Physiology and Endocrinology: Metabolism, health, and physiological processes

W245 Transition period concentrations of nonesterified fatty acids and β-hydroxybutyrate in dairy cows are not well correlated. Maris M. McCarthy*[1], Sabine Mann[2], Daryl V. Nydam[2], Thomas R. Overton[1], and Jessica A. A. McArt[2], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY.*

The objective was to use longitudinal data of blood nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) concentrations to describe the relationship between NEFA and BHBA in dairy cows during the periparturient period. Blood NEFA and BHBA concentration data collected from d −21 prepartum to 21 postpartum for 269 multiparous Holstein cows were recruited from 4 different studies carried out within our research groups. Of the 269 cows enrolled in the data set, 117 cows (43.5%) had at least 1 postpartum hyperketonemic event (BHBA ≥ 1.2 mmol/L), and 202 cows (75.1%) had at least 1 event of elevated postpartum NEFA (≥700 μEq/L) between 3 and 21 d in milk. Area under the curve (AUC) was used to investigate relationships between metabolites over time. Overall, Pearson correlation relationships between transition period NEFA and BHBA AUC were poor. There was a negative correlation between postpartum NEFA AUC and prepartum BHBA AUC, although the correlation coefficient was low (r = −0.26). A positive correlation existed between postpartum NEFA AUC and postpartum BHBA AUC; however, the correlation coefficient was low (r = 0.26), reinforcing a poor relationship between these metabolites during the periparturient period. Large variation was found between the day of maximum NEFA concentration within the first 21 d in milk and day of maximum BHBA concentration for the same time period. The mean and median days of maximum NEFA concentration were 6.8 and 6 d, respectively, whereas the mean and median days of maximum BHBA concentration were 9.6 and 8 d, respectively; however the range in days for both the mean and median day of maximum concentrations was very large. Overall, our data set indicates a poor relationship between blood concentrations of NEFA and BHBA during the periparturient period of dairy cows, suggesting that elevated concentrations of one should not be extrapolated to suggest elevated concentrations of the other metabolite.

Key Words: transition period, nonesterified fatty acids, β-hydroxybutyrate

W246 Associations of circulating haptoglobin with performance and metabolism in dairy cows during early lactation. Maris M. McCarthy*, Takashi Yasui, and Thomas R. Overton, *Department of Animal Science, Cornell University, Ithaca, NY.*

The objective of the current study was to clarify associations between the severity of systemic inflammation during the early postpartum period with performance and energy metabolism. Cows were assigned to categorical quartiles (Q; Q1 = 0.18–0.59, Q2 = 0.60–1.14, Q3 = 1.15–2.05, and Q4 = 2.06–2.50 g haptoglobin/L) based on the highest plasma haptoglobin concentration measured during wk 1 postpartum. Linear and quadratic contrasts were tested for all measurements, and data analyzed over time were subjected to repeated-measures ANOVA using PROC MIXED of SAS (v 9.3) and the REPEATED statement. There was a quadratic relationship ($P = 0.01$) on prepartum net energy for lactation (NE_L) intake such that cows in Q3 had lower prepartum NE_L intake compared with cows in the other Q (Q1 = 21.1, Q2 = 19.2, Q3 = 16.6, Q4 = 19.6 Mcal/d). There was a quadratic relationship ($P = 0.02$) on postpartum NE_L intake such that cows in Q3 also had lower postpartum NE_L intakes compared with cows in the other Q (Q1 = 36.3, Q2 = 34.6, Q3 = 29.8, Q4 = 33.7 Mcal/d). There was also a quadratic relationship ($P = 0.05$) with postpartum milk yield (Q1 = 42.6, Q2 = 40.1, Q3 = 34.2, Q4 = 37.4 kg/d), with similar relationships with 3.5% fat-corrected milk ($P = 0.02$) and energy-corrected milk ($P = 0.03$). There was a linear tendency ($P = 0.06$) for cows with increasing inflammation to have higher plasma glucose concentrations compared with cows with low inflammation. There was a similar linear relationship ($P = 0.04$) for plasma insulin and cows in Q3 and Q4 had higher postpartum insulin compared with cows in Q1 and Q2. There was a linear relationship ($P = 0.04$) for plasma NEFA and cows with increasing inflammation had lower NEFA (Q1 = 498, Q2 = 456, Q3 = 430, Q4 = 407 μEq/L). There was a quadratic relationship of inflammation ($P = 0.02$) with liver triglyceride content at d 7 postpartum and cows in Q3 had the lowest triglyceride content. There was also a quadratic relationship ($P = 0.02$) for liver glycogen and cows in Q3 had elevated liver glycogen content on d 7 postpartum compared with cows in other Q. Overall, cows with elevated haptoglobin in the first week after calving had reduced milk yields and alterations in metabolism; however, cows that have high early lactation haptoglobin (>1.14 g/L) had a diverse range of production responses and further investigation is warranted.

