
Monitoring of behavioral variables has been used to assess individual health and estrus presentation in dairy cows. The objective was to evaluate population dynamics of behavioral variables during postpartum to determine cut-off values associated with the subsequent occurrence of health events up to 21 DIM. Cows (n = 1,350) in an organic certified herd in CO, were affixed with CowManager tags (AABV, the Netherlands) for monitoring of rumination, eating, and active times (min/d). Health events were retrieved from on-farm software (PCDart, Raleigh, NC). Cows were categorized as healthy (HT) or diagnosed with at least one health event (DZ) within 21 DIM. Explanatory variables included behavioral variables summarized in 20 min intervals at 0, 1, and 2 DIM. Logistic regression and ROC curves analysis were used to calculate cut-off values maximizing sensitivity (Se, %) and specificity (Sp, %). Calving season, parity, and dystocia were included in the models as controlling variables when P<0.1. Overall, 17.2% of the cows were in the DZ category.Increments of the behavioral variables were associated with lower odds of disease presentation. For each 20 min/d increment in rumination at 0, 1, 2 DIM the odds of DZ decreased by 0.95 (95% CI: 0.92–0.98), 0.92 (0.9–0.95), and 0.90 (0.87–0.92), respectively. For each 20 min/d increment in eating time at 0, 1, 2 DIM the odds of DZ decreased by 0.89 (0.86–0.92), 0.85 (0.82–0.89), and 0.84 (0.8–0.88), respectively. For active time, the odds of DZ decreased by 0.96 (0.93–0.99), 0.94 (0.91–0.96), 0.95 (0.92–0.98), respectively. Eating time at 1 DIM had the greatest area under curve (AUC = 0.69), followed by eating (AUC = 0.68) and rumination time (AU = 0.64) at 2 DIM. The cut-off values that optimized Se and Sp at these points were 233.5 (Se = 65; Sp = 61), 285.9 (Se = 63.2; Sp = 61.3), and 438 (Se = 61.3; Sp = 52) min/d, respectively. AUC for active time was < 0.6 and Se and Sp < 50%. This study suggests that rumination time and eating time have discriminative value at early lactation for cows at risk of health disorders and these assessment points could be used for developing targeted prophylaxis after calving.

Key Words: behavior, disease, performance

M31 Consistency of lying time is associated with reduced serum nonesterified fatty acids of prepartum dairy heifers and cows. B. T. Menichetti*, J. M. Piñeiro, A. Garcia-Guerra, A. E. Relling, W. P. Weiss, and G. M. Schuenemann, 1Department of Veterinary Preventative Medicine, The Ohio State University, Columbus, OH, 2Department of Animal Science, Texas A&M AgriLife Extension Service, Amarillo, TX, 3Department of Animal Sciences, The Ohio State University, Columbus, OH, 4Department of Animal Sciences, The Ohio State University, Wooster, OH.

The objective was to assess the association of pre-partum lying time (LT) with serum nonesterified fatty acids (NEFA) in prepartum dairy heifers and cows. A total of 723 Holstein pregnant dairy heifers (n = 229) and multiparous cows (n = 494) from 3 dairy herds were enrolled at 14 ± 3 d before parturition (dpp). A cohort of 20 to 36 cows was enrolled monthly at each dairy herd and electronic data loggers (IceCube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual heifers and cows to assess their LT. To assess consistency of LT, the coefficient of variation (CV) of LT was computed for each individual animal by dividing the SD by mean LT within 7 d before blood NEFA collection and reported as an absolute ratio. Blood samples were collected from animals at 7 ± 3 dpp for serum NEFA concentration. Herd 1 regrouped animals 3 times per week while herds 2 and 3 regrouped animals once per week. PROC CORR procedure of SAS was used to assess the relationship between CV of LT and prepartum serum NEFA. Correlations were adjusted by parity, body condition score (BCS) at enrollment, season (winter, spring, summer and fall), and herd. Season, herd, parity, and BCS were associated with CV of LT (P < 0.05). Pregnant heifers had an overall mild (r = 0.28, P < 0.0001; herd 1: r = 0.13, herd 2: r = 0.52, and herd 3: r = 0.19) significant positive correlation between mean CV of LT and serum NEFA. Multiparous cows had an overall weak (r = 0.11, P = 0.01; herd 1: r = 0.07, herd 2: r = 0.13, and herd 3: r = 0.14) significant positive correlation between mean CV of LT and serum NEFA. The greater the CV of LT (<0.10 vs >0.30) within 7 d before blood collection, the greater the concentration of serum NEFA (302 µEq/L vs 450 µEq/L; P = 0.005). These findings provide evidence that consistency of LT of prepartum heifers and cows should be considered when troubleshooting metabolic problems at the herd level.

