161 The dynamics of BCS during the far-off and close-up period impacts postpartum diseases in Holstein cows. P. Melden-
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uguay, 4Bovine Practitioner, Buenos Aires, Argentina.

Body condition score (BCS) is a quick, cheap and reliable tool that helps determine fat reserves. BCS at calving and changes in BCS after calving have been consistently associated with milk yield, diseases, and fertility in dairy cows, yet, the dynamics of BCS during the dry period has been less studied. The objective was to assess the change in BCS within the far-off (dryoff to −21 d prepartum) and close-up (−21 d prepartum to calving) and its association with postpartum diseases. The study analyzed 22,000 lactations from 28 dairies from Argentina. BCS was assessed with a scale of a 1/4 unit from 1 (emaciated) to 5 (obese). BCS was carried out by 2 of the authors creating 4 groups. G1: cows that maintained or gained BCS during far-off and close-up; G2: cows that maintained or gained BCS during far-off and lost BCS during close-up; G3: cows that lost BCS during far-off and maintained or gained BCS during close-up; G4: cows that lost BCS during far-off and close-up. Diseases were compared among groups by conducting logistic regression. Dependent variable was the incidence of the disease (yes, no). Three models were run, comparing G1 vs G2, G1 vs G3, and G1 vs G4, correcting for year, BCS at calving, lactation and farm. Group was no. Three models were run, comparing G1 vs G2, G1 vs G3, and G1 vs G4; Table 1. The impact of losses of BCS during the far-off period was less dramatic (G1 vs. G3) than during the close-up period.

Key Words: BCS, postpartum disease, dry period

162 Incidence of subclinical and clinical ketosis in the California Central Valley: Similarities among commercial herds. M. Wukadinovich* and H. Rossow, University of California, Davis, Davis, CA.

Ketosis is a common metabolic disease in postpartum dairy cows due to negative energy balance at the onset of lactation resulting from low dry matter intake and disorders of energy metabolism. Ketosis results in hyperketonemia, hypoglycemia, decreased milk yield, and is often associated with other primary health disorders. To estimate the incidence of subclinical (SCK) and clinical ketosis (CK) in the California Central Valley, blood samples and milk production data were collected from 10 commercial dairy herds in Tulare, Kings, and Kern counties. In February 2018, an average of 17 multiparous Holstein cows from each herd were bled during wk 1 and wk 2 postpartum and whole blood was analyzed for glucose and acetoacetate (AcAc) levels using NovaMax meters (Nova Diabetes Care, Inc., Billerica, MA). Ketosis was defined as SCK at 1.0–1.4 mmol/L and CK > 1.4 mmol/L AcAc in whole blood. Previous lactation milk fat yield and 305-d equivalent milk yield were collected from DairyComp305 (Valley Ag Software, Tulare, CA). All data were analyzed using the Mixed Procedure of SAS with repeated measures by cow or a Generalized Linear Model Procedure (v. 9.4, SAS Institute 2015). Across all herds, average SCK was 12% (repeat cases 5%) and CK was 11% (repeat 61%). All repeat CK cases progressed from SCK in wk 1. Across herds, the lowest incidence of hyperketonemia (>1.0 mmol/L AcAc) was 0% and the highest incidence was 44%. Glucose and AcAc were inversely related (P < 0.001; R² = 0.12). Hyperketonemic cows produced 393 kg more milk (P = 0.1) and 44 kg more milk fat (P = 0.02) in their previous lactation compared with non-ketotic cows. Five dairies housed fresh cows in free stall corrals and 5 dairies housed fresh cows in free stall corrals with an attached exercise corral or in an open lot style corral. Cows with access to an exercise corral or housed in an open lot had lower blood AcAc levels compared with cows housed in free stalls (0.55 vs. 0.64 mmol/L; P = 0.02, R² = 0.17) and had a decreased recurrence of a ketotic event in the same lactation (5% vs. 26%). Therefore, factors affecting blood AcAc levels on these commercial herds were glucose, previous lactation milk and fat yield, and access to an exercise corral.

Table 1 (Abstr. 161).

