Adaptive responses of Mérinos d’Arles adult ewes submitted to nutritional and β-adrenergic challenges. E. González-García1, M. Alhamada1, N. Debuss2, J.-B. Menassol3, J. G. Vero3, B. Barbosa3, and F. Bocquier2, 1SELMET (Systèmes d’Élevage Méditerranéens et Tropicaux), INRA, Montpellier SupAgro, CIRAD, Univ Montpellier, Montpellier, France, 2SELMET, Montpelier SupAgro, CIRAD, INRA, Univ Montpellier, Montpellier, France, 3Universidad Estadual de Londrina (UEL), Centro de Ciências Agrárias, Londrina, Paraná, Brazil.

Shortage and refeeding situations lead to switches in metabolic pathways induced by undernutrition and body reserves (BR) replenishment cycles. In a 122-d experiment, we studied adaptive mechanisms in 36 adult non-pregnant Mérinos d’Arles ewes, selected with similar BW and BCS. Ewes were acclimated to diet ingredients (i.e., wheat straw, pelleted alfalfa and sugar beet pulp) and to the facility environment during 22 d. Then, the “diet challenge” (planes of nutrition; 12 ewes each) was applied during the first 50 d (normally fed, Control; underfed, Under; overfed, Over) and an individual monitoring (twice a week) of BW and energy metabolism was carried out. Then, a “reefeding challenge” was applied during the last 50 d (i.e., diets were adjusted using the same ingredients). The last day, the lipolytic activity was studied with a “β-adrenergic challenge” (4 mmol/ kg BW of isoproterenol) to the same ewes (BCS according to diet; i.e., Normal, Lean and Fat, respectively). The PROC MIXED with repeated measures of SAS package was used for data analyses. The NEFA response at each time after the β-adrenergic challenge was statistically calculated as well as the area under the concentration curve at 5 min. time intervals during 1 h. Anabolic or catabolic responses were accompanied by synchronized metabolic regulations, leading to contrasting metabolic and BR profiles. Average BW and BCS were higher and lower in Over and Under ewes, respectively, which was proportional to BR dynamics (higher and lower BR mobilization in Under and Over ewes, respectively). Higher plasma leptin were consistent with energy load (Over > Control > Under; overfed, Over) and lactose (P = 0.81) did not differ among treatments. Milk percentages of protein differed (P = 0.01) among treatments and percentages of milk solids (P = 0.04) and protein (P = 0.004) decreased linearly with increasing dose of RPG. Neither pre-feeding (P = 0.42) nor post-feeding (P = 0.57) concentrations of glucose differed among treatments; however, post-feeding glucose decreased (P = 0.01) from d 0 through 4. Pre-feeding insulin (P = 0.35) did not differ among treatments, but a post-feeding concave-up quadratic (P = 0.06) response of insulin was detected among treatments. Volume of the CL on d 10 did not differ (P = 0.49) among treatments. Milk urea nitrogen increased linearly (P < 0.001) with dose and pregnancy risk at first AI decreased linearly (P = 0.01) with increasing dose. Concentrations of progesterone increased (P < 0.01) from d 2 to 11 but were unaffected by treatment (P = 0.77). The pattern of progesterone on d 8 fit a 4th-order polynomial curve (R² = 0.97) with all concentrations during the 24-h period differing (P < 0.05) from the last sample concentration. We conclude that the rumen-protected glucose product did not affect progesterone concentrations.

Key Words: glucose, insulin, progesterone

Dose-frequency of prostaglandin F₂α treatment of dairy cows exposed to presynchronization and either 5- or 7-d Ovsynch program durations: Ovolatory, luteolytic, and pregnancy risks. J. S. Stevenson*, J. A. Sauls, L. G. D. Mendonça, and B. E. Voelz, Kansas State University, Manhattan, KS.