Key Words: prepartum, lying time, NEFA


The goal of this study was to use the metabolic health index (MHI) categorization to evaluate metabolism and productive performance of Holstein postpartum cows. Four hundred 21 cows were evaluated, 173 primiparous and 248 multiparous, from 26 herds in Paraná State, Southern Brazil. Each cow had a single blood sample collected between 0 to 21 DIM. Serum AST, GGT, BHB, NEFA, Ca, glucose, albumin, bilirubin, and cholesterol were evaluated. A MHI was calculated using albumin, bilirubin and cholesterol concentrations, as well as their general means and SD (Gallagher et al., 2019) within 3 classes of lactation week; between 0 to 7 DIM, 7 to 14 DIM, and 15 to 21 DIM. All cows were categorized as medium (mean MHI – 0.5 SD; n = 185 cows; MHI = 0.152), low (<mean MHI – 0.5 SD; n = 108 cows, MHI = −2.832) and high (>mean MHI + 0.5 SD; n = 128 cows; MHI = 2.17) groups, and each cow was categorized within its DIM range. Milk yield (kg), and fat and protein contents in the first test-day were recorded. Fat:protein ratio (FPR) was calculated and accumulated milk up to 30 and 100 DIM (kg) were recorded. Statistical analysis was conducted with GLM procedure from SAS. Cows in the low MHI group had higher BHB (1.00 vs. 0.64 mmol/L; P < 0.01), AST (104.3 vs. 84.3 U/L; P < 0.01), and NEFA (0.83 vs. 0.47 mmol/L; P < 0.01), and lower glucose (58.0 vs. 61.2 mg/dL; P < 0.01), and Ca (8.9 vs. 10.1 mg/dL; P < 0.01) than the high MHI group. It was observed higher (P < 0.01) milk yields at the first test-day for medium and high MHI groups than for low MHI group (39.1 and 37.9 vs. 35.2 kg/d, respectively). But there were no differences (P > 0.05) in accumulated milk up to 30 and 100 DIM, and milk fat content among MHI classes. It was observed lower (P < 0.01) milk protein content for low MHI group than for medium and high classes (3.10 vs. 3.21 and 3.26%), so these last 2 groups had lower FPR (1.18 and 1.19 vs. 1.26) than the high MHI. Our results suggest that there is an effect of the MHI class on milk yield in the first test-day, and there are effects on some blood metabolites which are not included in the MHI calculation.

Key Words: liver health, transition period

M33 Metabolic markers for purulent vaginal discharge and subclinical endometritis in dairy cows. O. Bogado Pascottini* and S. LeBlanc, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