Key Words: transition period, haptoglobin, metabolism

W247 Intestinal permeability and incidence of diarrhea in Holstein calves. Gemma Araujo[1], Cristina Yunta[1], Marta Terré[1], Alessandro Mereu[2], Ignacio Ipharraguerre[2], and Alex Bach*[3,1], *[1]Department of Ruminant Production, IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Caldes de Montbui, Spain, [2]Lucta S.A., Montornès del Vallès, Spain, [3]ICREA (Institució Catalana de Recerca i Estudis Avançats), Barcelona, Spain.*

Seventy-six newborn Holstein calves (44.4 ± 6.15 kg BW) were involved in this study from birth until 21 d of age. Within 2 h after birth, calves received 4 L of maternal colostrum via an esophageal tube. The following 3 meals consisted of 2 L of late colostrum (or transition milk). After that, calves were fed 1.5 L of milk replacer (22.9% CP, 20.1% fat) twice daily. Calves were considered diarrheic when showed fecal scores ≥3 for 3 consecutive days. Then, data from a random subset of 30 calves (45.9 ± 5.47 kg BW), 15 that never had diarrhea and 15 that had diarrhea were used to assess potential associations between intestinal permeability and incidence of diarrhea. On 0, 7, 14 and 21 d of life, intestinal permeability of calves was measured by dosing 2 markers (lactulose and D-mannitol) and assessing their concentration in serum by ultra-high performance liquid chromatography-mass spectrometry. Plasma IgG was measured at birth and at 6 and 24 h after first colostrum intake and efficiency of IgG absorption calculated. Plasma and colostrum IgG contents were determined by radioimmunoassay and bacterial load in colostrum samples by colony counting. Data were analyzed with a mixed-effects model for repeated measures. All diarrhea incidences occurred between 7 to 14 d of study. Overall colostrum quality was good, with an IgG content >50 mg/mL but total bacterial load was high (>100,000 cfu/mL). However, there were no differences in these 2 parameters between colostrums consumed by calves that did and those that did not incur diarrhea. Also, efficiency of IgG absorption was similar for all calves (~16%).

However, intestinal permeability was increased in diarrheic compared with healthy calves. Diarrheic calves had greater ($P < 0.01$) lactulose serum concentrations (15.3 ± 0.37 μg/mL) than healthy calves (8.4 ± 0.37 μg/mL). Furthermore, diarrheic calves tended ($P = 0.06$) to have a greater lactulose to D-mannitol ratio (1.26 ± 0.16) since birth until 21 d of life than healthy calves (0.81 ± 0.16). In conclusion, calves correctly immunized that develop diarrhea may be predisposed to suffer scours due to altered intestinal permeability right from birth.

Key Words: colostrum, intestinal integrity, scours

W248 Effects of realimentation on umbilical blood flow, fetal and placental measurements, and birth weight in nutrient-restricted pregnant ewes. Manuel Vasquez*, Kendall Swanson, and Kimberley Vonnahme, *North Dakota State University, Fargo, ND.*

Nutritional restriction (60% of total nutritional requirement) from d 50 to 130 applied in nulliparous ewes has shown to reduce umbilical blood flow (UBF; Lemley et al., 2012; AJP 302:R454-R467). We hypothesized that during restriction, UBF and fetal and placentome measurements would be less than in adequately fed ewes, but upon realimentation, ewes would have similar UBF as ewes that were never restricted. Second parity Dorset ewes were assigned either to an adequate nutrition group (CON, n = 7) or a restricted (60% of CON) group (RES, n = 8), from d 50 to 90 of gestation. On d 90, all ewes were fed 100% of nutritional requirements according to body weight. Ewe body weight and conceptus measurements via ultrasonography were recorded every 10 d from d 50 to 130 of gestation. Every 10 d, length and width from 10 random placentomes were averaged and then multiplied to obtain placentome area. Fetal biparietal and abdominal lengths were recorded. Doppler mode was used to obtain UBF, pulsatility index (PI), and resistance index (RI). At birth, lambs and placental measurements were obtained. The data were analyzed using the Proc Mixed procedure of SAS. Treatment and day were treated as fixed effects, ewe as random. By d 70, RES ewes were lighter ($P < 0.01$), and remained lighter than CON ewes throughout the experiment. While there were no treatment by day interactions or main effects of treatment ($P > 0.13$) for any measurements obtained by ultrasonography, there were some interesting observations. On d 80, UBF and placentome area were decreased, and PI, RI, and biparietal distance increased in RES vs CON ewes ($P \leq 0.05$; means separation of unprotected F test). On d 90, before the realimentation, all ultrasound measurements were similar. After realimentation, there was no effect of treatment on any of the ultrasound measurements. At birth, lambs and placental measurements were similar ($P > 0.43$). Perhaps the increased resistance indices and smaller placentome size on d 80 were a trigger to the dam to enhance UBF to the growing fetus. Further studies are needed to determine the impact of maternal age and parity in the face of nutrient restriction on UBF.

Key Words: ewe, pregnancy, blood flow

W249 Changes in insulin-like growth factor I and II profiles following anti-bPL antibodies infusions in six long-term-cannulated bovine fetuses at late gestation. Andrea Alvarez-Oxiley*[1], Noelita Melo de Sousa[2], Jean L. Hornick[2], Kamal Touati[2], and Jean F. Beckers[2], *[1]Facultad de Agronomia, Universidad de la Republica, Montevideo, Montevideo, Uruguay, [2]Faculty of Veterinary Medicine, University of Liege, Liege, Liege, Belgium.*