We hypothesized that neither duration of the Ovsynch (OVS) program nor dose-frequency of PGF₂α would change the proportion of cows with complete luteolysis and an additional GnRH treatment administered as part of a presynchronization (PRE) program (Double OVs: GnRH–7 d–PGF₂α–3 d–GnRH) would not alter the proportion of anovulatory cows starting OVS compared with a shorter PRE program with 1 GnRH treatment (PG-3-G: PGF₂α–3 d–GnRH). Lactating Holsteins (n = 405) milked 3 times daily were enrolled in a 2 × 2 × 2 factorial design (8 treatments) before first postpartum AI. Treatments were employed to test ovulatory, progesterone, and pregnancy outcomes to 3 main effects: (1) 2 PRE programs (PG-3-G vs. Double OVS) administered 7 d before OVS; (2) 2 OVS program durations (GnRH-1–5 or 7 d–PGF₂α–24 h–PGF₂α–32 h–GnRH-2–16 h–timed AI); and (3) 2 PGF₂α dose-frequency treatments (2 × 25 mg) 24 h apart vs. 1 dose (1 × 50 mg) of PGF₂α. The PRE treatments of PG-3-G and Double OVS had no effect on the proportion of cows with luteal function (progesterone [P4] > 1 ng/mL) at the onset of the OVS treatments (87.9 vs. 86.2%), respectively. Although ovulation risk was similar after GnRH-1 (65.8 vs. 60.2%), Double OVS cows had greater (P < 0.05) ovulatory responses than PG-3-G after GnRH-2 (95.6 vs. 90%), respectively. Two 25-mg doses of PGF₂α and 1 × 50-mg dose induced complete luteolysis (P4 < 0.4 ng/mL at 72 h) in both OVS durations, but the 1 × 50-mg dose was less (P < 0.05) effective in the 5-d program. More pregnancy per AI (P/AI; 49.2%) tended (P = 0.07) to occur in the PG-3-G cows in 7-d program.
compared with other treatment combinations (range: 32.4 to 37.4%: OVS × presynch). An OVS × PGF_{2α} dose-frequency interaction (P = 0.08) resulted in the 1 × 50-mg dosed cows in the 7-d program with the greatest (46.1%) P/AI and the least P/AI (30.6%) occurred in 1 × 50-mg dosed cows in the 5-d program. Complete luteolysis occurred less often in the 5-d program after the 1 × 50-mg dose, but both PGF_{2α} dose-frequency effectively induced luteolysis in the 7-d program and luteolysis was related to subsequent P/AI.

Key Words: luteolysis, ovsynch duration, PGF_{2α}, dose-frequency

M186 Relationship between air and vaginal temperatures in wild type and slick-haired Puerto Rican Holstein cows. H. L. Sánchez-Rodríguez and K. Domenech-Pérez, University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico.

Previous studies in cattle have reported a continuous significant increase in body temperature with air temperatures (AT) at or above 25–26°C. However, these studies were performed under environmental conditions that considerably differ from Puerto Rico’s tropical weather. The present study evaluated the response of lactating pregnant wild type (WT; n = 10; 2.0 ± 0.01 lactations; 161.4 ± 0.30 DIM; 18.8 ± 1.14 kg/d of milk) and slick-haired (SLICK; n = 10; 2.0 ± 0.01 lactations; 182.4 ± 0.30 DIM; 18.8 ± 1.14 kg/d of milk) Puerto Rican Holstein cows vaginal temperature (VT) to the islands AT values during August 2015. Cows were milked 2x/d (0200–0300 and 1400–1500 h; udders were washed before milking) and grazed the rest of the day in a paddock with natural shade available. The VT and AT were recorded every 5 min for 7 d. Data were averaged by hour for hair coat types’ VT comparisons (Proc GLIMMIX, SAS). To evaluate the AT and VT relationship (Proc GLM and REG, SAS), data were averaged to obtain one value by hair coat group every 5 min. The AT values ranged from 20.97 to 34.05°C. Daily VT values were greater for WT than SLICK cows (39.20 ± 0.04 and 38.77 ± 0.03°C, respectively; P < 0.0001). For WT cows, the relationship between AT and VT was best described by the quadratic curve: VT = −0.0063 AT^2 + 0.6026 AT + 30.162 (R^2 = 0.44; P < 0.0001). For SLICK cows the respective association was also best explained by a quadratic relationship where VT = −0.0063 AT^2 + 0.4061 AT + 32.442 (R^2 = 0.45; P < 0.0001). In general, these quadratic trends can be divided into 2 linear segments. The VT values linearly increased in both, WT (0.10°C per 1°C of AT; P < 0.0001; R^2 = 0.42) and SLICK cows (0.08°C per 1°C of AT; P < 0.0001; R^2 = 0.43) until the AT reached 30.5°C. After this critical value, VT was no longer affected by AT, for neither WT (P = 0.0602; R^2 = 0.006) nor SLICK cows (P = 0.6536; R^2 = 0.0003). After considerably higher AT values than those previously described as critical for thermoregulation in the literature, no further relationship was observed between the AT and VT, suggesting considerable adaptation to tropical weather.

Key Words: slick-haired cows, vaginal temperature, thermotolerance threshold

M187 Sodium propionate and sodium butyrate effects on histone deacetylase (HDAC) activity, histone H3 acetylation, and inflammatory gene expression in bovine mammary epithelial cells. L. Galoro da Silva*, B. Ferguson2, A. S. Avila3, and A. Faciolla1, 1University of Florida, Gainesville, FL, 2University of Nevada, Reno, NV, 3Universidade Estadual do Oeste do Parana, Marechal Candido Rondon, PR, Brazil.