This study aimed to compare serum markers for systemic inflammation (SI), and liver and energy metabolism in samples obtained —7, 1, 3, 5, 7, 14, 21, and 35 d relative to calving from healthy dairy cows and those diagnosed with purulent vaginal discharge (PVD) or subclinical endometritis (SCE) at 35 d postpartum. Measured metabolites in serum were total calcium (tCa), total protein, albumin, globulin, cholesterol, urea, glucose, gamma-glutamyl transferase, aspartate aminotransferase, glutamate dehy-
driogenase, β-hydroxybutyrate (BHB), nonesterified fatty acids (NEFA), haptoglobin (Hp), and insulin-like growth factor-1 (IGF-1). Holstein cows were classified healthy (neither PVD nor SCE; n = 38), PVD (n = 10) or SCE (n = 10) at 35 d postpartum. The cut-point for PVD was mucopurulent vulval discharge or worse, measured with Metricheck, and for SCE > 5% endometrial polymorphonuclear cells. PVD and SCE were mutually exclusive categories. The association of each blood serum metabolite with reproductive tract health classification was fitted in mixed linear regression models, accounting for repeated measures, sampling day, parity, BCS, and interactions of status and day. Serum haptoglobin was greater at 3, 5, 7, and 15 d postpartum for SCE and at 7 and 35 d postpartum for PVD in comparison to healthy cows. Albumin concentrations were lesser for PVD than healthy at 15 d postpartum and lesser for SCE than healthy at 35 d postpartum. The week before calving, SCE had lesser tCa than healthy cows, and at 7 and 15 d postpartum PVD had lower tCa than healthy cows. At 15 d postpartum, serum NEFA, BHB, and globulin were greater, and IGF-1 lower for SCE than PVD or healthy cows. For all other metabolites, no differences were found. Although PVD or SCE had more indication of postpartum SI (high Hp and low albumin) than healthy cows, markers of energy status were more compromised in SCE than in PVD or healthy cows. This supports the hypothesis that SCE is associated with maladaptation to postpartum metabolic demands and (sterile) SI rather than inflammation associated with uterine infection, as previously demonstrated by other authors for PVD.

**Key Words:** transition period, uterine disease, inflammation

**M34** Late gestation conditions leading to postpartal subclinical ketosis in dairy cows affects offspring growth and performance. N. A. Carpinelli1*, J. Halfen1,2, S. D. L. Ramirez3, and J. S. Osorio4, 1Dairy and Food Science Department, South Dakota State University, Brookings, SD, 2Núcleo de Ensino, Pesquisa e Extensão em Pecuária (NUPEEC), Universidade Federal de Pelotas, Pelotas, RS, Brazil, 3Universidad de la Salle, Bogotá, DC, Colombia.

Ketosis is a common disease associated with NEB in peripartal dairy cows, and the onset of this condition is linked to prepartal preconditions such as decreased intake and excessive fat mobilization. Likely the same prepartal endocrine peripheral changes that predispose cows to subclinical ketosis (SK) postpartum will affect fetal development and colostrum biosynthesis. Thus, the objective of this retrospective study was to evaluate the effects of maternal SK in peripartal dairy cows on offspring growth and development. Twelve Holstein dairy cows (n = 6/group) were monitored during the peripartal period. Cows were housed in bedded pack pens and fed the same close-up diet (0.63 Mcal/kg DM and 12.3% CP). After parturition cows were moved to a freestall barn and fed a lactation diet (0.73 Mcal/kg DM and 15.6% CP). Precision Xtra was used to measure blood BHB at 1, 3, 5, 7, 9, and 11 d postpartum and cows were classified as subclinically-ketotic (KET; > 1.4 mmol/L) or non-ketotic (NONKET; < 1.4 mmol/L). Daily calves were enrolled if calving difficult < 3, BW ≥ 32kg, single calf, colostrum quality ≥ 21% Brix refractometer, and 3.8 L of colostrum intake from the same dam. Calves born to dams in KET and NONKET groups were assigned accordingly. Calves were monitored from 1 to 8 wk of age. Calves were fed 2.8 L/d of milk replacer 2x/d from 1 to 6 wk, 1x/d in 7 wk, and weaned at 8 wk. Calves had ad libitum access to starter and water. The BW and withers height (WH) were measured weekly until wk 8. Health checks were performed daily. Data were analyzed using the MIXED procedure of SAS. Birth BW was greater (P = 0.05; 42.9 vs 37.9 kg) in KET calves than NONKET. However, a slower (P = 0.02) growth rate was observed in KET calves than NONKET from 1 to 8 wk (53.1 vs 56.2 kg). The latter was reflected in a lower (P = 0.05) weaning BW at 8 wk in KET than NONKET (713. vs 74.8 kg). Overall, offspring’s WH was not affected (P = 0.016) by maternal SK. Results suggest that prepartal endocrine and metabolic mechanisms predisposing dairy cows to postpartal SK may produce long-lasting effects on the offspring’s growth and development.

