Metabolic stress occurs in dairy cows when physiologic homeostasis is disrupted as a consequence of aberrant nutrient metabolism, chronic inflammation, and oxidative stress. Early lactation cows that suffer from metabolic stress are susceptible to health disorders that cause significant production losses. There is little information, however, regarding the occurrence and impact of metabolic stress during involution. The purpose of this study was to investigate well-known metabolic stress biomarkers at various time points during the early dry period when dairy cows are subjected to dramatic changes in physiologic homeostasis. A descriptive study was performed by collecting blood samples from the coccygeal vein of 27 healthy dairy cows at a commercial dairy herd. Sampling points were d −6, d 0, d +1, d +2, d +6, and d +12 relative to dry-off date. Serum samples were utilized to quantify biomarkers related to metabolic stress, including calcium, cholesterol, nonesterified fatty acids, β-hydroxybutyrate, albumin, haptoglobin, cortisol, reactive oxygen species, antioxidant potential, oxidative stress index, and isoprostanes. Data were checked for normality in SAS and analyzed by one-way ANOVA with Tukey’s adjustment for multiple comparisons. Within altered nutrient metabolism biomarkers, calcium and NEFA concentrations changed most from d 0 to d +2 during the sampling period. Haptoglobin is a biomarker of inflammation that gradually increased during involution with highest concentrations observed at d +12. In contrast, concentrations of albumin, a negative acute phase protein, were significantly lower at d +12 compared with other sampling points. All indicators of oxidative stress generally increased throughout most of the sampling period, suggesting altered oxidative stress states throughout early involution. Therefore, many of the biomarkers associated with metabolic stress changed during the transition from late lactation to the early dry period, but not to the same magnitude and duration reported previously in periparturient cows. Future studies should be directed toward assessment of other metabolic stress biomarkers and their effect on dairy cattle health and well-being during involution.

Key Words: metabolic stress, involution, inflammation

Genome-wide variation for visceral fat deposition in Holstein dairy cows. P. Melendez1, S. Poock1, P. Pinedo2, D. Manriquez3, S. Moore2, M. Lucy3, P. Pithua1, J. Neal1, and J. Taylor3,

The objective of this study was to assess genetic variation in visceral adiposity in US Holstein cows. The study included adult Holstein cows sampled from a slaughterhouse (Green Bay, WI) during September 2016. Only animals with a body condition score (BCS) between 2.75 and 3.25 were considered. The extent of omental fat at the level of the insertion of the lesser omentum over the pylorus area was assessed. A group of 100 Holstein cows with an omental fold <5 mm in thickness, and a second group of 100 cows with an omental fold ≥20 mm in thickness were sampled. A piece of muscle from the neck was collected from each cow into a sterile container for DNA extraction. Samples were submitted to a commercial laboratory for interrogation of genome-wide genomic variation using the Illumina BovineHD BeadChip. A genome-wide association analysis was performed to test potential associations between fat deposition and genomic variation. A univariate mixed linear model analysis was performed using GEMMA to identify SNP significantly associated with variation in visceral fat deposition. The chip heritability was 0.686 and the estimated additive genetic and residual variance components were 0.427 and 0.074, respectively. Eleven SNP defining 4 QTL regions were found to be significantly associated with visceral fat deposition ($P < 0.00001$). Two of the QTL were detected with 4 and 5 significantly associated SNP, respectively, while the QTL detected on BTA12 and BTA19 were each detected with only 1 significantly associated SNP. No enriched gene ontology terms were found within the gene networks harboring these genes when supplied to DAVID using either the Bos taurus or human gene ontology databases. We conclude that excessive omental fat in Holstein cows with similar BCS is not caused by a single Mendelian locus and that the trait appears to be at least moderately heritable; consequently, selection to reduce excessive omental fat is possible but would require the generation of predicted transmitting abilities from larger and random samples of Holstein cattle.

Key Words: visceral fat, subcutaneous fat, genome-wide association study (GWAS)

Acetoacetate induces hepatocytes apoptosis by the reactive oxygen species (ROS)-mediated MAPKs pathway in ketotic cows. X. Du, G. Liu, and X. Li*, College of Veterinary Medicine, Jilin University, Changchun, Jilin, China.

The objective of this study was to investigate hepatic oxidative stress and the apoptotic status of ketotic cows, and the mechanism of acetoacetate (AcAc)-induced hepatic oxidative damage in ketotic cows. Liver and blood samples were collected from healthy and clinically ketotic cows. Hepatocytes were isolated from calves and treated with 0.3, 1.2, 2.4 or 4.8 mM AcAc in presence or absence of antioxidant N-acetylcysteine (NAC), JNK inhibitor SP600125, p38MAPK inhibitor SB203580, and ERK activator SC3527, respectively. Compared with healthy cows, ketotic cows exhibited severe hepatic injury and oxidative stress. The blood concentration of the apoptotic marker CK18 M30 and the number of TUNEL-positive cells in the liver of ketotic cows were 1.19- and 2.61-fold, respectively, higher than the values observed in control cows. Importantly, the levels of phosphorylated c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38MAPK) were significantly increased but the level of phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2) was markedly decreased, which further promoted tumor protein 53 (p53) expression and inhibited nuclear factor E2-related factor 2 (Nrf2) expression. In vitro, AA treatment increased reactive oxygen species (ROS) content and further induced oxidative stress and apoptosis of calf hepatocytes. AA treatment increased the phosphorylation levels of JNK and p38MAPK and decreased the phosphorylation level of ERK, which could increase p53 and inhibit Nrf2 expression, nuclear localization and DNA-binding affinity, thereby inducing the overexpression of pro-apoptotic molecules Bax, Caspase 3, Caspase 9, PARP and inhibition of anti-apoptotic molecule Bel-2. Antioxidant NAC treatment or interference of MAPKs pathway could attenuate the hepatocytes apoptosis induced by AA. Collectively, ketotic cows display severe hepatic oxidative stress and the hepatic MAPK-p53/Nrf2 apoptotic pathway is over induced and partially mediated apoptotic damage.
Besides, AA triggers hepatocytes apoptosis via the ROS-mediated MAPKs pathway in ketotic cows.