Inflammation of the mammary gland is the most costly disease affecting the US dairy sector resulting in costs around $2 billion/year. Histone deacetylation (HDAC) inhibition has anti-inflammatory properties in rodents, and short-chain fatty acids (SCFA) are effective in inhibiting HDACs. We hypothesized that SCFA would inhibit inflammation via HDAC-dependent regulation of gene expression. We aimed to evaluate sodium propionate (SP) and sodium butyrate (SB) effects on HDAC activity, histone H3 acetylation, and inflammatory gene expression. Bovine mammary epithelial (MAC-T) cells were used as the lipopolysaccharide (LPS)-induced inflammatory model. Cells were cultured in basal medium and cell lysates were incubated with increasing doses of SP or SB (0 to 5 mM) for 2 h before incubation with HDAC substrates (2 h). HDAC activity was determined by fluorescence detection. Cells were also pretreated with SP or SB (0 to 3 mM) for 2 h, stimulated with LPS (1 µg/mL) for 2 h, and assessed for histone H3 acetylation by immunohotting. Next, cells were pretreated with SP or SB (1 mM) for 24 h, stimulated with LPS (1 µg/mL) for 2 h, and RNA was isolated. PCR array was used to examine the expression of 83 inflammatory genes and quantitative real-time PCR was used for gene validation. One-way ANOVA with Tukey post hoc analysis was used to assess significance (P ≤ 0.05). SP and SB dose-dependently and selectively inhibited class I HDAC activity, which differed between the SCFAs, where SB inhibited HDACs 2, 3, and 8, while SP inhibited HDACs 2 and 8. SP and SB dose-dependently increased histone H3 acetylation, differing between the SCFAs, where SB increased H3K9/14, H3K18 and H3K27 acetylation, while SP increased H3K9/14 and H3K18 acetylation. SCFAs increased the overall inflammatory gene expression in MAC-T cells. Under our experimental conditions, the findings suggest that SCFAs regulate epigenetic marks on nucleosomal DNA in addition to regulation of inflammatory gene events independent of HDAC activity in MAC-T cells.

Key Words: histone deacetylase inhibitor, inflammation, short-chain fatty acid

M188 Contribution of hormone-sensitive lipase to adipose tissue lipolysis and its regulation by insulin in periparturient dairy cows. J. De Koster1, R. Nelli1, C. Strieder-Barboza1, J. de Souza2, A. L. Lock2, and G. A. Contreras1*, 1Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI, 2Department of Animal Science, Michigan State University, East Lansing, MI.

The aim of this study was to determine how hormone-sensitive lipase (HSL) contributes to adipose lipolysis and how insulin regulates lipolysis in periparturient dairy cows. Subcutaneous adipose tissue (SCAT) samples were taken from multiparous dairy cows (n = 22) at 10 d prepartum (dry) and 10 d (fresh) and 24 d (lactation) postpartum. Adipose lipolysis was determined using a short-term in vitro explant culture (3 h). Basal lipolysis was determined without addition of reagents. Stimulated lipolysis was determined by β-adrenergic stimulation with isoproterenol (ISO, 10^-6 M). The inhibitory effect of insulin (1 µg/L) was determined on stimulated lipolysis. HSL contribution to basal lipolysis was determined by adding an HSL inhibitor, CAY10499 (CAY, 2 µM). Statistical analyses were performed in R using a mixed effect linear model. Basal lipolysis was higher in SCAT explants from dry cows (1.295 ± 0.12 nmol glycerol/10^6 adipocytes) compared with fresh cows (0.61 ± 0.14 nmol glycerol/10^6 adipocytes; P < 0.05). Inhibition of basal lipolysis by CAY was negligible in dry cows (−3.54 ± 0.05% of basal glycerol release), while in fresh and early lactation cows, CAY inhibited basal lipolysis by 36.05 ± 4.51% and 43.05 ± 4.83%, respectively (P < 0.05). ISO stimulated lipolysis in SCAT explants was not different across periods (P > 0.1). Inhibition of stimulated lipolysis by insulin was more pronounced in the dry period (−23.23 ± 3.45%) compared with the fresh
period (−9.64 ± 3.24, *P* < 0.05). Explants with larger adipocytes had higher basal lipolysis (*P* < 0.001) while adipocyte size did not influence lipolysis in explants cultured with CAY, ISO or insulin (*P* > 0.1). Our results demonstrate that the contribution of HSL to basal lipolysis is negligible in the dry period; however, HSL is the major driver of lipolytic responses in SCAT postpartum. Lower basal lipolysis in early lactation suggests that reduced lipogenesis is an important contributor to early lactation fatty acid release from SCAT. Loss of adipocyte sensitivity to the anti-lipolytic action of insulin develops in the early lactation period, which is suggestive for insulin resistance of the lipolytic activity.