**Key Words:** calves, ketosis, fetal programing

**M35** Effect of timing of prepartum vaccination relative to pen change of dairy cows on lying time and serum glucose, nonesterified fatty acids, and calcium at calving. B. T. Menichetti1, A. Garcia-Guerra2, J. Lakritz3, W. P. Weiss4, J. S. Velez1, D. Merchant1, and G. M. Schuennemann1, 1Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, 2Department of Animal Sciences, The Ohio State University, Columbus, OH, 3Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, 4Aurora Organic Farms, Boulder, CO.

The objective was to assess the effects of timing of prepartum vaccination (Enviracor J5 and Scourguard; Zoetis) relative to pen movements of gestating dairy cows on lying time (LT), energy status (plasma glucose and nonesterified fatty acids [NEFA]) and plasma calcium at calving. Briefly, pregnant multiparous Holstein cows (n = 296) from one dairy herd were randomly allocated into 1 of 3 treatment groups at 35 ± 3 d before anticipated parturition (dpp): 1) vaccination at 28 d and cow pen change at 21 d (V28-M21; n = 104), 2) vaccination and cow pen change at 28 ± 3 d (V28-M28; n = 96) and 3) vaccination and cow pen change at 21 ± 3 d (V21-M21; n = 96). All prepartum calves were loosed-housed in barns with free access to a contiguous dry-lot patio. Every other week, a group of 43 to 53 animals were enrolled and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual cows to assess their LT. Blood samples were collected at 28, 26, 21, 19, 14 dpp and at calving. Data were analyzed using MIXED procedure of SAS accounting for BCS, parity and cohort of enrollment. Regardless of treatment group, LT of prepartum cows ranged from 12 to 13.2 h/d. Cow movement with vaccination reduced LT (P = 0.0003) by 36 min/df for the first 3 d thereafter compared with cow movement alone. V21-M21 cows had higher concentrations of NEFA at 19 (70.1 µEq/L; P < 0.0001) and 14 dpp (161 µEq/L; P = 0.01) compared with V28-M28 (109 µEq/L and 130 µEq/L, respectively) or V28-M21 cows (102 µEq/L and 141 µEq/L, respectively) while no differences among groups were observed at calving. At calving, V28-M21 cows had higher (P = 0.04) glucose concentration (114.5 mg/dL) compared with V21-M21 cows (101.6 mg/dL) with V28-M28 cows (P = 0.004) compared with V21-M21 (37.3%) but did not differ from V28-M28 cows (24.8%). These findings suggest that vaccinating cows at 28 dpp and pen change at 21 dpp would benefit common prepartum changes in metabolism.

**Key Words:** prepartum, fatty acids, hypocalcemia

**M36** Blood biomarkers through an ex vivo LPS challenge under ketotic conditions in peripartal dairy cows. N. A. Carpinelli1*, J. Halfen1,2, F. Rosa1, E. Trevisi1, A. Minuti1, and J. S. Osorio1, 1Dairy and Food Science Department, South Dakota State University, Brookings, SD, 2Núcleo de Ensino, Pesquisa e Extensão em Pecuária (NUPEEC), Universidade Federal de Pelotas, Pelotas, RS, Brazil.

