MH; and 8) Stress at −7 h, 0 h and 7 h + MH. Steers were fitted with jugular catheters and rectal temperature (RT) probes on d −3, moved to stanchions, given a day to acclimate (d −2), baseline samples obtained on d −1, and the challenge was given on d 0. There were trt x time interactions or trt effects ($P < 0.05$) observed for cortisol (CORT), growth hormone (GH), insulin-like growth factor-I (IGF-I), and RT. Differences were observed ($P > 0.05$) in overall GH and IGF-I as well as concentrations before the MH challenge for trts that received multiple Stress challenges or Stress during and after the MH challenge. No differences were observed ($P > 0.05$) for change in GH and IGF-I concentrations after the MH challenge. Furthermore, no differences were observed ($P > 0.05$) in GH and IGF-I for trts that did not receive any Stress or Stress trt before the MH challenge. The results indicate the MH challenge altered GH and IGF-I in vaccinated calves regardless of the timing associated with the applied stress. However, the data also suggest that stressors can impact the overall regulation of GH and IGF-I when stressors occur during and after infection with MH even in animals protected by vaccination against MH.

Key words: cattle, immune, stress

400 Effects of plane of nutrition and 2,4-thiazolidinedione on insulin responses and adipose tissue gene expression in dairy cattle during late gestation. K. M. Schoenberg* and T. R. Overton, *Cornell University, Ithaca, NY.*

The objective was to determine effects of an insulin–sensitizing agent (thiazolidinedione, TZD) and dietary energy level on glucose and fatty acid metabolism during late gestation. Multiparous Holstein cows (n = 32) 50 d before expected calving date were assigned to 1 of 2 dietary energy levels for 3 wks (High, 1.52 Mcal/kg NEL; or Low, 1.34, Mcal/kg NEL) and received daily 4.0 mg TZD/kg BW (TZD) or Saline i.v. for the final 2 wk. Cows administered TZD had higher plasma glucose (62.5 vs. 59.6 mg/dL; $P = 0.03$) than Saline cows and cows fed the High diet had higher plasma insulin (35.1 vs. 25.3 μIU/mL; $P = 0.03$) compared with those fed the Low diet. All cows were subjected to an i.v. glucose tolerance test (GTT; 0.25 g dextrose/kg BW) and an insulin challenge (IC; 1.0 μg/kg BW) 110 min later. High cows tended to have a lower area under the curve (AUC) for plasma glucose during GTT (1895 vs. 2410 mg/dLx90 min; $P = 0.13$) than Low cows; however, Low cows had more negative NEFA AUC (–4838 vs –2137 μEq/Lx90 min; $P = 0.04$) and greater NEFA clearance rates (1.35 vs. 0.63%/min; $P = 0.01$) during GTT, suggesting differential responses of glucose and fatty acid metabolism in response to diet. During IC, TZD cows had more negative glucose AUC (–45.0 vs. –12.1 mg/dLx15 min; $P = 0.08$) than Saline, suggesting that TZD–treated cows had greater responses to insulin. Interactions of diet and TZD were only significant ($P = 0.04$) for NEFA responses to IC such that Low cows receiving TZD had a negative AUC (–80 μEq/L x 15 min), cows fed the High diet and treated with either saline or TZD had slightly positive AUC (65 and 67 μEq/L x 15 min, respectively), and cows fed the Low diet receiving Saline had the most positive AUC (517 μEq/Lx15 min). Cows fed the High diet had greater lipoprotein lipase mRNA expression (2.2 vs. 1.6; $P = 0.10$) and peroxisome proliferator–activated receptor–γ expression (2.4 vs. 1.3; $P = 0.02$) in adipose tissue collected by biopsy at the end of the study. These results indicate that energy level and insulin–sensitizing agents affect glucose and lipid metabolism during the dry period.

Key words: insulin, thiazolidinedione

401 Effects of overstocking on glucocorticoid production and analytes associated with energy metabolism. J. M. Huzzey*[1], D. V. Nydam[1], R. J. Grant[2], and T. R. Overton[1], *[1]Cornell University, Ithaca, NY, [2]W. H. Miner Institute, Chazy, NY.*