**Key Words:** lipolysis, adipose tissue, insulin sensitivity


During periparturient metabolic stress, excessive adipose lipolysis is a strong predisposing factor for postpartum complications. Linoleic acid is the most abundant polyunsaturated fatty acid (FA) in adipose tissue (AT) of dairy cows and is preferentially released during lipolysis. In the adipocyte, oxidized linoleic acid metabolites (OXLAM) are products of enzymatic and non-enzymatic pathways. Among OXLAM, 13-hydroxyoctadecadienoic acid (HODE) was identified as a lipolysis product, but its role in modulating lipid mobilization in AT is unclear. We hypothesize that 13-HODE reduces lipolytic response to adrenergic stimulation and enhances FA uptake in adipocytes. Subcutaneous AT explants collected from dairy cows (*n* = 7) at 10 ± 2 d postpartum and cultured bovine adipocytes (*n* = 4) were incubated with physiological concentrations of 13-HODE: 0 nM (CO), 50 nM (HL), or 200 nM (HH) for 4 h. To stimulate lipolysis, explants were then exposed to the β adrenergic agonist isoproterenol (BAS 0M; ISO, 10−6 M) for 3 h. Lipolytic responses were evaluated by measuring glycerol release. To evaluate the effect of 13-HODE on lipogenic capacity, adipocytes were treated with insulin (5 μg/mL, 1 h) and the rate of FA uptake was measured with the QBT FA uptake assay. The statistical model included the random effect of animal and the fixed effect of treatment, insulin or ISO stimulation and their interaction. Exposure of AT explants to HL and HH for 4 h did not induce a lipolytic response compared with CO (*P* = 0.62). Glycerol release upon adrenergic stimulation was reduced in HL+ISO and LL+ISO compared with CO+ISO (*P* < 0.05). Incubation with 13-HODE did not affect FA uptake in cultured adipocytes (*P* = 0.43). β-adrenergic agonist stimulation enhanced gene expression of 13-HODE targets GPR132 and testicular orphan nuclear receptor 4 (TR4), key regulators of lipid trafficking in adipocytes. These data suggest that 13-HODE is a modulator of lipolysis but not lipogenesis in AT. Future studies will evaluate the mechanisms by which 13-HODE signals to the AT to modulate lipolysis.

**Key Words:** osteopontin, adipose tissue macrophages, periparturient period


Lipolysis triggered adipose tissue remodeling (AT) in periparturient cows is characterized in part by macrophage infiltration and aggregation. In cows with metabolic diseases, characterized by intense and protracted lipolysis, macrophage accumulation in AT is exacerbated and may promote tissue dysfunction. In rodent models, lipolysis triggers macrophage recruitment coupled with increased expression of SPP1, the gene that encodes osteopontin. This chemotactic cytokine recruits cells expressing CD44 glycoprotein that include preadipocytes and macrophages. Our study evaluated the transcription of SPP1, CD44, and additional gene networks related to macrophage infiltration and AT remodeling using qPCR. Subcutaneous AT samples were collected from multiparous dairy cows (*n* = 21) at 10 d prepartum (dry), 10 d (fresh), and 24 d (lactation) postpartum. Data were analyzed in R using lognormal distributions and pairwise comparisons. SPP1 expression was higher in fresh compared with dry (*P* < 0.05). A positive relationship in SPP1 expression with large adipocytes (*P* < 0.05) suggested that cows with greater adiposity, those that are more prone to disease, recruit more macrophages. There was an increase in CD44 gene expression in lactation when compared with fresh (*P* < 0.01). This finding indicates that CD44, a receptor of SPP1, increases when lipolysis and SPP1 are higher. Expression of SIRPA, a mononuclear immune cell marker, was higher in dry compared with fresh and lactation (*P* < 0.01). Reduction of SIRPA in fresh and lactation indicates an increase in macrophage phagocytic activity. IL10 had a positive relationship with adipocyte volume in both fresh and lactation (*P* < 0.05). In human models, IL10 is responsible for the induction of osteopontin in a time- and dose-dependent manner. Upregulation of SPP1 alongside increases in IL10 indicate an association between osteopontin and AT macrophage recruitment in periparturient cows. Future studies will evaluate the potential of osteopontin as a biomarker for macrophage infiltration into AT.

**Key Words:** osteopontin, adipose tissue macrophages, periparturient period

M192  The adipocyte marker *FABP4* is most prominently induced by combined supplementation of ascorbic acid and bovine serum lipids in cultured bovine adipocytes. S. Jurek*1, M. A. Sandhu2, M. Kolisek3, G. Sponder1, and J. R. Aschenbach1.