Key Words: ketotic cows, acetoacetate, apoptosis

M77 Enhanced hepatic mitochondrial functional in dairy cows with mild fatty liver. Z. Shi, X. Li, G. Liu, and X. Li*, College of Veterinary Medicine, Jilin University, Changchun, Jilin, China.

The inevitable deficiency in nutrients and energy at the onset of lactation requires an optimal adaptation of the hepatic lipid metabolism to overcome metabolic stress. Fatty liver is one of the main health disorders after parturition. The regulation of hepatic lipid metabolism is largely dependent on mitochondria. The aim of this study was to investigate the changes in hepatic mitochondrial function in dairy cows with mild fatty liver. Liver and blood samples were collected from healthy (n = 15) and mild fatty liver (n = 15) cows. The mRNA levels of oxidative phosphorylation complexes (CO I-V) and the protein levels of the mitochondrial function regulators were detected by RT-qPCR and Western blotting, respectively, and analyzed with paired t tests. The number and volume of mitochondria in the liver were detected by transmission electron microscopy. The hepatic ATP content and mitochondrial DNA (mtDNA) were measured using biochemical kits and analyzed with Wilcoxon-signed-rank-test. Healthy cows were defined as less than 1% liver TG on wet weight basis and mild fatty liver cows were defined as 1–5% liver TG on wet weight basis. Compared with healthy cows, ultrastructural analysis revealed a significantly increased number and volume of mitochondria in the liver of mild fatty liver cows. Hepatic mtDNA was also markedly increased in mild fatty liver cows. These results indicate that mitochondrial function is enhanced in the liver of mild fatty liver cows. We further explored the molecular mechanisms involved. The protein levels of mitochondrial function regulators, PGC-1α and NRF1, were significantly increased in the liver of mild fatty liver cows. The mRNA levels of CO I-V regulated by PGC-1α were significantly higher in the liver of mild fatty liver cows than healthy cows. The ATP content was increased, which was consistent with the increase of CO I-V. In summary, our data show that the hepatic mitochondrial function of dairy cows with mild fatty liver is enhanced, and enhanced mitochondrial function may be the adaptive mechanism of dairy cows with mild fatty liver.

Key Words: mitochondrial function, adaptation mechanism, dairy cow with mild fatty liver

M78 Yeast culture alters volatile fatty acids production in a ruminal in vitro fermentation system. S. A. Armstrong*, S. S. Bascom, and D. J. McLean, Phibro Animal Health Corporation, Teaneck, NJ.

The objective of this study was to determine the effect of time of rumen fluid collection relative to feeding on gas production kinetics and volatile fatty acid (VFA) production for in vitro rumen fermentation. Three ruminally cannulated Holstein heifers were rumen fluid donors. Feed was removed from heifers 12 h before feeding, rumen fluid was collected from each heifer before feeding (0 h), and at 2, 4, and 6 h after feeding, repeated on 3 separate incubation days. Buffered rumen fluid (100 mL) was incubated in 250-mL bottles containing 1.4 g of dried TMR, in duplicate for each heifer at each collection time. All bottles were incubated for 24 h at 39°C and constant agitation (60 rpm), and capped with monitors to capture temperature and pressure every 15 min (RF1, Ankom Technology, Macedon, NY). Gas data were fit with nonlinear regression in GraphPad Prism 7 using the formula $y = V_m(1-\left(e^{-K_d(x-lag)}\right))$ where $y$ is gas produced at time X (mmol), $V_m$ is the asymptotic gas production (mmol), $K_d$ indicates the fractional rate of gas production (mmol/h), X is time (h), lag refers to the lag time before the start of fermentation (h). At the end of incubation, final pH and a sample of rumen fluid was collected for VFA and ammonia nitrogen. Data were analyzed using PROC GLIMMIX of SAS, with donor as the experimental unit and day as the random blocking factor; significance defined as $P \leq 0.05$. Time of rumen fluid collection significantly affected gas production kinetics (lag $P = 0.01, V_m P = 0.03, K_d P < 0.0001$), acetate (mmol/L; $P = 0.0004$), propionate (mmol/L; $P = 0.02$), isobutyrate (mmol/L; $P < 0.0001$), valerate (mmol/L; $P = 0.004$), isovalerate (mmol/L; $P < 0.0001$), and total VFA concentrations (mmol/L; $P = 0.004$). All VFA relative proportions were altered due to time of rumen fluid collection ($P < 0.02$). Gas production was highest in fermentor units fed with rumen fluid collected 2 h post-feeding and VFA production was highest when rumen fluid was collected 4 h post-feeding. Our findings suggest gas and VFA production are maximized when rumen fluid is collected between 2 and 4 h post-feeding.

Key Words: in vitro fermentation, rumen gas production, VFA

M79 Time of rumen fluid collection relative to feeding alters in vitro fermentation outcomes. S. A. Armstrong*, L. A. Pless1,2, A. N. Brewster1,2, and D. J. McLean+, 1Phibro Animal Health Corporation, Teaneck, NJ, 2Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR.