Studies were conducted to evaluate the changes in plasma concentrations of bovine fetal placental lactogen (bPL), insulin-like growth factor (IGF-I) and IGF-II following anti-bPL antibody infusion in fetal circulation. Abdominal aorta of bovine fetuses (n = 6) were chronically cannulated on the medial tarsal artery and infusion of rabbit anti-bPL IgG was performed during late gestation. From the first infusion, blood samples were collected from the fetus on a daily basis. Fetuses remained cannulated during the last 10 to 95 d of gestation. The time-series of hormonal data (bPL, IGF-I and IGF-II) were statistically analyzed for repeated measures as a split plot in time with number of anti-bPL infusions (one vs. more than one), dose of anti-bPL infused L (low, 4, 6, 8 mL) vs. H (high, 20 mL) and days after infusions (d 1 to d 7) as the fixed effects using the Mixed procedure of SAS (1998). IGF-I concentrations tended to reach a minimal concentration on d 3 after infusions (15.5 ± 3.4 ng/mL; $P = 0.056$). No changes were observed on IGF-II concentrations. The mean values of bPL on d 3 after infusion showed a significant increase. When low doses of anti bPL IgG were administered, no peak of binding in fetal serum was observed. When higher or repeated doses of anti bPL IgG were injected, a significant binding rate of the tracer (B/T) was recorded in the fetal serum during 1, 2 or several days. This high B/T can be due to circulating free anti bPL IgG; these rabbit IgG can remain in solution during the step of separation of free and bound fraction of the RIA and thus can bind part of the tracer and create an artifactual overestimation of the circulating bPL. However generally, concentrations of circulating bPL after anti bPL IgG injection were decreased justifying a transitory decrease in IGF-I concentrations. These data showed that following anti-bPL infusion, a transient decrease in IGF-I but not in IGF-II is observed. Our work suggests that PL somatogenic activities at the maternal-fetal interface are mediated through the IGF-I rather than IGF-II.

Key Words: IGF, bovine placental lactogen, fetus cannulation

W250 Effects of different feeding intensities during the first weeks of rearing on the metabolic status and on the circulating concentrations of adiponectin in dairy calves until 110 days of age. Julia Kesser[1], Miriam Hill[1,2], Christian Koch[2], Marion Piechotta[3], Jürgen Rehage[3], Klaus Eder[4], Hassan Sadri[1], Ute Müller[1], and Helga Sauerwein*[1], *[1]Physiology and Hygiene Group, Institute for Animal Science, University of Bonn, Bonn, Germany, [2]Lehr- und Versuchsanstalt Neumühle, Münchweiler an der Alsenz, Germany, [3]Clinic for Cattle, University of Veterinary Medicine Hannover, Hannover, Germany, [4]Animal Nutrition, University of Giessen, Gießen, Germany.*

Dairy calves are commonly reared at restrictive levels of feeding, but greater allowances of feed intake may yield beneficial effects for both animal welfare and later milk production. We aimed to test the hypothesis that different feeding levels in early life will continue to affect the circulating concentrations of metabolically relevant hormones beyond the time of differential feeding. After receiving colostrum for the first 3 d of life, 57 German Holstein calves were randomly allocated to 3 groups fed either restrictively (r) with milk replacer (MR) (group MRr: 130 g MR/L, 6 L/d, n = 20), or MR ad libitum (al) (MRal: 160 g MR/L, n = 17) or whole milk (MI) al (MIal, n = 20). All calves received colostrum from their dams for the first 3 d of life, and were then fed according to their group regimen from d 1 – 27 (phase (P) 1). Thereafter all calves were fed according to the MRr plan and were gradually weaned from d 56 - 70 (P2). Calves were further observed for the subsequent P3, i.e., d 71 – 109. All calves had free access to hay, water and concentrate and received a TMR in P3. Blood samples were collected on d 0 (before colostrum feeding), and on 10 other d covering P1-P3 to assess the concentrations of glucose, NEFA, leptin and adiponectin (bovine specific ELISA). Data were analyzed using the linear mixed model (SPSS). Differences ($P < 0.05$) between the groups were largely limited to the

time of differential feeding (P1): glucose was greater in MIal than in MR fed groups (glucose: 1.1-fold), insulin was greater in MRal and MIal than in MRr (2.3-fold), whereas NEFA were lower in MRal than in MRr (0.65-fold). Leptin was not different between the groups at any time. Adiponectin concentrations did not differ between groups in P1, but tended ($P < 0.1$) to lower values in MRr as compared with MRal and MIal in P2. In P3 the MRal group had higher values ($P < 0.05$) than MRr and MIal, thus supporting continued effects of the differential feeding. In view of the insulin-sensitizing effects of adiponectin, feeding intensity in early life might thus affect insulin sensitivity at older ages.

Key Words: adiponectin, dairy calves, insulin sensitivity

W251 Mitochondrial DNA copy numbers in blood cells during early and late lactation in dairy cows. Lilian Laubenthal, Michael Hölker, Karl-Heinz Südekum, Helga Sauerwein, and Susanne Häussler*, *University of Bonn, Institute of Animal Science, Bonn, Germany.*