<table>
<thead>
<tr>
<th>Model</th>
<th>Milk fever</th>
<th>RFM</th>
<th>Metritis</th>
<th>Ketonisis</th>
<th>Mastitis</th>
<th>Lame</th>
<th>Culling</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 vs. G2</td>
<td>0.89</td>
<td>0.76</td>
<td>0.68</td>
<td>0.75</td>
<td>0.78</td>
<td>1.02</td>
<td>0.86</td>
<td>0.82</td>
</tr>
<tr>
<td>0.72–1.12</td>
<td>0.63–0.92</td>
<td>0.54–0.86</td>
<td>0.54–1.06</td>
<td>0.68–0.90</td>
<td>0.83–1.27</td>
<td>0.74–1.00</td>
<td>0.74–0.92</td>
<td></td>
</tr>
<tr>
<td>G1 vs. G3</td>
<td>1.22</td>
<td>0.85</td>
<td>1.20</td>
<td>0.85</td>
<td>0.92</td>
<td>0.95</td>
<td>0.84</td>
<td>0.97</td>
</tr>
<tr>
<td>0.93–1.60</td>
<td>0.68–1.06</td>
<td>0.68–1.84</td>
<td>0.60–1.20</td>
<td>0.79–1.09</td>
<td>0.74–1.22</td>
<td>0.70–1.00</td>
<td>0.86–1.11</td>
<td></td>
</tr>
<tr>
<td>G1 vs. G4</td>
<td>1.02</td>
<td>0.74</td>
<td>0.91</td>
<td>1.02</td>
<td>0.80</td>
<td>0.89</td>
<td>0.66</td>
<td>0.72</td>
</tr>
<tr>
<td>0.65–1.60</td>
<td>0.53–1.04</td>
<td>0.59–1.41</td>
<td>0.54–1.92</td>
<td>0.62–1.03</td>
<td>0.60–1.30</td>
<td>0.50–0.86</td>
<td>0.58–0.89</td>
<td></td>
</tr>
</tbody>
</table>
order. Calibration was per manufacturer’s standards, as was that milk was centrifuged to milk sera for testing with the ChemWell-T 460 analyzer, and whole milk was tested using the Fossomatic. Thresholds of 200 and 300 µM/L for classifying cows as ketotic (K) vs. non-ketotic (N) were tested for test agreement and accuracy. Results were 374 runs on ChemWell and 352 runs on Fossomatic (one outlier run excluded). ChemWell results: mean correlation with standards 0.991 (range 0.970 to 0.999). 200 µM/L threshold: 97.6% agreement (K vs. N, Kappa 0.848 very good) with standards, if spiked milks considered “gold standard.” Sensitivity (sens) 99.1%, specificity (spec) 82.4%, 300 µM/L threshold: 98.7% agreement (K vs. N, Kappa 0.956, very good), sens 98.4%, spec 100%. Fossomatic results: mean correlation with standards 0.920 (range 0.784 to 0.984). 200 µM/L threshold: 85.8% agreement (K vs. N, Kappa 0.355 fair) with standards, if spiked milks considered “gold standard,” sens 94.6%, spec 35.8%. 300 µM/L threshold: 91.5% agreement (K vs. N, Kappa 0.656, good), sens 96.0%, spec 66.7%. The FTIR Fossomatic BHB testing requires recalibration multiple times per day and results are not as accurate as those with the clinical pathology analyzer, but are more accurate than many other milk high throughput diagnostic tests currently in the dairy industry. Continued improvement in BHB milk calibration standards for the Fossomatic is needed.

**Key Words:** β-hydroxybutyrate (BHB), milk, laboratory

### 164 Association between hyperketonemia during the first 10 days postpartum and productive parameters throughout lactation in dairy cows. Z. Rodriguez*, J. Lukach, E. Wynands, P. Cecilio Ferro, G. Cramer, and L. Caixeta, Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.

Hyperketonemia (HYK) is frequently observed in high-producing dairy cattle and has been associated with suboptimal health and performance. Traditionally, HYK has been defined by blood β-hydroxybutyrate (BHB) of >1.2 mmol/L, although cows vary in their clinical response to HYK. Thus, our objective was to evaluate the association between HYK occurring in early lactation with milk yield and productive performance in Holstein dairy cows throughout an entire lactation. We hypothesized that elevated BHB in the first 10 d postpartum is not necessarily detrimental to all cows. Blood BHB was measured in 1,108 cows from 6 herds in Minnesota between 3 and 10 DIM. Cows were followed for their entire lactation, until next calving or culling. Health and performance data were collected from herd management software. In addition to classification based on blood BHB (HYK+ and HYK−), cows were further classified into remaining (REM, cows that reach subsequent calving) or culled (CUL, cows that were sold or died during lactation). Cox proportional hazard was performed to analyze time to pregnancy, adjusting for herd and parity effects. Milk yield was analyzed by ANOVA accounting for repeated measures and adjusting for herd effect; separate models were created for primiparous and multiparous cows. Disease events and DIM at culling were applied to all models and removed using backward stepwise elimination. Overall prevalence of HYK was 11.2% (range 3.2% to 20.3%). Although the hazard ratio (HR) for pregnancy by 300 DIM for HYK+ (REM and CUL combined) cows was 0.64 (95% CI: 0.47 – 0.86) when compared with HYK− (REM and CUL combined) cows, the REM HYK+ HR was 5.65 (95% CI: 4.65 – 6.87) times higher than CUL HYK− cows. Similarly, no difference was observed when comparing milk production for combined HYK+ and HYK− multiparous cows. However, REM HYK+ cows produced, on average, 5.1 kg/day (95% CI: 3.47 – 6.11) more milk than the CUL HYK+ cows. These findings suggest that HYK does not affect all cows in a similar fashion.