The objective of this study was to determine the effect of time of rumen fluid collection relative to feeding on gas production kinetics and volatile fatty acid (VFA) production for in vitro rumen fermentation. Three ruminally cannulated Holstein heifers were rumen fluid donors. Feed was removed from heifers 12 h before feeding, rumen fluid was collected from each heifer before feeding (0 h), and at 2, 4, and 6 h after feeding, repeated on 3 separate incubation days. Buffered rumen fluid (100 mL) was incubated in 250-mL bottles containing 1.4 g of dried TMR, in duplicate for each heifer at each collection time. All bottles were incubated for 24 h at 39°C and constant agitation (60 rpm), and capped with monitors to capture temperature and pressure every 15 min (RF1, Ankom Technology, Macedon, NY). Gas data were fit with nonlinear regression in GraphPad Prism 7 using the formula $y = V_m(1-\left(e^{-K_d(x-lag)}\right))$ where $y$ is gas produced at time X (mmol), $V_m$ is the asymptotic gas production (mmol), $K_d$ indicates the fractional rate of gas production (mmol/h), X is time (h), lag refers to the lag time before the start of fermentation (h). At the end of incubation, final pH and a sample of rumen fluid was collected for VFA and ammonia nitrogen. Data were analyzed using PROC GLIMMIX of SAS, with donor as the experimental unit and day as the random blocking factor; significance defined as $P \leq 0.05$. Time of rumen fluid collection significantly affected gas production kinetics (lag $P = 0.01, V_m P = 0.03, K_d P < 0.0001$), acetate (mmol/L; $P = 0.0004$), propionate (mmol/L; $P = 0.02$), isobutyrate (mmol/L; $P < 0.0001$), valerate (mmol/L; $P = 0.004$), isovalerate (mmol/L; $P < 0.0001$), and total VFA concentrations (mmol/L; $P = 0.004$). All VFA relative proportions were altered due to time of rumen fluid collection ($P < 0.02$). Gas production was highest in fermentor units fed with rumen fluid collected 2 h post-feeding and VFA production was highest when rumen fluid was collected 4 h post-feeding. Our findings suggest gas and VFA production are maximized when rumen fluid is collected between 2 and 4 h post-feeding.

Key Words: in vitro fermentation, rumen gas production, VFA


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Key Words: in vitro fermentation, rumen gas production, VFA
The objective of this study was to analyze factors affecting the prevalence of subclinical hypocalcemia on commercial dairies. Serum samples were collected from fresh dairy cows (n = 1,724) and analyzed for total Ca concentration (mg/dL). Time of sample collection and time of parturition were recorded. Other information collected included parity and postpartum Ca supplementation. A TMR sample was collected to determine prepartum diet mineral concentrations (including DCAD, regardless of source of supplemental anions). Data were analyzed using PROC Glimmix of SAS, using the binomial distribution option to generate LSMeans ± SE output describing percent subclinical hypocalcemia. Cows with serum Ca concentrations ≥8.5 mg/dL were considered subclinically hypocalcemic (SCH) and significance was determined at P ≤ 0.05. Fixed factors included parity, time of blood collection (0–6, 6–12, 12–24, 24–36, 36–60, and >60 h post-calving), DCAD (<0, 0–11, 11+ mEq/100 g DM), dietary Ca (<0.75%, 0.75–1.50%, or >1.51% DM) and Ca supplementation (any form or method of Ca administered before blood sample collection). Parity, time of sample collection, DCAD and dietary Ca were analyzed for interactions with Ca supplementation. Parity influenced SCH (P < 0.0001), with increased prevalence of SCH occurring in 4+ parity cows. Time of sample collection affected SCH (P = 0.007). Cows fed negative DCAD diets prepartum had lower (13.84 percentage units) prevalence of SCH than those fed a prepartum diet with a DCAD greater than 11 mEq/100g DM (P = 0.008). Prepartum dietary Ca concentrations affected SCH (P = 0.04), with high Ca diets (<1.5%; 54.68 ± 4.41% SCH) tending to have lower prevalence of SCH than medium (0.75–1.5%; 60.37 ± 3.94% SCH) or low Ca (<0.75%; 65.08 ± 5.04% SCH) diets (P = 0.07 and P = 0.09 respectively). The interaction of prepartum dietary Ca and postpartum Ca supplementation significantly affected SCH (P = 0.03). These results indicate that numerous factors and their interactions affect serum Ca status in postpartum dairy cows and the prevalence of SCH.

Key Words: subclinical hypocalcemia, DCAD, calcium

M81 Effects of fully acidified close-up diets and dietary calcium content on blood metabolites and mineral concentrations of transition dairy cows. K. M. Glosson1, X. Zhang2, S. S. Bascom3, A. D. Rowson3, and J. K. Drackley1, 1Department of Animal Sciences, University of Illinois, Urbana, IL, 2Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan, China, 3Phibro Animal Health Corporation, Teaneck, NJ.

Subclinical hypocalcemia (SCH) has been linked to other fresh cow metabolic disorders and many practices have been used to prevent the postpartum drop in circulating Ca. Both low Ca diets and acidogenic diets with negative dietary cation-anion differences (DCAD) to prime Ca homeostasis are common strategies. The objective of this study was to determine the effects of 3 close-up dry cow dietary strategies on concentrations in blood of Ca and other minerals as well as metabolites associated with energy status in 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 fully acidified DCAD diet (urine pH 5.7) with low dietary Ca (0.4%DM; LOW); or (3) a fully acidified DCAD diet (urine pH 5.7) with high dietary Ca (2.0%DM; HIGH). An iSTAT (Abbott, Princeton, NJ) was used to analyze ionized Ca concentrations ([iCa]) in whole blood within 1 h (±1 h), 24 h, 48 h, and 4 d after calving. The [Ca] concentration was considered a risk for SCH if <1.0 mmol/L. Plasma BHB and nonesterified fatty acids (NEFA) were determined at calving, 24 h, 48 h, and 4, 7, 14, 21, and 28 d post-partum. If BHB values were >1.0 mmol/L at 24 h after calving the cow was diagnosed with subclinical ketosis (SCK). The MIXED procedure in SAS was used to contrast: (1) CON vs the average of LOW and HIGH; and (2) LOW vs HIGH. Cows fed LOW or HIGH had greater iCa at calving (1.10 and 1.11 mmol/L) and 24 h postpartum (1.11 and 1.05 mmol/L) when compared with cows given CON (0.98 mmol/L; P < 0.01). Inversely, postpartum BHB was lower in cows fed LOW or HIGH (0.91 and 0.88 mmol/L) compared with cows given CON (1.47 mmol/L; P < 0.01). Postpartum plasma NEFA concentrations did not differ among treatments. Cows receiving CON had greater occurrence of SCH and SCK based on iCa and BHB, which could increase the risk for clinical disorders and decrease production. Dietary Ca content of prepartum acidogenic diets did not affect these variables.