The objective of this study was to determine if overstocking alters energy metabolism and glucocorticoid production. Four groups of 10 dry Holstein cows (~60 d prepartum) were exposed to 2 treatments: Control (1 lying stall/cow and 0.67m linear feed bunk (FB) space/cow) and Overstocked (0.5 stalls/cow and 0.34m FB/cow) in a replicated 2 × 2 crossover design with 14–d treatments. Plasma NEFA, glucose and insulin were measured from blood sampled every 2 d of each treatment and during an intravenous glucose tolerance test (GTT: 0.25g dextrose/kg BW) performed on d 13. Feces, collected every 2 d, were analyzed for fecal cortisol metabolites (FCORT). Plasma cortisol response to an intravenous ACTH challenge (0.125 IU ACTH/kg BW) was measured on d 14. Data from individual cows were averaged to create a group mean and all statistical analyses used group as the experimental unit. Average DMI per cow was greater during the overstocked treatment relative to the control period (15.9 vs. 14.9 kg/d, $P < 0.001$). NEFA and glucose concentrations were higher during the overstocked treatment (0.11 vs. 0.09 mEq/L and 65 vs. 64 mg/dl respectively, $P < 0.05$); however, when stratified by parity these responses were limited to heifers ($P < 0.01$). Overstocking had no effect on insulin concentration during the treatment period ($P > 0.20$) while FCORT tended to be higher (19 vs. 16 ng/g fecal DM, $P \leq 0.14$) during overstocking. During the GTT, cows took longer to return to basal glucose concentration (55.1 vs. 51.5 min, $P = 0.05$), tended to have greater area under the curve estimates for glucose (2837 vs. 2630 mg/dl × 120 min, $P = 0.06$), had lower peak insulin concentrations (201 vs. 260 μIU/L, $P = 0.02$), and tended to have a reduced rate of NEFA decline from circulation (1.4 vs. 1.9 μEq/L per min, $P = 0.1$) following the overstocked treatment. Cortisol production after administration of ACTH was not affected by stocking density treatment ($P > 0.48$). Overstocking alters energy metabolism. These effects seem to be mediated through changes in insulin production rather than insulin resistance; the role of glucocorticoids in influencing these effects is still unclear.

Key words: overstocking, energy metabolism, cortisol

402 Effect of milking frequency and feeding level in early lactation on metabolites in grazing dairy cows. J. K. Kay*, C. V. C. Phyn, A. G. Rius, S. R. Morgan, T. M. Grala, and J. R. Roche, *DairyNZ, Hamilton, New Zealand.*

Study objectives were to investigate the effect of milking frequency (MF) at 2 feeding levels (FL) in early lactation on plasma metabolite content. Multiparous Holstein-Friesian cows (n = 120), grazing a generous pasture allowance (residuals of 1,600 kg DM/ha) and milked twice daily (2X) from calving until 34 ± 6 DIM were allocated to one of 4 treatments in a 2 × 2 factorial arrangement. Treatments were 2 MF (2X or once daily; 1X) and 2 FL (UnRes: 15 kg DM/cow/d or Res: 9kg DM /cow/d) for 3 wk. After treatment, all animals were offered a generous pasture allowance and milked 2X for 20 wk. Individual blood samples collected weekly from 2 wk pre- until 20 wk post-treatment were analyzed for NEFA, BHBA, glucose, aspartate aminotransferase (AST) and glutamate dehydrogenase (GDH). Differences were significant when $P < 0.05$. During the treatment period plasma glucose decreased with pasture restriction and increased with reduced MF. A MF x FL interaction indicated that increases in glucose during 1X milking were greater in Res compared with UnRes cows. One to 8 wk post-treatment, glucose remained less in Res cows and greater in cows milked 1X; however, by 9–20 wk post-treatment there was no effect of MF or FL. Plasma NEFA and BHBA increased with pasture restriction and decreased with reduced MF. When cows were milked 1X, the decrease in NEFA with reduced MF was greater in UnRes than Res cows, while the decrease in BHBA was greater in Res than UnRes cows. Post-treatment (1–8 wk) there was no effect of MF on NEFA or BHBA, however NEFA was greater in UnRes compared with Res cows. By 9–20 wk post-treatment neither NEFA nor BHBA were affected by FL. Liver function enzymes (AST and GDH) increased during pasture restriction but were not affected by MF. These metabolite data are consistent with the previously reported decrease in milk production and improved LWT with reduced MF. In summary, results indicate reducing MF improves energy balance in both restricted and unrestricted grazing cows and may have implications for management strategies when pasture availability is limited.