During the transition period most high-yielding dairy cows suffer from negative energy balance due to decreased energy intake and increased energy demands required for milk synthesis. Mitochondria are the main sites of energy metabolism in mammalian cells and their number varies depending on age, sex, organ, and physiological or pathological conditions. Mitochondria exhibit their own genome, the mitochondrial DNA (mtDNA), and its copy numbers reflect the abundance of mitochondria within a cell. In the course of lactation, environmental, physiological, and energetic conditions alter. We hypothesized that these changes may influence the number of mtDNA/cell in dairy cows and thus investigated the number of mtDNA copies in blood during early and late lactation. German Holstein cows (n = 21; BCS: 3.0 ± 0.1) were fed according to their requirements. Estimated total energy requirements were calculated by adding the requirements for maintenance and milk production. Blood samples from the jugular vein were collected 3 and 35 wk postpartum. Genomic DNA was extracted from whole blood using a commercially available kit. Based on the amplification of the 12S rRNA (mtDNA target gene) and the β-globin (reference gene) to normalize the DNA content in each sample, mtDNA copy number was assessed by a multiplex qPCR. Data (mean ± SEM) were analyzed by the pairwise Student's *t*-test (SPSS 22). In early lactation the number of mtDNA (87.4 ± 15.5 copies/cell) was about twice as much as in late lactation (46.0 ± 3.7; $P = 0.008$). In early lactation energy demands are increased compared with late lactation, as indicated by the estimated total energy requirements for wk 3 (132 ± 8.26 MJ NE_L) and wk 35 (103 ± 5.77 MJ NE_L). The greater energy demands in early lactation were accompanied by elevated mtDNA copy numbers in peripheral blood when compared with late lactation. The observed changes meet the expectation that the metabolic load during early lactation requires more mitochondria. Peripheral blood thus forms a suitable matrix to assess the cellular content of mitochondria and the cellular energetic status of dairy cows when tissues are not accessible.

Key Words: mtDNA copy number, dairy cow, lactation

W252 Mitochondrial DNA copy number in liver, mammary gland, and adipose tissue of early lactating dairy cows. Lilian Laubenthal, Michael Hölker, Karl-Heinz Südekum, Helga Sauerwein, and Susanne Häussler*, *University of Bonn, Institute of Animal Science, Bonn, Germany.*

With the onset of lactation, energy requirements rapidly increase in high-yielding dairy cows. To adapt to lactation, energy metabolism needs to be regulated and coordinated among the key organs, namely adipose tissue (AT), liver, and mammary gland. Mitochondria are the main site for energy production in mammalian cells and their number depends on the energy demand and physiological state of each individual. Mitochondria have their own DNA and therefore the abundance of mitochondria in a cell is reflected by the copy number of mitochondrial DNA (mtDNA). Age-related differences of mtDNA are known for mice and humans, in which mtDNA varies between different tissues. However, little is known about mtDNA copy numbers in dairy cows; we thus aimed to provide an overview of mtDNA copy numbers in liver, subcutaneous (sc) AT and mammary gland of lactating dairy cows. Lactating German Holstein cows (n = 21; BCS: 3.0 ± 0.1) were fed according to their requirements. Liver, mammary gland and subcutaneous (sc) AT from the tailhead region were sampled during early lactation (3 wks postpartum), in which the estimated total energy requirement was 132 ± 8.26 MJ NE_L. Biopsies were immediately snap frozen after sampling. Genomic DNA was extracted using commercially available kits and the number of mtDNA copies/cell was quantified by a multiplex qPCR, targeting the 12S rRNA gene and using b-globin as reference gene. Tissue-specific differences were examined by Student's *t*-test (SPSS 22). Data are presented as means ± SEM. The number of mtDNA copies/cell in liver (360 ± 22.3 copies/cell) was 7.7-fold and 5.3-fold higher ($P < 0.001$) than in scAT (46.7 ± 2.32 copies/cell) and mammary gland (68.3 ± 4.66), respectively. Moreover, mammary gland contained 1.5-fold more mtDNA copies/cell than scAT ($P = 0.001$). The differences in mtDNA content observed between the organs investigated herein are presumably reflecting their metabolic activity during the first weeks of lactation with liver playing a key role.

Key Words: mtDNA copy number, dairy cow, liver

W253 Lipolysis induces adipose tissue macrophage infiltration in lactating dairy cows. G. Andres Contreras*[1], Kyan Thelen[2], Courtney L. Preseault[2], Sarah E. Schmidt[2], and Adam L. Lock[2], *[1]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, [2]Department of Animal Science, Michigan State University, East Lansing, MI.*

Excessive rates of lipolysis during periods of negative energy balance (NEB) are associated with increased susceptibility to disease. Lipolysis increases adipose tissue macrophage (ATM) populations. Depending on their phenotype, ATMs modify inflammatory processes and alter adipose metabolic functions. Classically activated ATMs (M1) are pro-inflammatory while alternatively activated ATMs (M2) promote inflammation resolution. The objective of this study was to evaluate changes in ATM trafficking and phenotype in healthy cows during feed restriction-induced NEB. Lactating multiparous dairy cows (DIM 119–210) were fed a common diet to meet nutrient requirements during a 14d preliminary period (d1 to 14) and then randomly assigned to one of 2 feeding protocols: ad libitum (AL; n = 6) or feed-restricted (FR; n = 7). Caloric intake was reduced in FR cows for 4 d (d15 to 18) to achieve a targeted NEB of −15 Mcal/d. Omental and subcutaneous adipose tissue samples were collected to harvest stromal vascular cells (SVC) on d11 and d18. Data were analyzed in a mixed model with treatment and day as fixed effects and cow as a random effect. FR cows reached a NEB of −13.5 ± 1.9 Mcal/d inducing a lipolytic response (NEFA on d18: FR = 0.52 mEq/L; AL = 0.17 mEq/L; $P < 0.01$), while AL animals remained in positive energy balance (3.2 ± 2.2 Mcal/d). Flow cytometry analysis revealed that at d18, FR increased the infiltration ratio of $CD68^+$, a specific ATM surface marker, in omental SVC (FR = 2.85 ± 0.39; AL = 1.25 ± 0.35; $P = 0.01$), while the expression ratio of CD14, an M1 marker, remained unaltered ($P = 0.86$). Adipose tissue from FR cows

exhibited an increased expression of the macrophage-related gene SIRPA ($P = 0.01$), but no change in M1 genes CCL2 and TNFα (both $P > 0.49$). Additionally, compared with AL, FR upregulated the expression of M2 specific genes IL10 and ARG1 (both $P < 0.01$). This finding contrasts with the predominately M1 phenotype observed previously in ATMs from clinically diseased cows. These results provide evidence for an active role of ATMs during NEB in ruminants and emphasize changes in their inflammatory phenotype during lipolytic periods.