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*FABP4* is a marker for adipocyte differentiation. It is induced during in vitro transdifferentiation of bovine pre-adipocytes to adipocytes by the presence of bovine serum lipids (BSL) in the absence of fetal bovine serum (FBS). The intention of this study was to test whether ascorbic acid (AA) has an influence on transdifferentiation and *FABP4* expression. Subcutaneous adipose tissue was collected from calves. After induction of differentiation, adipocytes were incubated with or without AA (40 μL/mL), BSL (10 μL/mL) and/or FBS (10%) for 14d. The accumulation of non-polar lipids was evaluated by Nile-red fluorescence and normalized to DAPI fluorescence. Stem cell markers and *FABP4* were investigated by quantitative RT-PCR and immunohistochemistry. Statistics was conducted by 2-way ANOVA. Results: The development of lipid droplets was promoted (*P* < 0.001) by BSL in absence of FBS. The mRNA expression of *ENG* was reduced in all treatments compared with pre-adipocyte values, with lowest values in BBS-free media (*P* < 0.05). *FABP4* expression was highest in BSL and lowest in BBS-free media compared with pre-adipocyte values and most BBS-treated groups (*P* < 0.05). In the presence of AA, the mRNA expression of the adipocyte marker *FABP4* increased 208, 30 and 144 times in media containing BSL, BBS and BSL+FBS, respectively (*P* < 0.05). In the absence of AA, only BSL increased the mRNA expression of *FABP4* (75 times) above pre-adipocyte values (*P* < 0.05). The differential mRNA expression of
M193  Effects of fully acidified close-up diets and dietary calcium content on in vitro innate immune function in transition dairy cows. X. Zhang1, K. M. Glosson*2, S. S. Bascom3, A. D. Rowson3, and J. K. Drackley2,1Institute of Animal Nutrition, Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Sichuan Agricultural University, Chengdu, Sichuan, China, 2University of Illinois, Department of Animal Sciences, Urbana, IL, 3Phibro Animal Health Corp., Teaneck, NJ.

A negative dietary cation-anion difference (DCAD) dry cow diet has been used to reduce the risk of clinical and subclinical hypocalcemia (SCH). Innate immune system functions, phagocytosis (P) and oxidative burst (OB) activities of neutrophils (N) and monocytes (M), are believed to be influenced by Ca, which may contribute to immune dysfunction in freshening cows with SCH. The objective of this study was to determine the effects of 3 close-up dry cow dietary strategies on the innate immune system of multiparous Holstein dairy cows (n = 81) during the transition period, blocked by parity and calving date. Cows were assigned to 1 of 3 treatments during the close-up period, blocked by parity and calving date. Cows were assigned to 1 of 3 treatments during the close-up dry period (−28 d to calving): 1) a positive DCAD diet with low dietary Ca (0.4% DM; CON); 2) a negative DCAD diet with low dietary Ca (0.4% DM; LOW); or 3) a negative DCAD diet with high dietary Ca (2.0% DM; HIGH). Urine was sampled for 3 consecutive days at the start of the close-up period and then every second day until calving to monitor urine pH. Urine samples at −21, −14, −7, +1, +2, and +7 d relative to calving were analyzed for mineral and creatinine concentrations. Urine volume, calculated using creatinine and BW, was estimated for mineral excretion. The MIXED procedure in SAS was used to contrast: 1) CON vs the average of LOW and HIGH; and 2) LOW vs HIGH, with a fixed effect of treatment, random effect of block, and sample as the repeated variable. Prepartum urine pH for cows fed LOW or HIGH averaged 5.7 and cows fed CON remained above 8.0. The excretion of Ca in urine prepartum was greater in cows receiving LOW or HIGH (8.4 and 13.4 g/d) than in cows receiving CON (1.0 g/d; P < 0.01). Cows fed the HIGH diet had greater urinary Ca excretion than those given LOW (P < 0.01). After calving there was no statistical difference in urine volume or urinary Ca concentration averaged over the postpartum samples, but Ca excretion remained greater in cows receiving LOW or HIGH (0.68 and 0.42 g/d) when compared with cows fed CON (0.19 g/d; P = 0.04). In conclusion, cows given LOW and HIGH successfully created a Ca sink in the prepartum period to increase the requirement of Ca before calving. Higher dietary Ca did not affect urinary pH but increased urinary Ca excretion.

Key Words: adipoocytes, FABP4

M194  Effects of fully acidified close-up diets and dietary Ca content on urinary mineral excretion in transition dairy cows. K. M. Glosson*1, X. Zhang2, S. S. Bascom3, A. D. Rowson3, and J. K. Drackley1, 1University of Illinois, Department of Animal Sciences, Urbana, IL, 2Institute of Animal Nutrition, Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Sichuan Agricultural University, Chengdu, Sichuan, China, 3Phibro Animal Health Corp., Teaneck, NJ.