Blood samples were collected at −30, −15, 5, 14 and 30 DIM to evaluate biomarkers of metabolism, inflammation, and oxidative stress at 5 DIM.
blood samples were collected for an ex vivo LPS challenge conducted at 0 (control), 0.01 (low dose), and 5 μg of LPS/mL (high dose) for 3.5h. After LPS challenge, the samples were centrifuged and plasma was obtained for biomarker analysis. The MIXED procedure of SAS was used to analyze the data. A Group × Day (G × D; P < 0.01) was observed in postpartal DMI, where lower DMI was observed in KET cows than NONKET during 1 and 2 wk postpartum. There was a trend (P = 0.15) for greater milk yield in NONKET cows than KET (37.3 vs 32.4 kg). A G × D (P < 0.01) was observed in NEFA, which resulted in a trend (P ≤ 0.10) for greater NEFA in KET than NONKET at 14 and 30 DIM. There was a trend (P = 0.06) for G × D in haptoglobin, where greater (P < 0.01) haptoglobin was observed in NONKET vs KET at 5 DIM. In the ex vivo LPS challenge, a trend (P = 0.06) for Group × LPS was observed in myeloperoxidase (MPO), where MPO tended (P = 0.09) to increase with greater concentrations of LPS in KET while no change (P = 0.29) was observed in NONKET. A Group × LPS (P < 0.01) was observed in IL-1β, where greater (P = 0.05) IL-1β was observed in KET than NONKET at high LPS. These results confirm that ketosis plays an important role in the effective immune response to additional diseases that peripartal dairy cows may endure soon after calving.

Key Words: ketosis, metabolism, lipopolysaccharide

M37  Assessment of the effects of intrauterine dextrose on clinical cure rate, body condition score, and concentration of β-hydroxybutyrate and haptoglobin in postpartum dairy cows diagnosed with clinical metritis. A. A. Barragan*, J. Hamilton1, E. Hovingh1, M. Martinez1, L. Byler1, S. Bas2, J. Zug1, and S. Haan3. 1Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA, 2Phytobiotics Futterzusatzstoffe GmbH Bvd, Villa Maria, Córdoba, Argentina, 3Zugstead Farm, Mifflintown, PA.

The objective of this study was to assess the effects of intrauterine dextrose (50%) infusion on clinical cure rate, body condition score (BCS), β-hydroxybutyrate (BHB) and systemic inflammation (haptoglobin; HP) in dairy cows diagnosed with clinical metritis (CM). Cows (n = 351) from a dairy farm located in Pennsylvania were screened at 7 ± 3 DIM using a Metricheck device to assess vaginal discharge. Cows that presented a fetid red-brownish watery vaginal discharge (n = 53) were classified as CM cows, blocked by parity and randomly assigned to 1 of 2 groups: 1) CONV (n = 27): 2 injectable (SC) administrations of ceftiofur (6.6 mg/Kg; Excede, Zoetis Inc.) 72 h apart; and 2) DEX (n = 26): 3 intrauterine infusions of dextrose (50%; 1 L/cow) 24 h apart. Furthermore, cows that presented a normal vaginal discharge at 7 ± 3 DIM (i.e., dense clear discharge; healthy group; HLT; n = 27) were randomly selected and matched by parity to CONV and DEX cows. Cows were re-screened at 14 ± 3 DIM and 21 ± 3 DIM to assess clinical cure rate. Body condition score was assessed and blood samples (i.e., BHB, HP) were collected at enrollment and at 14 ± 3 DIM and 21 ± 3 DIM. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized complete block design. There was no difference in clinical cure rate at 14 ± 3 DIM (CONV = 50.27 ± 12.49%; DEX = 45.9 ± 12.37%) and 21 ± 3 DIM (CONV = 88.05 ± 6.59%; DEX = 88.51 ± 6.35%) between CONV and DEX groups. HLT cows tended to have a higher BCS at 21 ± 3 DIM compared with cows in the CONV and DEX groups (CONV = 3.05 ± 0.09 pts.; DEX = 2.96 ± 0.09 pts.; HLT = 3.25 ± 0.10 pts.). There was no difference in BHB concentrations between study groups. Overall, cows treated with DEX had higher HP concentrations compared with HLT cows (CONV = 119.91 ± 23.52 μg/mL; DEX = 153.06 ± 22.75 μg/mL; HLT = 71.52 ± 23.91 μg/mL). These results suggest that, although intrauterine infusion of dextrose may achieve similar clinical cure rates as a conventional antibiotic treatment, the treatment may not be as effective on decreasing systemic inflammation in CM cows.