**Key Words:** ketosis, reproductive performance, milk yield

### 165 High concentrations of fatty acids induce hepatic lipid accumulation by activating endoplasmic reticulum stress in dairy cows with severe fatty liver. Y. Zhu and X. Li*, Jilin University, Changchun, Jilin, China.

Disruption of endoplasmic reticulum (ER) homeostasis is intrinsically linked with lipid metabolism disorder in humans and mice. Whether ER homeostasis is affected in cows with fatty liver is unknown. The aim of this study was to investigate ER status and the potential role of ER stress in the progression of fatty liver in dairy cows. Liver and blood samples were collected from cows diagnosed as healthy (n = 15) or with severe fatty liver (n = 15). Hepatocytes were isolated from calves and treated with various concentrations of fatty acids and/or taurocholate. Hepatocytes were observed using an electron microscope, while TUNEL staining was used to detect apoptosis. The results indicated that the activation of ER stress in response to increasing doses of fatty acids and/or taurocholate significantly weakened these effects. Overall, results indicate that an activated ER stress and the ensuing UPR in response to increased influx of fatty acids into hepatocytes are causative of severe fatty liver in dairy cows.

**Key Words:** fatty liver, endoplasmic reticulum stress, unfolded protein response

### 166 Herbal formula CHF03 attenuates high-fat diet-induced nonalcoholic fatty liver disease by regulating nuclear factor-KB in mice. Y. Cui*1, R. Chang1, T. Zhang2, X. Zhou3, Q. Wang1, H. Gao1, L. Hou1, and C. Xu1, 1College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang, China, 2Beijing University of Agriculture, Beijing, China, 3China Animal Health and Epidemiology Center, Laboratory of Zoonosis, Beijing, China.

Nonalcoholic fatty liver disease (NAFLD) is a hepatic ailment with a rapidly increasing incidence due to dietary hyper nutrition and subsequent obesity. Discovering effective natural materials and herbs can provide alternative and complementary medical treatments to current chemical pharmaceuticals. To develop an effective natural agent for NAFLD, we formulated a combination of 10 herb mixtures and observed lipid-lowering efficacy and to investigate the preventive effects of a compound of Chinese Herbal Formula (CHF03) on a high-fat diet (HFD) induced model of NAFLD in mice in vitro and in vivo. The CHF03 groups were fed with HFD and orally administered 10 g/kg CHF03 once a day orally. HE staining was performed to analyze pathologic changes of the liver; a Transmission Electron Microscopy assay was performed to measure the ultrastructural alterations of the liver.
mitochondria, and Western blotting was performed to detect the expression of gene proteins related to lipid metabolism and inflammation. To further examine the safety of CHF03, the composition of CHF03 was analyzed by liquid chromatography-mass spectrometry (LC/MS) and acute toxicity and maximum tolerable dose was performed in mice. The results of histomorphological and ultra-structural changes showed that CHF03 could effectively inhibit the occurrence of non-alcoholic fatty liver induced by high fat. CHF03 attenuated high-fat diet-induced oxidative stress, which was confirmed by measuring the level of oxidative stress markers (GSH, GSH-Px, MDA, SOD and CAT). The preventive effects of CHF03 against lipogenesis and inflammatory were mediated by the inhibition of protein acetyl-CoA carboxylase (ACC1), fatty acid synthase (FAS) and nuclear factor kB (NFkB). Moreover, CHF03 dose-dependently inhibited lipid accumulation and gene expressions involved in lipogenesis and related regulators (SREBP-1c, CPT1, FAS, ACC1, ApoA and NFkB) and prevented the PA-induced lipid accumulation in hepatocytes. Additionally, CHF03 inhibited the PA-induced increase in the expression, nuclear localization, and transcriptional activities of NFkB. These results suggest that CHF03 to suppress HFD-induced NAFLD in part through the activation of NF-kB.