A negative dietary cation-anion difference (DCAD) dry cow diet strategy has been used to reduce the risk of clinical and subclinical hypocalcemia. The acidogenic diet creates compensated metabolic acidosis in late gestating cows, monitored through urinary pH. The objective of this study was to determine the effects of 3 dietary strategies for close-up dry cows on mineral excretion of multiparous Holstein dairy cows (n = 81) through the transition period. Cows were assigned to 1 of 3 treatments during the close-up dry period (−28 d to calving): 1) a positive DCAD diet with low dietary Ca (0.4% DM; CON); 2) a negative DCAD diet with low dietary Ca (0.4% DM; LOW); or 3) a negative DCAD diet with high dietary Ca (2.0% DM; HIGH). Urine was sampled for 3 consecutive days at the start of the close-up period and then every second day until calving to monitor urine pH. Urine samples at −21, −14, −7, +1, +2, and +7 d relative to calving were analyzed for mineral and creatinine concentrations. Urine volume, calculated using creatinine and BW, was estimated for mineral excretion. The MIXED procedure in SAS was used to contrast: 1) CON vs the average of LOW and HIGH; and 2) LOW vs HIGH, with a fixed effect of treatment, random effect of block, and sample as the repeated variable. Prepartum urine pH for cows fed LOW or HIGH averaged 5.7 and cows fed CON remained above 8.0. The excretion of Ca in urine prepartum was greater in cows receiving LOW or HIGH (8.4 and 13.4 g/d) than in cows receiving CON (1.0 g/d; P < 0.01). Cows fed the HIGH diet had greater urinary Ca excretion than those given LOW (P < 0.01). After calving there was no statistical difference in urine volume or urinary Ca concentration averaged over the postpartum samples, but Ca excretion remained greater in cows receiving LOW or HIGH (0.68 and 0.42 g/d) when compared with cows fed CON (0.19 g/d; P = 0.04). In conclusion, cows given LOW and HIGH successfully created a Ca sink in the prepartum period to increase the requirement of Ca before calving. Higher dietary Ca did not affect urinary pH but increased urinary Ca excretion.

Key Words: adipoocytes, FABP4

M195  Impacts of reducing urine pH prepartum by altering dietary cation-anion difference on physiological and productive responses of Holstein × Gir dairy cows. R. O. Rodrigues1, R. F. Cooke2, S. M. B. Rodrigues1, L. N. Bastos1, V. F. S. Camargo1, K. G. S. Gomes1, and J. L. M. Vasconcelos*1, 1São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu/SP, Brazil, 2Department of Animal Science, Texas A&M University, College Station, TX.

This study compared physiological and productive parameters in 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate containing traditional anionic salts to reduce urine pH to 7.0 (CON; n = 17), or an anionic supplement (Animate, Phibro Animal Health, Teaneck, NJ) to reduce urine pH to 6.0 (SUPP; n = 17). Nonlactating, multiparous, pregnant cows were ranked by parity, body weight (BW), and body condition score (BCS), and assigned to receive SUPP or CON for 21 d before expected calving. Cows were maintained in single drylot pens with ad libitum access to corn silage, and individually received their prepartum concentrate once daily before calving. Cows from both treatments completely consumed their concentrate allocation within 30 min after feeding. Cow BW and BC were recorded weekly, urine pH measured every 3 d, and blood samples collected on d −21, −14, −9, −6, and −3 relative to expected calving. Cows were moved to an adjacent drylot pen with ad libitum access to water and a total-mixed ration after calving (d 0), and were milked twice daily. Cow BW and...
BCS were recorded weekly, and daily milk production recorded until 30 d in milk (DIM). Blood samples were collected before each milking during the initial 5 DIM, and at 6, 9, 16, 23, and 30 DIM before the morning milking. Based on actual calving dates, cows receiving SUPP or CON for (mean ± standard error) 19.2 ± 1.2 and 19.0 ± 0.9 d, respectively. Urine pH was less (P < 0.01) in SUPP vs. CON cows during the last 15 d of gestation (6.12 vs. 7.15, respectively). Milk yield during the experimental period was greater (P = 0.04) in SUPP vs. CON cows (by 14%). Serum concentrations of fatty acids were greater (P ≤ 0.01) in SUPP vs. CON cows 3 d before and at calving (by 52 and 22%, respectively), whereas SUPP cows had less (P ≤ 0.03) serum glucose and cortisol concentration at calving (by 23 and 27%, respectively). Hence, SUPP decreased prepartum urine pH to 6.0 in Holstein × Gir dairy cows without depressing concentrate intake, and increased milk yield compared with CON.