Key Words: metritis, dextrose, inflammation

M38  The effect of vaccination with a Mannheimia haemolytica subunit vaccine on milk yield in lactating dairy cows. M. Overtorn* and M. Armfelt, Elanco Animal Health, Greenfield, IN.

Many herds in the US use a Mannheimia vaccine in lactating dairy cows as an aid in reducing the impact of Bovine Respiratory Disease (BRD). A prospective, randomized clinical trial was conducted to evaluate milk production changes associated with the administration of a Mannheimia haemolytica subunit vaccine (MHSV; Nuplura PH, Elanco Animal Health, Greenfield, IN). A total of 972 lactating Holstein dairy cow were randomized into 1 of 3 study groups on a Midwestern dairy. Daily milk production during the 7 d before treatment was averaged and used as the baseline for comparison. Lactation Group (1, 2, or 3*), DIM, and 7-d pretreatment average milk production (41.7 kg) was not different across the groups. Cows were treated at the end of the morning milking on vaccination day according to group assignment of (MHSV), Saline, or Negative Control (no injection). Cow-level milk production was then averaged for vaccination day (Day 0) and the following 2 d. The 3-d post-treatment milk average was then compared with the baseline 7 d average milk yield for each group. The association between vaccination with MHSV and subsequent milk production changes while controlling for explanatory factors was compared with both the Saline and Negative Control groups using ANOVA and fitting least squares using JMP Pro 14.3.0. Negative Control cows served as the referent value. Saline group’s decline in milk was 0.25 kg greater than Negative Control cows but the difference was not significant (P = 0.57). The Nuplura group’s decline in milk was 0.7 kg more than the Negative Control, P = 0.02, but the Saline Nuplura declines were not significantly different (P = 0.17). On Day 1, the Nuplura group loss was 1.18 kg more than the Negative Control cows (P < 0.01) but the Saline group’s loss of 0.13 kg was not different from Negative Control (P = 0.92). On Day 0 and Day 2, there were no significant differences between groups.

Key Words: Mannheimia vaccine, dairy cow, bovine respiratory disease


OmniGen-AF (OGAF; Phibro Animal Health, Teenack, NJ) is a feed additive with demonstrated benefit on improving cellular immunocompetence of stressed animals. OmniGen Pro (OGPRO, Phibro Animal Health) is a new product built on the OmniGen foundation, developed to maintain the principles of OGAF while improving gastrointestinal tract function and integrity. This study aimed to confirm the similar immunological effects of these 2 products. Fifteen Dorset ewes (39 kg BW) were randomly assigned to 1 of 3 diets: control (no additive), OGAF (6 g OGAF/head/d), and OGPRO (6.75 g OGPRO/head/d). Ewes were fed 227 g of grain, with the additives, and ad libitum hay for 23 d. On d 20, ewes were IV-injected with dexamethasone (DEX) at a rate of 0.4 mg/kg BW/day for 3 d. D 0, 20 and 23 blood draws were used to quantify whole blood gene expression of IL-8, IL-1β, and D-Selectin (gene expression), nor for neutrophil functional activity before or after the DEX challenge. OmniGen increased IL-8 expression was greater for OmniGen-fed ewes at d 20 (P = 0.05) and 23 (P < 0.01). Pre-DEX challenge, IL-8-Rβ gene expression, L-selectin protein abundance, and neutrophil functional activity did not differ (P > 0.05) between treatment groups. Following the DEX challenge, OmniGen increased IL-8-Rβ gene expression (P = 0.02), L-selectin protein abundance (P = 0.03), neutrophil phagocytosis (P < 0.01) and oxidative burst activity (P < 0.01). No differences were observed (P > 0.05) between OGAF and OGPRO for L-selectin (gene expression and protein abundance), IL-8-Rβ (gene expression), nor for neutrophil functional activity before or after the DEX challenge.
DEX. This study confirms OGAF and OGPRO have similar beneficial effects on preventing the immunosuppressive effects of dexamethasone.