Key words: milking frequency, nutrition, grazing

403 Insulin-glucose clamps and intramammary LPS challenge: cross reactions between metabolism and mammary immune response. M. C. M. B. Vernay, L. Kreipe, H. A. van Dorland, R. M. Bruckmaier, and O. Wellnitz*, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Insulin, a central regulator of carbohydrate and fat metabolism, influences the immune system. The aim of this study was to evaluate the effects of a 3-d hypoglycemia and hyperinsulinemia/euglycemia on the bovine mammary immune system. Seventeen midlactating, non pregnant, anestric dairy cows received an insulin infusion (HypoG; n=5; constant plasma hypoglycemia of 2.32 ± 0.3mmol/l), an euglycemic hyperinsulinemic clamp (EuG; n=6; insulin infusion rate: 0.62mU/kg/min), or saline solution (control; n=6) for 56 h. 48 h after the start of infusion two udder quarters were challenged with lipopolysaccharide (LPS). Only significant results ($P \leq 0.05$) are shown. Intramammary LPS challenge induced an insulin resistance indicated by an increase of plasma insulin (between 32 and 252μU/L) in all groups, while glucose remained stable in controls, glucose infusion rates in EuG had to be markedly reduced (from 2.9 to 0.9 mmol/kg/min), and insulin infusion rates in HypoG had to be increased (from 0.2 to 0.9 mU/kg/min) to maintain constant glucose levels. AUC of plasma insulin was 333 in control, 875 in EuG, and 529 in HypoG. Hourly measurements of SCC showed increases to $>10^6$ cells/mL in LPS treated quarters without differences between groups. mRNA abundance of immune parameters in mammary tissue biopsies before and 8 h after LPS administration was quantified by qRT-PCR: LPS induced an increased expression (between 2.1 and 8.4 crossing points) of tumor necrosis factor-α, interleukin (IL)-8, −1β, and −10, and serum amyloid A (SAA). IL-1β, IL-10, and SAA were higher expressed (difference between 2.2 and 3.6 crossing points) in LPS treated quarters of EuG than of HypoG. In conclusion, intramammary LPS challenge induces insulin resistance characterized by increased insulin release independently of insulin and glucose plasma concentrations before challenge. Increased plasma insulin occurs concomitantly with changes of the mammary immune response to LPS based on mRNA expression of measured immune factors. The results indicate cross-reactions between insulin resistance and cytokine release in the bovine mammary gland.

Key words: insulin, mammary immunity, intramammary LPS challenge

404 Insulin sensitivity in tropically adapted cattle selected for residual feed intake. G. L. Shafer*[1,2], A. W. Lewis[1], L. C. Caldwell[2], A. N. Hafla[2], G. E. Carstens[2], T. D. A. Forbes[3], T. H. Welsh Jr[2], and R. D. Randel[1], *[1]Texas AgriLife Research, Overton, [2]Texas AgriLife Research, College Station, [3]Texas AgriLife Research, Uvalde.*

Residual feed intake (RFI) identifies animals requiring less feed to achieve the same performance. This study evaluated the effect of a glucose (G) challenge on efficient (L) and inefficient (H) tropically adapted yearling bulls and heifers. Bonsmara heifers (n = 24) and Santa Gertrudis bulls (n = 16) were tested at different times and data analyzed separately. Animals were infused with a 50% dextrose solution at 0.5 mL/kg BW by catheter. Blood was collected at −5, 0, (heifer: 5), 10, 15, 20, (bull: 30), 40, 60, 80, 100, 120, 140, 160, and 180 min relative to challenge. Insulin (I) was determined by RIA and G by colorimetry. Repeated measures ANOVA were conducted using the MIXED model of SAS for analysis of RFI, time, and their interactions on I, G and insulinogenic index (IND). Time to peak I and half-life of G were analyzed using GLM. In bulls, time affected ($P < 0.001$) I and G. RFI did not affect ($P > 0.05$) I peak or peak time in bulls. L and H bull I peaks were (mIU/mL) 50.6 ± 13.3 and 67.7 ± 13.3, respectively and I peak times (min) were 46.2 ± 23.1 and 81.2 ± 23.1, respectively. RFI did not affect ($P > 0.05$) G half life in bulls. IND was affected by RFI ($P < 0.05$), but not time. L and H bull IND ($\Delta I/\Delta G$) were 0.17 ± 0.02 and 0.26 ± 0.02, respectively. Among heifers time affected ($P < 0.0001$) I and G. There was no RFI x time interaction ($P > 0.05$) for I or G. RFI did not affect ($P > 0.05$) I peak or peak time. L and H heifers had I peaks (mIU/mL) of 108.0 ± 12.0 and 75.5 ± 12.6, respectively and I peak times (min) were 16.6 ± 1.0 and 18.6 ± 1.0, respectively. RFI did not affect ($P > 0.05$) G half life in heifers. L and H heifer G half lives were (mg/dL) 80.0 ± 3.1 and 77.0 ± 3.2, respectively and G Half life times (min) were 33.2 ± 2.5 and 36.9 ± 2.6, respectively. IND was affected by RFI ($P < 0.05$), but not time. L and H heifer IND ($\Delta I/\Delta G$) were 0.44 ± 0.02 and 0.29 ± 0.03, respectively. L heifers had a higher I response than H heifers. The opposite response was seen in bulls. There may be differences in energy metabolism between genders and breeds. Further research will be required to explain the opposite results of Bonsmara heifers and Santa Gertrudis bulls.

Key words: insulin, glucose, residual feed intake