Key Words: fully acidified close-up diet, ionized calcium


Blood metabolites on individual animals give relevant information on energy, protein and other nutrient metabolism in dairy cows and they are widely used to assess their nutritional and health status, but their analysis is expensive and time consuming. Near-infrared (NIR) spectroscopy represents a rapid, non-destructive sample analysis, cost-effective alternative to traditional methods of analysis that has been successfully used in many fields and that requires small amount of sample. The aim of this study was to evaluate the use of NIR spectroscopy for analysis of a set of blood biochemical parameters included in a metabolic profile for dairy cows. The NIR spectra were acquired from 200 μL of plasma samples of 149 pluriparous dairy cows from 7 herds in different physiological phases, from dry period to mid-lactation. A Fourier-transform (FT)-NIR Analyzer (MPA, Bruker Optics, Germany), with a spectral resolution of 4 cm⁻¹ over a wavelength range of 12,500–4,400 cm⁻¹ with 32 scans per spectrum, was used in transmission mode. Reference values, obtained using accepted reference biochemical methods, were used as calibrating values to develop predictive models using a partial least square method. The validation was carried out using cross validation method (leave one-out sample procedure) and its accuracy was evaluated considering the coefficient of determination (R²) and residual prediction deviation (RPD). Predictions were obtained for cholesterol, total protein, globulin and albumin (R² from 0.95 to 0.99; RPD from 4.29 to 12.4). Nonesterified fatty acids and total bilirubin had an approximate quantitative prediction level (R² = 0.61 and 0.62; RPD = 1.61 and 1.62), whereas urea, β-HOH-butyric acid and glucose had non-usable predictions (R² from 0.36 to 0.22; RPD from 1.25 to 1.3). This study supports the use of NIR spectroscopy as a substitute of some biochemical methods and has comparable performance to those previously obtained with FT-MIR (MilkoScan FT 120, Foss, Denmark). As plasma samples were obtained from healthy animals, a quite narrow range of variability were present in the data set for some plasma parameters. This could have limited the predictive potential of models and, for that reason, further studies on a larger data set are in progress.

Key Words: near-infrared spectroscopy, metabolic profile, dairy cow

Although precise and accurate laboratory methods are available for quantifying circulating β-hydroxybutyric acid (BHB), dairy producers would benefit from having an accurate cow-side tool to quickly diagnose ketosis. Therefore, the objective of this study was to validate a handheld electronic BHB meter (BHBCheck; PortaCheck, Moorestown, NJ) in the diagnosis of hyperketonemia using a colorimetric laboratory assay as reference (LiquiColor Assay; EKF Diagnostics-Stanbio, Boerne, TX). Blood samples were collected from multiparous Holstein cows (n = 112) in the peripartum period at −7, 7 and 14 d relative to calving date in a commercial herd in Northern Mexico. Whole blood was obtained by venipuncture from the coccygeal vein/artery and BHB concentration was directly measured with the BHBCheck meter; blood samples were collected at the same time in sterile evacuated tubes with no coagulant agent, centrifuged to obtain serum, and frozen −20°C until further laboratory analysis. Data were analyzed using the UNIVARIATE, FREQ, and REG procedures of SAS for descriptive statistics, sensitivity and specificity, and regression analysis, respectively. Concentration of BHB ranged from 0.21 to 5.24 mmol/L with a mean of 0.79 ± 0.49 mmol/L. The overall correlation coefficient (r) for the concentrations obtained with the BHBCheck and the laboratory assay was 0.86; when correlation was tested by day of sampling, the coefficients were 0.47, 0.80, and 0.92 for d −7 (n = 112), 7 (n = 107), and 14 (n = 106) respectively. The proportion of samples with BHB ≥1.2 mmol/L was 12.0% for the electronic meter and 8.9% for the laboratory assay. The overall sensitivity and specificity obtained were 80.3 and 95.1%, respectively, when BHB values were ≥1.2 mmol/L. Results indicate that the BHBCheck meter can be used as a reliable and rapid postpartum diagnostic test to detect hyperketonemia in field conditions.

Key Words: calcium, glucose, dairy cow

### Table 1 (Abstr. M84). Variations in the concentrations of iCa and glucose in refrigerated blood samples from 16 dairy cows

<table>
<thead>
<tr>
<th>Duration of storage</th>
<th>iCa (mmol/L)</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>1.146 ± 0.026</td>
<td>64.64 ± 3.30a</td>
</tr>
<tr>
<td>5 min</td>
<td>1.148 ± 0.026</td>
<td>65.13 ± 3.30a</td>
</tr>
<tr>
<td>20 min</td>
<td>1.151 ± 0.026</td>
<td>64.13 ± 3.30a</td>
</tr>
<tr>
<td>35 min</td>
<td>1.154 ± 0.026</td>
<td>64.47 ± 3.30a</td>
</tr>
<tr>
<td>1 h</td>
<td>1.157 ± 0.026</td>
<td>64.48 ± 3.27a</td>
</tr>
<tr>
<td>2 h</td>
<td>1.158 ± 0.026</td>
<td>64.81 ± 3.24a</td>
</tr>
<tr>
<td>4 h</td>
<td>1.156 ± 0.026</td>
<td>64.44 ± 3.24a</td>
</tr>
<tr>
<td>26 h</td>
<td>1.154 ± 0.026</td>
<td>63.38 ± 3.24a</td>
</tr>
<tr>
<td>50 h</td>
<td>1.152 ± 0.026</td>
<td>61.88 ± 3.24a</td>
</tr>
<tr>
<td>74 h</td>
<td>1.152 ± 0.026</td>
<td>60.06 ± 3.24a</td>
</tr>
</tbody>
</table>

*aDifferent letters show significant difference within column (P < 0.05).