Key Words: lipolysis, adipose tissue macrophages, negative energy balance

W254 Longitudinal characterization of the gene expression of key components of the mTOR signaling and ubiquitin proteasome system in skeletal muscle of dairy cows during the periparturient period and subsequent lactation. Yi Yang[1], Helga Sauerwein*[1], Sven Dänicke[2], Jürgen Rehage[3], and Hassan Sadri[1], *[1]Institute of Animal Science, Physiology and Hygiene Group, University of Bonn, Bonn, North Rhine-Westphalia, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Braunschweig, Lower Saxony, Germany, [3]Clinic for Cattle, University for Veterinary Medicine, Foundation, Hannover, Lower Saxony, Germany.*

At the onset of lactation when voluntary feed intake is insufficient to cover the total needs for maintenance and lactation, dairy cows need to mobilize body reserves. Besides fat, skeletal muscle protein is also degraded. Muscle is the main labile source of amino acids (AA) and may thus partition AA to protein syntheses in other organs, mainly the mammary gland, for gluconeogenesis and for generating ATP. The mammalian target of rapamycin (mTOR) and ubiquitin-proteasome system (UPS) are considered as the major regulators of protein synthesis and protein degradation, respectively. We hypothesized that the transcript abundance of key components of mTOR signaling and of 2 major muscle-specific E3 ubiquitin ligases, MuRF1 (muscle RING-finger protein-1) and atrogin-1 in skeletal muscle will change throughout late pregnancy and the subsequent lactation period. From 14 German Holstein cows, muscle tissue (*M. semitendinosus*) were obtained for biopsy on d −21, 1, 21, 70, 105, 182, 196, 224, and 252 relative to calving. The target mRNAs were quantified by qPCR. Data were analyzed by the MIXED procedure of SAS and results are reported as LSM with P-values adjusted by the method of Tukey-Kramer. The mRNA abundance of mTOR increased ($P < 0.05$) from d −21 to d 1, followed by a decline toward pre-partum values by d 180 and then increased thereafter. A 6-fold increase in eukaryotic initiation factor 4E-binding protein mRNA was observed from d −21 to d 1, and then a gradual decrease until d 105 with relatively stable values thereafter. The encoding ribosomal protein S6 kinase mRNA decreased ($P < 0.0001$) during lactation. The abundance of MuRF1 mRNA increased (3.6-fold) from d −21 to d 1, declined to nearly pre-partum values by d 105 and then remained unchanged. The mRNA abundance of atrogin-1 followed almost a similar trend as that of MuRF1; a 2.2 fold increase was noted from d −21 to d 1, and then a decrease until d 21 and unchanged values thereafter. In conclusion, our data show that key components of mTOR and UPS are upregulated at the level of the mRNA on d 1, suggesting a greater rate of protein turnover in muscle around calving.

Key Words: skeletal muscle, mTOR, ubiquitin-proteasome system

W255 Comparison of fractional gluconeogenesis estimates in sheep determined with D_2O administered via vein or rumen and by intravenous infusion of $^{13}C_6$-glucose. Cornelia C. Metges*[1], Solvig Görs[1], Gürbüz Das[1], Umang Agarwal[2], and Brian J. Bequette[2], *[1]Institute of Nutritional Physiology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, [2]Dpt. Animal and Avian Sciences, University of Maryland, College Park, MD.*

In ruminants, the main precursor for GNG is ruminal propionate which fundamentally differs from gluconeogenic precursors of monogastrics. The D_2O method has been used to measure fractional gluconeogenesis (GNG) in humans. We aimed to determine whether the route of D_2O administration (rumen (IR) or jugular vein (IV)) affects D enrichment of rumen fluid, ruminal propionate and estimates of fractional GNG. In addition, we compared these GNG estimates with estimates derived from $^{13}C_6$-glucose infusion. Four sheep (23.5 ± 1 kg BW), equipped with a rumen fistula and a jugular vein catheter, were fed a pelleted ration (35 g/kg BW and d; 9 MJ ME/d) at 2-h intervals. Water was offered ad lib. Sheep were given 2 boli of 7 g D_2O (99.2 atom% (AP) D/kg BW) at 0800 and 1200 h either IR (method 1) or IV (method 2), or received continuous IV infusion of $^{13}C_6$-glucose (0.15 g/h) for 10 h (method 3) in a crossover design with 1 wk separating tracer administrations. Ruminal D enrichments were measured by isotope ratio mass spectrometry whereas ruminal propionate and plasma glucose D enrichment was measured by GC-MS. Fractional GNG was calculated from the ratio of D enrichment at C-5 of glucose (labeling via GNG only) to that at C-2 (labeling during GNG and glycogenolysis). The ^{13}C-mass isotopomer enrichment of plasma glucose was determined by GC-MS and GNG was calculated. Statistical comparison of GNG estimates was made with repeated measures ANOVA using PROC MIXED of SAS. Rumen fluid D enrichment attained a plateau 6 h after the first bolus (IR: 1.51; IV: 1.43 APE; $P > 0.1$). The D enrichment of ruminal propionate as a precursor for GNG showed faster labeling with the IR route (3 h: $P < 0.10$; 6 h: $P < 0.05$). However, the plateau enrichments of rumen fluid D and propionate D did not differ ($P > 0.10$). GNG estimates derived from IR and IV routes of D_2O administration did not differ ($P = 0.83$) which resulted in an overall GNG estimate of 58.8%. In contrast, GNG estimate derived from $^{13}C_6$ glucose dilution was at 72.8%, which did not differ from the other 2 methods ($P > 0.13$). Thus all 3 methods yield similar estimates of GNG in ruminants.