Key Words: nonalcoholic fatty liver disease, herbal formula, hepatocytes


Accelerometers can be used to gauge rumination activity (RumAct). The purpose of this study was to determine if hypocalemia affected RumAct in older periparturient cows fed a high or low DCAD diet to induce or prevent periparturient hypocalemia. Twenty-six Holstein cows entering their 3rd or greater lactation were assigned to an Anion Supplemented (AS) or No Anion precalving diet and fed behind Calan gates. After calving, all cows were fed the same lactation diet. DMI were determined for each cow from 14 d before calving until d 5 of lactation. Blood was sampled daily the wk before calving, at calving, and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 10 d after calving. Urine sample pH determined before calving was significantly decreased in cows fed the AS diet (6.99 ± 0.16 vs. 8.26 ± 0.06). The effects of diet, d around calving and their interaction on plasma Ca, DMI, and RumAct were assessed by the Mixed Procedure of SAS, with d around calving as the repeated measure. Cows fed AS diet had significantly higher blood Ca concentrations from 24 h before calving thru the first 36 h of lactation (P < 0.05). Four No Anion cows developed milk fever necessitating IV Ca treatment. On the 1st and 2nd d of lactation, cows fed AS diet consumed significantly more DM (3.85 kg on d 1, P < 0.01; 3.39 kg on d 2, P < 0.025) than cows fed the No Anion diet. RumAct decreased in all cows at parturition. Diet had no effect on 24 h RumAct until the day before calving. During that 24-h period, cows fed AS spent 457 min ruminating while No Anion cows spent only 356 min ruminating; a 22% decrease (P = 0.005). RumAct was greater in cows fed anions during the 1st and 2nd 24 h periods of lactation with anion fed cows ruminating 127 and 115 more min/d during the 1st and 2nd 24 h periods after calving respectively (P < 0.002). RumAct data were also compiled into min of RumAct per 2 h period. Cows fed AS had significantly greater RumAct (P < 0.05) than No Anion cows during 13 of the 24 2-h periods between 12 h before calving thru the first 36 h after calving. Milk fever cows had extended periods where the rumination rate / 2 h was undetectable, even after treatment restored blood Ca concentrations to normal levels.

Key Words: rumination, anions, hypocalemia

168 Effects of carprofen in a pain management protocol in dairy cows with abomasal displacement undergoing left flank omentopexy. H. Meyer1, S. Kaestner2, and J. Rheage*1, 1Clinic for Cattle, University of Veterinary Medicine Hannover, Hannover, Germany, 2Clinic for Small Animals, University of Veterinary Medicine Hannover, Hannover, Germany.

Aim of the study was to investigate the efficacy of the nonsteroidal anti-inflammatory drug carprofen for pain management during and after surgical correction of left sided abomasal displacement (LDA) and possible adverse effects on integrity of abomasal mucosa. The blinded study included 24 lactating Holstein cows with LDA corrected by left flank omentopexy. Cows were randomly assigned to one of 2 treatments, carprofen (C; n = 12; 1.4 mg/kg of Rimadyl, Zoetis, IV) or placebo (P; saline; n = 12) one h before and 72 h after surgery. Local paralumbar nerve blocks and infiltration of the incision line (160 mL Procaine 2%) induced anesthesia. Cows were investigated from the day before until 4 d after surgery. Daily feed intake and milk yield were assessed, and behavior monitored by video recordings. Heart and respiratory rate, body temperature, mean arterial blood pressure as well as blood concentrations of glucose, lactate, β-hydroxybutyrate, nonesterified fatty acids and cortisol were determined in regular pre-set intervals. Macroscopic fecal examination, hemoFec testing for occult fecal blood, analysis of pepsinogen in serum and hematological parameters were carried out to determine adverse effects on abomasal mucosa. Results were evaluated in a mixed model with repeated statement (SAS statistical package; fixed effects: group, time, random effect: cow). A significantly decreased mean cortisol response was detected in cows of group C during the entire period from 20 min before the start of the surgical procedure until 10 h after the operation was completed (time × group P < 0.05). According to results of video recordings after surgery in average cows of C spent significantly (P < 0.05) more time ruminating and presented less pain associated behavior (determined by visual analog scale and multiple pain discomfort scale) than cows of P. Based on macroscopic fecal examination, blood count, the hemoFec-Test and serum pepsinogen concentrations, there was no indication for adverse effects on integrity of the abomasal mucosa. The study demonstrated that the use of carprofen in pre-emptive multimodal pain management increased the wellbeing of the patients without evoking adverse effects.