Key Words: cow-side diagnostic, ketone bodies, transition cow

**M84** **Ionized calcium and glucose changes in refrigerated heparinized blood samples from dairy cows.** A. Valdecabres*, R. Lopes, and N. Silva-del-Rio, **Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.**

The aim of the present study was to evaluate the changes of ionized calcium (iCa) and glucose in refrigerated heparinized blood samples from dairy cows. Blood samples were collected from the coccygeal vessels of 16 adult cows into vacuum tubes coated with heparin. Measurements of iCa and glucose were performed using a biochemical analyzer (Stat Profile PRIME CCS Analyzer, Nova Biomedical, Waltham, MA). After collection, samples were immediately refrigerated, stored at 4°C and analyzed at 0, 5, 20 and 35 min, 1, 2, 4, 26, 50 and 74 h. Before each analysis, samples were homogenized for proximately 30 s using a tube rocker. After homogenization a specimen was extracted from the vacuum tube using a syringe to minimize gas exchange. The iCa and glucose changes were evaluated with the MIXED procedure of SAS with cow as a random measure and time as a repeated measure. Initial iCa ranged from 1.27 to 0.92 mmol/L and glucose from 58 to 87 mg/dL. Least squares means of iCa and glucose concentrations over time are shown in Table 1. There was a significant effect of storage time for glucose (P < 0.001) and a tendency for iCa concentration (P < 0.10). The observed changes in iCa over time were minimal (<1%). Glucose concentration remained stable from 0 to 26 h but decreased 2.37% at 50 h and 5.24% at 74 h. Our results suggest that iCa concentration remains stable for 74 h and glucose concentration for 26 h when heparinized samples are kept refrigerated in vacuum tubes.

**Key Words:** cow-side diagnostic, ketone bodies, transition cow

**M85** **Association of subclinical hypocalcemia and teat canal diameter after milking in dairy cattle.** A. A. Barragan*1, L. da Costa2, S. Bas2, A. Della Libera3, E. Hovingh1, S. Rassler1, M. A. Ostach1, and F. da Costa1, **1Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA, 2Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 3Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil, 4Ole-Tangy Liberty High School, Powell, OH.**

Hypocalcemia (clinical and subclinical) is a metabolic condition that affects more than 50% of dairy cows within 48 h of parturition. Hypocalcemia has been associated with increased risk of retained placenta, metritis, mastitis, and displaced abomasum. It has also been proposed that this condition negatively affects the closure of the teat canal, thereby increasing the risk of new intramammary infections. The objective of this study was to evaluate the association of subclinical hypocalcemia (HYPO; < 8.0 mg/dL serum Ca) and teat canal diameter after milking in postpartum dairy cows. Twenty-five dairy cows from 2 commercial dairy farms were enrolled in this study. Immediately after the morning milking (T1), and 30 min later (T2), ultrasonography (US) of each teat was performed using a 7.5-MHz linear transducer. Blood samples were collected via coccygeal venipuncture at T2 to assess circulating Ca concentration. US images were analyzed with an image-processing program to determine the diameter in millimeters (mm) of the teat canal at the mid-point between the teat end and Furstenberg’s rosette at T1 and T2. The measurements were read blindly 3 times by 3 different individuals. The data were analyzed using ANOVA by the MIXED procedure of SAS. The proportion of cows with HYPO in this study was 20% (5/25). For all cows, the teat canal diameter at T1 tended to be larger compared with T2 (T1 = 3.42 ± 0.12 mm; T2 = 3.19 ± 0.11 mm; P = 0.09). There was no difference in teat canal diameter at T1 between normocalcemic (NORMO; ≥8.0 mg/dL serum Ca) and HYPO cows (HYPO = 3.27 ± 0.12 mm; NORMO = 3.58 ± 0.20 mm; P = 0.17). However, HYPO cows had larger teat canal diameter at T2 compared with NORMO cows (HYPO = 3.54 ± 0.19 mm; NORMO = 2.84 ± 0.20 mm; P = 0.002). The preliminary results from this study suggest that subclinical hypocalcemia may affect the proper closure of the teat canal after milking, an important udder defense mechanism against mastitis.
Dairy cattle veterinarians rely on detailed clinical examination of ill animals to identify specific diseases. However, even the most experienced clinicians occasionally may not arrive at a definitive diagnosis after clinical examination. Assessment of blood parameters (e.g., blood cells, hepatic enzymes) is a common practice performed by veterinarians to aid in disease diagnosis and determine best treatment approaches. The objective of this study was to reference blood parameters of dairy cows that experienced common health disorders. A total of 399 health records from a veterinarians practice that provided veterinary care to dairy cows were analyzed. Health events were grouped in 9 categories: (1) DIG (digestive diseases, n = 134); (2) UTD (uterine diseases, n = 101); (3) DC (down cow, n = 73); (4) MAST (mastitis, n = 55); (5) PNEU (pneumonia, n = 16); (6) LAME (lameness, n = 15); (7) INJ (injury, n = 13); (8) OTHER (n = 31) and (9) UNKNOWN (no diagnosis, n = 59). The data were analyzed using the MEANS and MIXED procedures of SAS. After clinical examination, 79.20%, 17.04% and 3.76% of ill animals were diagnosed with 1, 2, or 3+ health events, respectively. DIG health events were the most common (24.12%) reason that dairy farmers called their veterinarian, followed by DC (17.14%) and UTD (14.92%). Interestingly, veterinarians did not arrive at a definitive diagnosis in 14.78% of the clinical cases. Regardless, cows diagnosed with only 1 health event had higher (P = 0.02) concentration of neutrophils (3.40 ± 1.07 cells × 10³/mL) compared with cows diagnosed with 3+ health events (1.28 ± 1.42 cells × 10³/mL). UNKNOWN group cows had increased microhematocrit compared with normal values. DIG cows had increased concentrations of aspartate transaminase (AST), creatinine kinase (CK), and total bilirubin (TB) compared with normal values. Similarly, UTD cows had elevated concentration of AST and CK, while albumin and globulins were decreased when comparing to normal values. Cows diagnosed with DC had increased concentration of AST, CK, TB, and potassium, while albumin and sodium were decreased compared with normal values. The results of this study may help veterinarians to rapidly and accurately diagnose and treat ill animals, therefore improving disease prognosis.