Key Words: ruminants, gluconeogenesis, stable isotope-labeled tracer

W256 Propionate and cyclic AMP induced bovine PCK1 gene transcription is concurrently mediated by CRE and HNF4α binding elements. Qian Zhang, Stephanie L. Koser, and Shawn S. Donkin*, *Purdue University, West Lafayette, IN.*

Cytosolic phosphoenolpyruvate carboxykinase (PCK1), a key gluconeogenic enzyme, is controlled at the transcriptional level. Our objective was to determine regulatory elements within the bovine PCK1 promoter that control transcription in response to cyclic AMP (cAMP), glucocorticoids, and propionate (PROP). Putative DNA promoter transcription factor protein binding sequences were identified for cAMP response element (CRE) at −94 to −87 and for Hepatic Nuclear Factor 4α (HNF4α) at +68 to +72 (HNF4α1) and −1078 to −1074(HNF4α2). To test control of transcription, the wild-type (WT) bovine PCK1 promoter (−1238 to +221) was ligated to a luciferase reporter gene and transfected into H4IIE cells followed by incubation with 2.5 m*M* PROP, 1 m*M* cAMP (cAMP), 5 μ*M* dexamethasone (DEX) or their combinations. The functionality of CRE, HNF4α1, and HNF4α2 cis-regulatory elements was determined using deletion mutations of the core transcription factor binding regions within the PCK1 promoter DNA. The deletion mutations tested were HNF4α1$^-$; HNF4α2$^-$; CRE$^-$; HNF4α1$^-$/HNF4α2$^-$; CRE$^-$/HNF4α1$^-$; CRE$^-$/HNF4α2$^-$; and CRE$^-$/HNF4α1$^-$/HNF4α2$^-$. H4IIE cells were transfected with the promoter-reporter constructs and exposed to treat-

ments for 23 h. Luciferase activity was measured in the cell lysates as a direct proxy for bovine PCK1 promoter activity. Within each construct, treatment effect was expressed as the fold change of luciferase activity relative to base media control (n = 3 cell preparations). Analyses of variance of the data were performed using the Proc Mixed procedure of SAS 9.3. Exposure to cAMP, DEX, cAMP+DEX, PROP, cAMP+PROP, cAMP+DEX+PROP induced ($P < 0.05$) expression of the WT promoter relative to no addition controls by 2.0, 2.3, 3.9, 6.0, 7.3, 14.4 ± 1.4 x respectively. A similar pattern was observed for each single mutant bovine PCK1 promoter. Responses to cAMP, DEX, PROP and their combinations were abolished for mutations lacking both HNF4α1 and CRE binding sites indicating that these elements act synergistically to control bovine PCK1 transcription.

Key Words: promoter, transcription factor binding site

W257 Hepatic mRNA expression of genes related to somatotropic axis and metabolism of dairy cows treated with recombinant bovine somatotropin during the periparturient period.
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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 4 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.

Key Words: periparturient cow, recombinant bovine somatotropin, hepatic gene expression

W258 A direct method is not as effective as an indirect method for determination of fatty acids from bovine placental tissue.
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Quantification of fatty acids (FA) in reproductive tissues including the placenta is important to understand the influence of FA on reproductive function. Two methods of FA determination (direct and indirect) are commonly used, but their comparative efficacy to quantify FA in bovine tissue is not known. Therefore, we compared the efficacy of the 2 methods of FA determination in bovine placental tissue. Placenta (fetal cotyledon) samples from 13 dairy cows were collected within 5 h of calving, before placental release, snap frozen and stored. Tissues (0.05 g) were assigned in duplicate to either direct methylation (samples directly subjected to methylation process with no extraction step [direct method]) or indirect methylation (samples first subjected to FA extraction and then methylated [indirect method]). The indirect method was approximately 6 h long that involved an 18-step procedure, whereas the direct method took 3 h and had only 10 steps. Briefly, frozen tissue samples were pulverized under liquid nitrogen and methylated either directly, without extraction, or indirectly, after extraction. The FA methyl esters were then injected for gas chromatographic analysis. Fatty acid data were analyzed using the Mixed procedure of SAS. Forty-five different FA were identified from the placental tissue, of which 32 FA were significantly higher in the indirect method. Moreover, the unsaturated-long chain FA of our interest, i.e., oleic, linoleic, α-linolenic, eicosapentaenoic and docosahexaenoic acid were significantly higher ($P < 0.0001$) in the indirect method than in the direct method (0.42, 0.08, 0.008, 0.02 and 0.05 vs. 0.30, 0.06, 0.005, 0.01 and 0.04 mg per 50 mg sample, respectively). In addition, the total amount of FA, saturated FA, polyunsaturated FA, omega-3 and −6 FA were also higher ($P < 0.0001$) with the indirect method than the direct method. Even though, the direct method is shorter and faster, it reduced the amount of FA extracted. We conclude that the indirect method has higher efficacy and should be preferred over the direct method for extraction of FA from placental tissue.