**Key Words:** OmniGen, immunocompetence, immunosuppression

**M40**  Low expression of sirtuin 1 in the dairy cows with mild fatty liver alters hepatic lipid metabolism. Y. Li*, H. Ding*, S. Feng*, J. Li*, X. Wang*, J. Wu*, Y. Liang*, and J. J. Loo*, 1Anhui Agricultural University, Hefei, Anhui, China, 2University of Illinois at Urbana-Champaign, Urbana, IL.

This study aimed to investigate the effect of hepatic steatosis on mRNA and protein abundance of the sirtuin 1 (SIRT1), along with the target mRNA and protein expressions and activities of related to lipid metabolism in liver tissue. Lactating Holstein multiparous cows (days postpartum 70 ± 12) of the same breed, age and having similar milk production characteristics and body condition scores were selected from a commercial dairy farm. Control cows (n = 6, parity 3.0 ± 0.2, milk production 287 ± 27 kg/d) and mild fatty liver cows (n = 6, parity 2.3 ± 1.5, milk production 20 ± 6 kg/d) were retrospectively selected based on liver triglycerides (TG) content (% wet liver)(control = 0.45, fatty liver = 7.65, 5–10 was considered as mild fatty liver). Dairy cattle were slaughtered for collecting liver tissue samples and processed as part of the normal work of a commercial abattoir. One-way ANOVA (with Dunnett’s post hoc test) and a 2-tailed Student’s t-test were performed to compare the control group, fatty liver cows and the control group. Fatty liver cows had greater concentrations of cholesterol and TG along with the typically vacuolated appearance and greater lipid droplets in the liver. Furthermore, fatty liver cows had greater mRNA and protein abundance related to hepatic lipid synthesis (SREBP-1c, ACSL, ACC and FAS) and lipid transport (L-FABP, ApoE, LDLR and MTTP) (P < 0.05). However, they had lower mRNA and protein abundance associated with fatty acid β-oxidation (SIRT1, PGC-1α, PPARα, RXRa, ACO, CPT1, CPT2 and LCAD) (P < 0.05). Besides, mRNA abundance and enzyme activity of Cu/Zn SOD, CAT, GSH-Px and Mn-SOD decreased and mRNA and protein abundance of Nrf1 and TFAM decreased (P < 0.05). Lower enzyme activities of SIRT1, PGC-1α, Cu/Zn SOD, CAT, GSH-Px and Mn-SOD (P < 0.05) and concentration of GSH (P < 0.05) were observed in fatty liver cows. Overall, the data suggest that low SIRT1 expression associated with hepatic steatosis promotes hepatic fatty acid synthesis and inhibits fatty acid β-oxidation. Hence, SIRT1 might be a novel therapeutic target for coping with fatty liver in dairy cows.

**Key Words:** dairy cow, SIRT1, fatty liver

**M41**  Validation of putative target genes of nuclear factor erythroid 2-related factor 2 (NRF2) in bovine. H. Ford*, M. Bionaz, and S. Busato, Oregon State University, Corvallis, OR.