Key Words: carprofen, analgesia, cows

169 Impaired hepatic autophagic activity in dairy cows with severe fatty liver. X. Du, G. Liu, and X. Li*, Jilin University, Changchun, Jilin, China.

The ability of liver to respond to changes in nutrient availability is essential for the maintenance of metabolic homeostasis. Autophagy is a conserved catabolic process that mobilizes intracellular nutrients to meet energy requirements in the event of nutrient deficiency. Dairy cows with severe fatty liver generally have a severe negative energy balance (NEB). Therefore, the aim of this study was to investigate the hepatic autophagy status in dairy cows with severe fatty liver. Liver and blood samples were collected from healthy (n = 15) and severe fatty liver (n = 15) cows. Liver tissue was biopsied and serum samples were collected on 3 consecutive days. Dairy cows with severe fatty liver displayed significant hepatic lipid accumulation. Activities of liver injury indicators (aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, and gamma-glutamyl transferase) were all greater in cows with severe fatty liver. The blood concentrations of haptoglobin and serum amyloid A were also markedly higher in dairy cows with severe fatty liver. The mRNA expression of autophagosomal formation related gene ULK1 was lower in the liver of dairy cows with
severe fatty liver than in healthy cows. However, the expressions of *Becn1*, vacuolar protein sorting 34 (*Vps34*), autophagy-related gene (*ATG*) 3, *ATG5*, *ATG12*, were comparable between these 2 groups. More importantly, the ubiquitinated proteins, protein expressions of sequestosome-1 (*SQSTM1*, also called p62) and microtubule-associated protein 1 light chain 3 (*MAP1LC3*, also called LC3)-II were significantly higher in the liver of dairy cows with severe fatty liver than in healthy cows. Moreover, transmission electron microscopy observation showed increased number of autophagosomes in the liver of dairy cows with severe fatty liver. Taken together, these results indicate that dairy cows with severe fatty liver display liver damage, systemic inflammation and impaired hepatic autophagic flux. Furthermore, impaired autophagic flux may result in liver damage and inflammation and further promote the occurrence and development of fatty liver. This study also demonstrates that the hepatic adaptive capacity is impaired in dairy cows with severe fatty liver.

**Key Words:** fatty liver, autophagic activity, dairy cow

170 **A fluorescence resonance energy transfer approach to determine intracellular zinc bioavailability in bovine mammary epithelial cells.** R. Mohan*, F. Rosa, and J. S. Osorio, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

Zinc is a key micronutrient involved in many cellular processes and biological pathways including oxidative stress and inflammation. Therefore, we evaluated the effect of the intracellular bioavailability of Zn in bovine mammary epithelial alveolar cells (MacT) incubated at 0, 10, and 50 μM concentrations of Zn. Before transfection, MacT cells were cultivated in high glucose Dulbecco modified Eagle’s medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and Fungizone Antimycotic. The plasmid used in this study was the peZinCh-NB (Addgene) designed to detect intracellular Zn through a fluorescence resonance energy transfer (FRET) technology. Cells were seeded 24 h before transfection at 30,000 cells/well in a 96-well plate. Additional cells were seeded at 300,000 cells/well in a 6-well plate for gene expression analysis. Cells were transfected with the transfection reagent Lipofectamine 3000 at 0.3 μL/well and at 150 ng/well of plasmid in a reduced serum medium (OptiMEM) deprived of FBS. Transfected cells were treated for 24h in triplicates with 0, 10, and 50 μM Zn. An inverted fluorescent microscope for live imagining (EVOS FL Auto) equipped with a motorized scanning stage, and an environment-controlled chamber at 37°C and 5.0% of CO2 was used to take 4 pictures/well at 4× magnification at 0, 12, and 24 h post-treatment. Quantification of Zn and cell viability were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS. Overall intracellular availability of Zn increased \( (P < 0.01) \) in cells incubated with both 10 μM and 50 μM of Zn as early as 12 h post-treatment. At 12 and 24 h post-treatment, the greatest \( (P < 0.01) \) Zn intracellular bioavailability was observed with 50 μM of Zn, when compared with 10 μM of Zn and control. The cell viability at 24 h was similar \( (P > 0.26) \) across treatments with 81.59, 80.92, and 75.06% for control, 10 μM and 50 μM of Zn, respectively. These preliminary data indicate that intracellular Zn can be detected via a fluorescent protein system in real-time in bovine cells. To expand on these effects, gene expression analysis will be performed.

**Key Words:** zinc, bovine cells, fluorescent protein