Key Words: extraction, methylation, bovine placenta

W259 The effect of prepartum diets supplemented with oilseeds on maternal and newborn calf plasma fatty acid profile.
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Long-chain polyunsaturated fatty acids (PUFA) have important roles during pregnancy, both in the dam and the fetus. However, limited information is available regarding the transfer of specific PUFA from dam to fetus in cattle. Our objective was to examine the effects of oilseed (oilseed vs. no oilseed) and type of oilseed (canola vs. sunflower) supplementation during late gestation on the fatty acid (FA) profile of maternal and newborn calf plasma. Pregnant Holsteins were assigned to 1 of 3 diets containing 8% rolled sunflower (SUN, high in linoleic acid; 8 cows) or canola seed (CAN, high in oleic acid; 7 cows) on dry matter (DM) basis, or no oilseed (CON; 7 cows), for the last 35 ± 2 d of gestation. Blood samples were collected within 3 h after calving from dam and newborn calf to determine FA profile. Data were analyzed using the Mixed procedure of SAS. The proportion of total saturated FA (TSFA) was higher in CON (46.8 ± 3.7) fed cows than in those fed

oilseeds (37.5 ± 2.7). Feeding SUN increased the proportion of total PUFA and linoleic acid (47.8 ± 2.5 and 45.5 ± 2.9) relative to those fed CAN (39.5 ± 2.7 and 36.0 ± 2.9, respectively). Moreover, SUN fed cows had higher TPUFA:TSFA ratio (1.29 ± 0.06) than those fed CAN (0.99 ± 0.06) or CON (0.88 ± 0.06). However, oilseed or type of oilseed supplementation during prepartum period did not affect FA profile of calves. Correlation analysis in CON treatment indicated that calf and dam TSFA were positively associated (R = 0.79, $P = 0.03$). Total monounsaturated FA (TMUFA, R = −0.66, $P = 0.10$) and oleic acid (OLA, R = −0.68, $P = 0.08$) in calf were negatively correlated with dam TSFA in CON treatment. Calf OLA (R = 0.68, $P = 0.05$) and TMUFA (R = 0.67, $P = 0.06$) in SUN treatment had positive association with dam total n-6. There was no relationship between dam and calf FA profile in CAN treatment. In summary, oilseed and type of oilseed supplemented during late gestation affected maternal FA profile but not that of newborn calf. Moreover, oilseed supplementation and the type of oilseed supplemented during prepartum period affected the association between maternal and newborn calf FA.

Key Words: sunflower seed, canola seed, fatty acid profile

W260 Examining peripheral activity of catechol-O-methyltransferase (COMT) in Holstein cows following artificial insemination. Rachel M. Wilson*, Christa L. Gilfeather, Caitlin G. Hart, and Caleb O. Lemley, *Mississippi State University, Mississippi State, MS.*

The objective was to determine peripheral activity of COMT in pregnant versus non-pregnant lactating Holstein cows. Cows (n = 22) were synchronized using the Ovsynch plus CIDR protocol and bred via artificial insemination on d 0. Cows were retrospectively classified as pregnant (n = 4) or open (n = 14) based on rectal palpation at d 35 post-insemination. Moreover, cows were classified as rebred (n = 4) if they showed signs of estrus and were re-inseminated before rectal palpation. Blood samples were collected on d 0, 4, 8, 12, 16, 20, and 24 post-insemination. Plasma was separated by layering 3 mL of blood onto 3 mL of Ficoll and centrifuged at 400 × *g* at room temperature for 30 min. One mL of erythrocytes were collected and stored at −80°C. Peripheral activity of COMT was determined by incubating erythrocyte cell homogenates with 2 m*M* s-adenosyl methionine, 3 m*M* cysteine, 10 m*M* $MgCl_2$, and 1 m*M* of the COMT specific substrate 6–7-dihydroxycoumarin. Samples and substrates were incubated at 37°C for 30 min and enzyme reactions were stopped by adding 0.5 *M* HCl, 10% $NaNO_2$, 10% $NaMoO_4$, and 1 *M* NaOH to each sample. Lastly, the disappearance of 6–7-dihydroxycoumarin was determined by measuring the amount of light absorbed at 510 nm using a Spectra Max Plus plate reader. Activity of COMT was analyzed using repeated measures ANOVA of the MIXED procedure of SAS and the model statement included day, pregnancy status, and their respective interaction. Activity of COMT was increased ($P < 0.01$) on d 16 post-insemination compared with all other days in cows classified as open or pregnant. Activity of COMT was not different ($P = 0.87$) throughout the sampling period in cows classified as rebred. Moreover, activity of COMT was increased ($P < 0.01$) on d 16 in pregnant cows versus rebred cows. Therefore, peripheral activity of COMT in lactating Holstein cows, which is involved in catechol-estrogen metabolism, may be altered by pregnancy status as well as days post-insemination.