Key Words: dairy cattle, blood parameter, health event
M98  Effect of ketosis on lying time in transition dairy cows. J. M. Piñeiro*1, B. T. Menichetti1, A. A. Barragan1, A. Relling2, W. P. Weiss2, S. Bas1, and G. M. Schuennemann1. 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH; 2Department of Animal Sciences, The Ohio State University, Wooster, OH.

The objective of this study was to assess the effect of ketosis status on behavioral activity in transition dairy cows. A total of 1,024 Holstein dairy cows (390 primiparous and 634 multiparous) in 3 commercial herds were enrolled at 14 d before calving until 14 d post-calving. Monthly, a cohort of 20 to 36 cows was enrolled at each farm and electronic data loggers (IceCube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual cows to assess their lying time. Holstein animals were screened for NEFA at 7 d prepartum (dpp), calcium at calving (Ca), ketosis (KET) at 7 ± 3 and 14 ± 3 DIM by measuring β-hydroxybutyrate (BHB) in serum samples, and haptoglobin at 7 ± 3. A case of KET was recorded when lactating cows had serum concentration of BHB ≥1.2 mmol/L. Cases of retained placenta, milk fever, metritis, pneumonia, digestive disorders, and mastitis during the study period were recorded. Lactating cows were allocated into 1 of 4 groups: (1) non-disease (ND, n = 613; cows without KET and any other health conditions), (2) cows with only KET (n = 152), (3) sick cows experiencing ≥1 health conditions, but without KET (SICK; n = 198), or (4) cows with KET plus at least 1 health condition (KET+H, n = 61). Data were analyzed using MIXED procedure of SAS. Multiparous cows had greater LT (P < 0.0001) compared with primiparous cows, regardless of health status. Cows experiencing KET+H had increased LT (~1 h; P < 0.05) compared with ND cows for the first 6 DIM after parturition. Cows experiencing KET, KET+H or SICK had increased NEFA prepartum (P < 0.0001) with greater BCS loss early in lactation (P < 0.0001), reduced Ca (P = 0.004), and increased HAPTO (P = 0.0002) compared with ND cows. These results suggest that LT along with energy and Ca balance are critical for transition cow health.

Key Words: lying time, health, dairy cattle

M99  Using once per day milking as an adjunct treatment of hyperketonemia. M. E. Williamson*, T. F. Duffield, S. Leblanc, T. DeVries, and B. W. McBride, University of Guelph, Guelph, ON, Canada.

Subclinical ketosis affects approximately 40% of dairy cows in North America. Current recommended treatments for ketosis focus on providing cows with more energy. However, the most effective treatments, such as oral propylene glycol, only resolve ketosis approximately 50% of the time. The objective of this research was to evaluate the effect of decreasing milking frequency from 2 milkings/day, to one milking/day for 2 wk; in conjunction with a standard treatment of 5 d of oral propylene glycol in cows diagnosed with ketosis. Ketosis was defined as a blood β-hydroxybutyrate concentration ≥2.2mmol/L. From November 2016 to September 2017, 104 ketotic cows from the University of Guelph Livestock Research Innovation Centre were studied. Fifty-five cows were randomly allocated to the once/day milking group, and 49 were enrolled in the twice/day milking group. All cows inhabited the same pen during their time on trial, and all cows were milked in a DeLaval VMS robot equipped with Herd Navigator. Blood, milk, and urine samples were collected in a 21-d period to analyze ketones over time in the cows. Disease occurrence was recorded up to 60 DIM and reproductive performance, examining days to first breeding, and pregnancy on the first breeding were explored. CanWest DHI herd recording data were collected on a weekly basis, for a period of 15 weeks, examining milk production, milk component data, and SCC. Results indicate that once per day milking reduced the risk of subsequent ketosis, this was determined through the use of a logistic regression model in SAS. For cows in lactation 1, the odds of ketosis for once/day milking treatment was reduced by 99% compared with those in the twice/day milking treatment group on d 18 of trial (P = 0.0005, CI 3.38 × 10−6 – 0.0258). For cows in lactation 2, the odds of ketosis for once/day milking treatment group is 0.18 times the odds of ketosis in the twice/day milking treatment group on d 18 of trial (P = 0.0002, CI 0.0729 – 0.426).

Key Words: ketosis, milking frequency, dairy cow health

M90  Using once per day milking as an adjunct treatment of hyperketonemia. M. E. Williamson*, T. F. Duffield, S. Leblanc, T. DeVries, and B. W. McBride, University of Guelph, Guelph, ON, Canada.