Key Words: anionic supplementation, dairy cow, prepartum


Blood total calcium in the adult cow is closely regulated. Subclinical hypocalcemia (SCHCa) were in this study defined as blood Ca concentration below 2.2 mmol/L (8.9 mg/dl). The prevalence of subclinical hypocalcemia in dairy cows at dry off is unknown. The objective of this study was to determine the prevalence of subclinical hypocalcemia at drying off in dairy herds in France and Denmark. Additionally, to identify risks factors related to the drying-off management procedures leading to the emergence of hypocalcemia at this stage. This is a multicenter prospective cohort study including 381 dairy cows from 37 herds in France and 345 cows from 21 herds in Denmark. Biochemical parameters were assessed at 2 blood samplings; one in mean (SD) 0.9 (0.7) h (BS1) after the last milking and another in mean (SD) 9.2 (1.3) h later (BS2). Data on feeding and management practices at cow level were collected. Specifically, the interaction between dry-off method and incidence of SCHCa at BS2 was assessed using descriptive statistics and ANOVA. A gradual dry off implied no prior change in either milking or feeding; 180 and 154 cows were dried off abruptly in France and Denmark respectively. Gradual dry off implied any change in these practices; 201 and 175 cows were dried off gradually in France and Denmark respectively. Calcium concentrations at BS1 and BS2 and relative variation between BS1 and BS2 are shown in Table 1. Cows dried off gradually in France had an incidence of SCHCa at BS2 at 17%, in Denmark 21.8%. Cows dried off abruptly had a lower incidence of SCHCa at BS2 in France 5.5% and in Denmark 2.7%. Relative risk (RR) for SCHCa at BS2 is 3.85 (CI95% 2.18:6.78) (P < 0.0001) for cows dried off gradually compared with abruptly. Reduction of blood Ca concentrations after dry-off occurs with varying incidence across countries. A gradual dry off increases the likelihood that cows will have subsequent subclinical hypocalcemia.

Table 1 (Abstr. M196). Mean [minimum; maximum] calcium concentration (mmol/L)

<table>
<thead>
<tr>
<th>Country</th>
<th>BS1</th>
<th>BS2</th>
<th>BS1 − BS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>2.4</td>
<td>2.3</td>
<td>−1.7%</td>
</tr>
<tr>
<td></td>
<td>[1.6; 2.9]</td>
<td>[1.6; 2.7]</td>
<td>[−33.3; 31.3]</td>
</tr>
<tr>
<td>Denmark</td>
<td>2.4</td>
<td>2.3</td>
<td>−4.0%</td>
</tr>
<tr>
<td></td>
<td>[1.9; 3.2]</td>
<td>[1.5; 3.1]</td>
<td>[−37.5; 13.6]</td>
</tr>
</tbody>
</table>

Key Words: dry off, subclinical, hypocalcemia

M197  Hepatic gluconeogenesis is differentially altered by choline and methionine in bovine primary hepatocytes.  T. L. Chandler*, S. J. Bertics1, B. A. Barton2, and H. M. White1, 1University of Wisconsin-Madison, Madison, WI, 2Balchem Corp., New Hampton, NY.

Coordinated expression patterns of genes controlling gluconeogenesis in primary hepatocytes suggested increased capacity for gluconeogenesis with increasing choline, but not methionine. The objective of this experiment was to quantify glucose export and cellular glycogen in primary bovine hepatocytes exposed to increasing concentrations of choline chloride (CC), d,l-Met (DLM), and added fatty acids (FA). Primary hepatocytes isolated from 3 Holstein calves were maintained as monolayer cultures for 24 h before treatment with CC (61, 128, 2028, 4528 μM) and DLM (16, 30, 100, 300 μM), with or without a 1 mM FA cocktail in a 4 × 2 factorial design. Treatments were applied in triplicate to basal medium with 5.5 mM glucose and 1.25 mM sodium pyruvate. After 24 h, medium was collected to quantify glucose, and cells were harvested to isolate glycogen and quantify DNA. Total glucose, comprised of medium glucose and glucose exported from cells, and total cellular glycogen were normalized to corresponding total DNA to account for differences in cell plating density between calves, before averaging the normalized values within triplicates. Data were expressed relative to the lowest CC and DLM treatment without FA within each cell prep before data were analyzed using PROC MIXED of SAS 9.4 with linear and quadratic contrasts in a model with fixed effect of treatment, corresponding interactions, and random effect of calf. Interactions were not significant; therefore, only main effects are discussed. Fatty acid treatment did not affect medium glucose (P = 0.98) or cellular glycogen (P = 0.29). Increasing CC linearly decreased (P = 0.0019) relative glucose in medium by up to 13.5%, and linearly (P > 0.0001) and quadratically (P < 0.0001) increased relative cellular glycogen by up to 26.7%. Medium glucose (P = 0.86) and cellular glycogen (P = 0.69) were not affected by DLM. Increased cellular glycogen with increasing CC, and a lack of change in medium glucose or cellular glycogen with increasing DLM, supports previous gene expression data. Differential effects of CC on cellular glucose export and cellular glycogen should continue to be investigated.