Key Words: catechol-O-methyltransferase, erythrocytes, pregnancy

W261 IGF-1 concentrations during early pregnancy in suckled Nellore beef cows. Rogerio F. G. Peres*[1], Ky G. Pohler[3], Hugo B. Graff[2], Adnan D. P. Rodrigues[1], Michael F. Smith[3], Duane H. Keisler[3], and Jose L. M. Vasconcelos[1], *[1]Departamento de Produção Animal, Faculdade de Medicina Veterinária e Zootecnia-UNESP, Botucatu, São Paulo, Brazil, [2]Agropecuária Fazenda Brasil, Barra do Garças, Mato Grosso, Brazil, [3]Department of Animal Sciences, University of Missouri, Columbia, MO.*

The objective of this experiment was to evaluate temporal changes in serum IGF-1 concentrations in Nellore postpartum cows and its effect on the pregnancy rates to TAI. Cows (n = 1208) from 2 farms with different grass quality (Farm1 = high quality forage; n = 931, BCS 2.81 ± 0.01; Farm2 = low quality forage; n = 277, BCS 2.97 ± 0.03) with 53 ± 6.2 d postpartum were supplemented with mineral premix (110 g/cow/day) throughout the study. Cows underwent an estrous synchronization protocol with TAI. Blood was sampled on D-11 (first day of protocol), D0 (TAI), and D30 (pregnancy diagnosis) and analyzed for IGF-1 concentrations (all cows). Concentrations of IGF-1 on Days −11, 0 and 30 were analyzed using PROC MIXED including farm, BCS, pregnancy status on D30 and cow within pasture as a random effect. PROC GLM was used to evaluate IGF-1 concentrations effect on pregnancy rates. The pregnancies to TAI were 48.2% (449/931) and 45.5% (126/277) for Farm1 and 2, respectively. IGF-1 concentrations were greater ($P < 0.05$) in all days in Farm1 compared with Farm2 ($P < 0.05$). IGF-1 concentrations decreased during the study on Farm1 (D-11: 145.0 ± 2.4a, D0: 125.2 ± 2.9b and D30: 98.6 ± 1,8c ng/mL, $P < 0.05$). On farm 2 the IGF-1 concentrations were greater on D-11 compared with D0 and D30 (D-11: 73.0 ± 1.5a, D0: 65.1 ± 1.5b, D30: 63.0 ± 1.4b ng/mL, $P < 0.05$). There was a linear negative association of IGF-1 concentrations on pregnancy rates ($P < 0.05$). There was no difference in IGF-1 concentrations on D-11 and D0 between pregnant and open cows. Cows in Farm1 that became pregnant (96.0 ± 1.9a ng/mL) had less IGF-1 concentrations on D30 compared with nonpregnant cows (102.4 ± 1.9b ng/mL). In Farm 2 pregnant (60.0 ± 1.7b ng/mL) cows tended ($P < 0.06$) to have less IGF-1 concentration than nonpregnant cows (63.7 ± 1.5b ng/mL). These data show that Nellore cows have a decrease in IGF-1 concentrations within 30 d of the first insemination and pregnant cows had less IGF-1 concentration on D30 compared with nonpregnant cows. Although the farm with better quality forage had cows with increased IGF-1 concentrations compared with cows in the farm with low quality forage, in both farms pregnant cows had a decrease in IGF-1 after TAI. Further research is needed to understand mechanisms by which less IGF-1 is associated with pregnancy in beef cows. FAPESP Project #2014/03209–0.

W262 Circulating anti-Müllerian hormone (AMH) in Holstein and Jersey breeds, at different physiological states and in dam-daughter pairs. E. O. S. Batista*[1,2], C. Collar[1], N. Silva-Del-Rio[1], P. D. Carvalho[4], J. P. Verstegen[3], P. S. Baruselli[2], M. C. Wiltbank[4], and A. H. Souza[1], *[1]University of California, Tulare, CA, [2]University of Sao Paulo, Sao Paulo, SP, Brazil, [3]Mofa Global, International Center for Biotechnology, Wisconsin, WI, [4]University of Wisconsin, Wisconsin, WI.*

Our aim was to investigate circulating levels of the anti-Müllerian hormone (AMH) in Holstein and Jersey breeds at different physiological states. A second objective was to study possible associations between circulating AMH in dam-daughter pairs. Mature cows and heifers were located in 2 commercial facilities in Central-California. Blood sample for AMH analysis was taken from Holstein (Mature cows = 141; pregnant Heifers = 408) and Jersey (Mature cows = 148; pregnant Heifers =

123) breeds and measured with the MofA-Global bovine fertility assay. Data were analyzed by the GLIMMIX and CORR procedures of SAS. Circulating AMH differed ($P < 0.01$) across cattle breeds (Holstein cows = 243.9 pg/mL; Holstein heifers = 237.8 pg/mL vs Jersey cows = 312.2 pg/mL; Jersey heifers = 334.4 pg/mL). Despite of cattle breed, circulating AMH seem to be increased in cows with greater days in milk ($P = 0.01$); but was not influenced by level of milk production in lactating cows ($P = 0.92$) or even parity number ($P = 0.68$). In virgin heifers, stage of pregnancy did not influence circulating AMH ($P > 0.10$) in both Holstein and Jersey breeds. Interestingly, there was a significant although somewhat low correlation of circulating AMH in dam-daughter pairs (Holsteins: n-pairs = 116, r = 0.18, $P < 0.01$; Jerseys: n-pairs = 106, r = 0.22, $P < 0.01$). In conclusion, AMH results are specific within major dairy breeds and some important nuisance variables may need to be taken into account when interpreting AMH results. The significant correlation between circulating AMH in dams and their respective daughters might allow for selection of this important heritable trait.

Key Words: anti-Müllerian hormone (AMH), dairy breed, heritability