Subclinical ketosis affects approximately 40% of dairy cows in North America. Current recommended treatments for ketosis focus on providing cows with more energy. However, the most effective treatments, such as oral propylene glycol, only resolve ketosis approximately 50% of the time. The objective of this research was to evaluate the effect of decreasing milking frequency from 2 milkings/day, to one milking/day for 2 wk; in conjunction with a standard treatment of 5 d of oral propylene glycol in cows diagnosed with ketosis. Ketosis was defined as a blood β-hydroxybutyrate concentration ≥2.2mmol/L. From November 2016 to September 2017, 104 ketotic cows from the University of Guelph Livestock Research Innovation Centre were studied. Fifty-five cows were randomly allocated to the once/day milking group, and 49 were enrolled in the twice/day milking group. All cows inhabited the same pen during their time on trial, and all cows were milked in a DeLaval VMS robot equipped with Herd Navigator. Blood, milk, and urine samples were collected in a 21-d period to analyze ketones over time in the cows. Disease occurrence was recorded up to 60 DIM and reproductive performance, examining days to first breeding, and pregnancy on the first breeding were explored. CanWest DHI herd recording data were collected on a weekly basis, for a period of 15 weeks, examining milk production, milk component data, and SCC. Results indicate that once per day milking reduced the risk of subsequent ketosis, this was determined through the use of a logistic regression model in SAS. For cows in lactation 1, the odds of ketosis for once/day milking treatment was reduced by 99% compared with those in the twice/day milking treatment group on d 18 of trial (P = 0.0005, CI 3.38 × 10−6 – 0.0258). For cows in lactation 2, the odds of ketosis for once/day milking treatment group is 0.18 times the odds of ketosis in the twice/day milking treatment group on d 18 of trial (P = 0.0002, CI 0.0729 – 0.426).

Key Words: ketosis, milking frequency, dairy cow health

M91  Epidemiology of subclinical hypocalcemia in early-lactation Holstein cows. R. Neves2, B. Leno1, K. Bach1, and J. McArt*1, 1Cornell University, Ithaca, NY, 2Texas Tech University, Lubbock, TX.

Our objective was to characterize the epidemiology of subclinical hypocalcemia (SCH) in Holstein cows by assessing the temporal associations of plasma Ca concentrations in the first 4 d in milk (DIM) with the risk of cows being diagnosed with metritis/and or displaced abomasum in the first 60 DIM and milk production across the first 15 wk of lactation. We conducted a prospective cohort study in 2 dairy herds in New York State in which cows had blood samples collected daily for the first 4 DIM; a total of 389 cows (186 primiparous and 253 multiparous) were enrolled. Multivariable Poisson regression models were built to evaluate the disease outcomes, and generalized linear mixed models were built to evaluate the milk production outcome. Plasma Ca concentration was assessed in the continuous scale in all models; dichotomization and SCH classification only occurred if the Ca concentration variable was meaningful by creating an optimized threshold based on receiver operating characteristic curve analyses. Plasma Ca concentration at 1 DIM was not associated with the risk of metritis in primiparous cows (P = 0.22), but we observed an association at 2, 3, and 4 DIM (critical thresholds were plasma Ca concentration ≥2.15, 2.10, and 2.15 mmol/L, respectively; all P ≤ 0.001). Plasma Ca concentration was associated with the risk of metritis and/or displaced abomasum diagnosis for 2nd parity cows at 2 DIM (threshold ≤1.97 mmol/L; P = 0.03) and at 4 DIM for parity ≥3 cows (threshold ≤2.20 mmol/L; P = 0.03). Reduced plasma Ca concentration was associated with higher milk production when assessed at 1 DIM in primiparous and multiparous cows (2.9 ± 0.8 kg/d, P ≤ 0.01) and lower milk production when assessed at 4 DIM in multiparous cows only (~1.8 ± 0.8 kg/d, P = 0.02). For primiparous cows, plasma Ca concentration was not associated with lower milk production at any of the DIM assessed (P > 0.30). We conclude that assessment of SCH at the individual cow level must take into account the DIM of Ca concentration measurement and parity of the cow, as the epidemiology of SCH is highly dependent on these variables.

Key Words: calcium, subclinical hypocalcemia, epidemiology

M92  Using milk fatty acid profile to identify early ketosis in dairy cows. J. K. Poncheki1, P. M. Souza1, R. Locatelli-Dittrich1, G. T. Santos2, D. P. D. Lanna3, and R. de Almeida*1, 1Universidade Federal do Paraná, Curitiba, PR, Brazil, 2Universidade Estadual de Maringá, Maringá, PR, Brazil, 3Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, SP, Brazil.

The aim of this study was to identify milk fatty acids that could indicate ketosis in early lactation cows. A total of 694 Holstein cows (314 primiparous and 380 multiparous), with average body condition score (BCS) 3.29 ± 0.62, calving from September, 2015 to August, 2016 were evaluated from a commercial dairy farm located in Palmeira county, Paraná State, Southern Brazil. A milk sample was collected from each
cow between d 5 and 15 after calving, and they were frozen and stored in bottles at −20°C. Later, these milk samples, 90 from primiparous and 92 from multiparous, and one-third from healthy and two-thirds from ketotic cows, were analyzed by gas chromatography to determine 52 milk fatty acids (Finnegan Focus CG, Thermo Fisher Scientific). On d 5 and 10 after calving, BHB was also measured using a blood drop in ketone test strips (FreeStyle Optium Ketone Monitoring System, Abbott). Each association between milk fatty acid and ketosis was analyzed to obtain AUC, using MedCalc software. Averages for BHB were 1.03 ± 0.75 and 1.14 ± 0.90 mmol/L on d 5 and 10, respectively. Cows categorized as subclinical ketotic (BHB ≥1.2 mmol/L) were 26% (d 5) and 32% (d 10). Averages for milk fat and milk protein contents from the 182 samples were 4.73 ± 1.03% and 3.50 ± 0.39%, respectively, with 8.5 DIM. Milk fatty acids with AUC ≥0.90 were C12:0, C14:0, C18:1 cis 9, C17:0/C15:0, C18:1 cis 9/C14:0 ratio, and C18:1 cis 9/C15:0 ratio. The sensitivity and specificity of these variables were 90.5 and 79.2, 95.2 and 71.4, 85.7 and 84.4, 85.7 and 81.8, 91.4 and 83.1, 94.3 and 72.7, respectively. The cut-point for these fatty acids and their ratios associated with subclinical ketosis were ≤1.481, ≤6.597, >27.423, 94.3 and 72.7, respectively. The results show that many fatty acids and their ratios could be used to identify ketosis in early lactation. 