Key Words: glucose, glycogen, fatty acid


Choline and methionine can influence carbon metabolism in bovine hepatocytes. Pyruvate carbon was traced through pathways of gluconeogenesis to elucidate the ability of choline or methionine to alter carbon flux. Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h. At 24 h glucose-free medium was applied and treatments of choline chloride (CC; 0, 0.01, 0.1, or 1.0 mM) and d,l-Met (DLM; 0, 0.1, or 0.3 mM) were added in a factorial design along with 1.0 mM FA, reflecting the blood FA profile at calving. After 21 h, 1.25 mM [2-14C]pyruvate was added to medium and CO2 collected after a 3-h incubation. Cells were harvested to quantify glycogen and 14C enrichment. Parallel treatments were incubated without radiolabeled substrate for 24 h to quantify medium glucose. Data were normalized to DNA, expressed relative to a no FA control within each cell prep, and analyzed by PROC MIXED (SAS 9.4) with fixed effects of CC, DLM, their interaction, and random effect of calf. Contrasts evaluated for CC were 0 mM vs. (0.01, 0.1, 1.0 mM) and linear contrast
Methionine supply in vitro alters cell proliferation, metabolism, and production of reactive oxygen species in ruminal microglial cells undergoing oxidative stress. I. Martínez-Cortés*, J. Stanton2, J. Muñoz-Gutiérrez3, and J. J. Loor1, 1University of Illinois, Urbana, IL, 2University of Georgia, Athens, GA, 3University of Wyoming, Laramie, WY.

Microglia are monocytes (MC) that function as resident macrophages in the central nervous system (CNS). The MC are the most important immune defense in the CNS, responding rapidly to infection in part by releasing pro-inflammatory cytokines and reactive oxygen and nitrogen species (ROS, RNS). Methionine (Met) is the first-limiting amino acid for milk protein synthesis and also serves as substrate for the anti-inflammatory response (IL-10). However, expression of genes associated with glutathione synthesis and peroxidase activity were upregulated (GPX1 and GSR) in the MET cows. That idea is supported by the upregulation of lactate dehydrogenase in response to feeding MET suggesting increased metabolic activity, which agrees with the greater overall phagocytosis and oxidative burst in those cows. It indicates that compared with MC not pretreated with Met, 10 or 20 μM Met prevented an increase in IL-6 even after H2O2 challenge. Similar to feeding MET suggesting increased metabolic activity, which agrees with the greater overall phagocytosis and oxidative burst in those cows. Nevertheless, expression of genes associated with glutathione synthesis and peroxidase activity were upregulated (GPX1 and GSR) in the MET cows. Overall, the data suggest that enhanced supply of methionine improves immune function of PMNL at least in part through molecular mechanisms associated with energy metabolism, glutathione metabolism, and inflammatory responses.

Key Words: neutrophil function, phagocytosis, oxidative burst

M200  Methionine supply during the periparturient period alters transcriptome profiles and enhances function of polymorphonuclear leukocytes in Holstein cows. H. Dai*1,2, F. Batistel2, R. R. C. Yambao2, A. A. Elolimy2, C. I. M. Garces2, J. M. Arroyo3, C. Parys4, X. Shen5, and J. J. Loor2, 1Nanjing Agricultural University, Nanjing, China, 2University of Illinois, Urbana, IL, 3University of the Republica, San José, Uruguay, 4Evonik Nutrition & Care GmbH, HanauWolfgang, Germany.

Polymorphonuclear leukocytes (PMNL) are key cellular components of the immune system and represent the first line of defense against pathogens or other stressors, especially during the periparturient period. The objective was to examine the role of methionine supply during the transition period on PMNL function and mRNA abundance of genes associated with inflammation, oxidative stress, and metabolism. Multiparous Holstein cows were used in a block design and assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH, Germany). Metpron was fed from −28 to 30 d relative to parturition at a rate of 0.09% and 0.10% of the dry matter intake during the prepartum and postpartum period, respectively. Rate ensured a ratio of lys to Met in the metabolizable protein close to 2.8:1. Blood for PMNL extraction and in vitro oxidative burst and phagocytosis was collected at −10, +10, and +21 d relative to parturition. Data were analyzed by using the mixed procedure of SAS considering block as random effect and treatment, time and its interaction as fixed effect. Although the glucose transporter SLC2A1 did not differ, there was an overall upregulation of lactate dehydrogenase in response to feeding MET suggesting increased metabolic activity, which agrees with the greater overall phagocytosis and oxidative burst in those cows. That idea is supported by the upregulation of various components of the Toll-like receptor cascade and both the pro-inflammatory (TLR2, TLR4, NFKB1, and IL1B), and anti-inflammatory response (IL10). Despite greater overall plasma concentration of myeloperoxidase, a microbical enzyme, in MET cows, no differences were detected for genes associated with oxidative stress response (MPO, NOX1, SOD1, SOD1, and NOS2). However, expression of genes associated with glutathione synthesis and peroxidase activity were upregulated (GPX1 and GSR) in the MET cows. Overall, the data suggest that enhanced supply of methionine improves immune function of PMNL at least in part through molecular mechanisms associated with energy metabolism, glutathione metabolism, and inflammatory responses.

Key Words: central nervous system, inflammation, amino acid metabolism, and oxidative stress.