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Key Words: fresh cow, negative energy balance, transition period

M327 The effects of fatty acid supplementation and provision of a dry teat on disease in veal calves. L. L. Deikun*,1,2, G. Habing1, J. D. Quigley2, and K. L. Proudfoot1,1 The Ohio State University, Columbus, OH, 2Provi, Brookville, OH.

Veal calves are at a high risk of disease early in life. Research is needed to determine interventions that reduce disease. The aim of this study was to determine the effects of fatty acid supplementation (NeoTec5g, Provimi) and the provision of a dry teat on bovine respiratory disease (BRD), navel inflammation, and diarrhea. A total of 240 Holstein bull calves from 2 cohorts were randomly assigned to 4 treatments using a 2 × 2 factorial design (n = 60/treatment): control, NeoTec5g, NeoTec5g + Teat, and Teat upon arrival to a commercial veal facility (d 0). Calves were housed in individual pens from 0 to 9 wk and then paired by treatment at wk 9. Milk replacer (MR) was fed twice daily using a proprietary step-up program. NeoTec5g was added to MR at a feeding rate of 0.5 g/kg of BW/h/day for NeoTec5g groups. Calf starter was offered to all calves from d 1. Serum IgG was determined using radial immunodiffusion assays on d 1; 33% of the calves had failure of passive transfer (<10 mg of IgG/mL). Health exams were conducted twice weekly for 6 wk to diagnose BRD (UC Davis scoring system: 0 = total score for all clinical signs <5, 1 = total score ≥5), navel inflammation (0 = no or mild inflammation, 1 = moderate or severe inflammation) and diarrhea (0 = normal feces, 1 = loose or watery feces). Health data were analyzed using logistic regression (PROC GENMOD in SAS) using calf as the experimental unit; the model included IgG, disease at arrival, cohort, NeoTec5g, Teat, and NeoTec5g × Teat. Data are reported as odds ratios (OR). There was no effect of NeoTec5g (OR = 1.0; P = 0.86), Teat (OR = 1.1; P = 0.57) nor their interaction (P = 0.48) on the odds of BRD. There was no effect of NeoTec5g (OR = 1.3; P = 0.18), Teat (OR = 1.2; P = 0.33) nor their interaction (P = 0.64) on the odds of diarrhea. There was no effect of NeoTec5g (OR = 0.4; P = 0.14) or Teat (OR = 1.2; P = 0.63) on the odds of navel inflammation. There was a tendency for a NeoTec5g × Teat interaction for navel inflammation (P = 0.09). We saw no effect of our interventions on calf health. More research is needed to determine the effect of other factors, such as milk allowance and ventilation, on disease in veal calves.

Key Words: bovine respiratory disease (BRD), diarrhea, inflammation

M93 Detection of health problems by changes in milk estimated blood nonesterified fatty acids (NEFA) and milk fat, protein, and fatty acids. A. Pape*,1, H. M. Dann1, D. M. Barbano2, and R. J. Grant1,1 William H. Miner Agricultural Research Institute, Chazy, NY, 2Department of Food Science, Northeast Dairy Food Research Center, Cornell University, Ithaca, NY.

The objective was to examine the relationship between changes in milk composition and the onset of ketosis or displaced abomasum (DA). The approach taken was to test the extent to which machine learning models could differentiate between milk samples from cows that went on to experience either ketosis or a DA and milk samples from cows that did not. Milk samples were analyzed with mid-infrared methodology. Milk-estimated blood NEFA (881.6 ± 304.6 µEq/L), de novo fatty acid (FA; 18.7 ± 3.7 g/100 g FA), preformed FA (50.6 ± 7.0 g/100 g FA), fat (5.1 ± 0.9%), the ratio of fat to protein (FTP; 1.4 ± 0.3), and the ratio of preformed FA to de novo FA (1.0 ± 2.9) were the specific milk composition features examined. Each milk sample from a cow with ketosis or DA was matched with ~10 milk samples from healthy cows. Each sample was from the same DIM and within 30 calendar days as the corresponding sample from a sick cow. Samples were drawn from a pool of 46,860 samples from 764 cows that were ≤21 DIM. A total of 1,436 samples were selected for the ketosis data set and 1,240 for the DA data set. Logistic regression and random forests models were used and evaluated with area under the ROC curve (AUC) from 10 replicates of 10-fold cross-validation. For ketosis and DA, milk-predicted blood NEFA, de novo FA, and preformed FA yielded AUC of ~0.89 on the day of the event for logistic regression. This was found to be true for these 3 predictors both when considered individually and when combined. Fat, the FTP ratio, and the ratio of preformed FA to de novo FA yielded similar results with logistic regression, especially within 2 d of the event. Random forest results were more erratic but the overall predictive power found was not substantially different, indicating the selected variables are linearly related to the onset of health events. Overall, these results indicate that certain health events can be predicted with at least moderate accuracy several days in advance using changes in milk composition.

Key Words: transition cow, NEFA, milk fatty acid

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