Finding ways to ameliorate the oxidative stress experienced by high-producing dairy cattle in the periparturient period is one of the many challenges faced by researchers and producers focusing on dairy production. The nuclear receptor transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) has been studied in non-ruminant models and may be utilized in ruminants to reduce oxidative stress through the upregulation of genes associated with antioxidant defense and cell survival. This is extremely important in the mammary gland where heightened oxidative stress can cause cell death and reduce milk production. Despite this, research on NRF2 in ruminants is limited and there is a need to identify the genes affected by NRF2 modulation and the associated biological outcomes. In this study, the aim was to validate in bovine a set of 7 putative NRF2 target genes as established in monogastric animals. This was achieved by using a combination of 10 μM of the NRF2 agonist sulforaphane (SFN), 100 nM of the NRF2 antagonist brusatol (BRU), NRF2-translation inhibition via Morpholino, and an untreated control in immortalized bovine mammary epithelial cells (MAC-T). RNA was extracted for RT-qPCR. Genes upregulated by SFN were GSR, KEAP1, NFE2L2, GCLC and NQO1 while treatment with BRU resulted in significant downregulation of GSTM1 and NQO1, but upregulation of GCLC and NFE2L2. Furthermore, treatment of cells with Morpholino resulted in downregulation of GSTM1 and GPX1, and NQO1 but upregulation of GCR, GCLC, and KEAP1. Data indicated that transcription of GSTM1 and GPX1 requires a basic activation of NRF2 but is not induced by it. Transcription of GCLC and NFE2L2 is partly controlled by NRF2 but the regulation is complex, likely involving other transcription factors. The data revealed that NQO1 is the only transcript among the ones tested that is tightly regulated by NRF2 (i.e., true NRF2 target). All the other putative NRF2 targets are likely controlled by additional transcription factors in bovine mammary cells. Our data highlighted the problem of using in ruminant target genes previously identified in monogastric animals and, thus, the need to identify reliable NRF2 targets in bovine.

**Key Words:** NRF2, cow, RT-qPCR

**M42**  Whole-transcriptome analysis of nuclear factor erythroid 2-related factor 2 (NRF2) modulation in bovine mammary cells. H. Ford*, M. Bionaz, and S. Busato, Oregon State University, Corvallis, OR.

The nuclear factor erythroid 2-related factor 2 (NRF2) has been well studied in non-ruminant models and has gained increased attention for its role as a potential target in promoting antioxidant defense and cell survival. The role of NRF2 in bovine and other ruminant models is not well studied. Given the extreme metabolic changes and associated oxidative stress encountered by dairy cattle as they transition from pregnancy to lactation, there is a clear need to better understand the role of NRF2 in ruminants. To accomplish this, MACT cells were treated with sulforaphane (SFN), a putative NRF2 activator, brusatol (BRU), a NRF2 inhibitor, and Morpholino (MOR), a synthetic oligonucleotide inhibitor of NRF2 translation. After 24h of treatment, both BRU and MOR decreased > 80% while SFN increased > 8-fold NRF2 activation as assessed via a gene reporter assay. RNA was extracted, prepared for sequencing using the QuantSeq kit (Lexogen), and sequenced using HighSeq 3500 (Illumina). The RNA-Seq data were normalized by RPM and statistically analyzed via ANOVA using JMP Genomics (SAS). Functional analysis of differentially expressed genes (DEG; FDR < 0.05) compared with an untreated control was performed using DAVID and the Dynamic Impact Approach. The 943 DEG by BRU are associated with overall inhibition of metabolism, especially amino acid and energy metabolism. MOR had 421 DEG associated with activation of inflammatory and immune responses while inhibiting collagen and extracellular matrix. The 502 DEG by SFN are associated with induction of disulfide bonds, glycoproteins and selenoamino acid metabolism, but associated with inhibition of metabolism of other amino acids, ATPase activity, and intracellular trafficking. There were more DEG with opposite effects between BRU and SFN than MOR and SFN (151 vs. 48), indicating that BRU and SFN are modulating not only NRF2. Our study provided the first characterization of NRF2 role in bovine mammary cells highlighting a role of NRF2 that goes beyond the oxidative stress response.

**Key Words:** NRF2, cow, RNA-Seq


Alpha-1-acid glycoprotein (AGP) is an anti-inflammatory acute-phase protein which is negatively associated with feed intake in postpartum...
dairy cows. Our objective was to determine associations between AGP and other biomarkers in transition dairy cows, and to evaluate the utility of AGP for predicting postpartum disease risk. Plasma samples (n = 2,086) from 434 Holstein cows in 6 studies were analyzed for AGP, glucose, insulin, free fatty acids (FFA), β-hydroxybutyrate (BHB), and haptoglobin on d −21, −12 ± 3, −3, 1, 3, 7 ± 1, 14 ± 1, and 21 ± 1 relative to parturition. A model was constructed with linear and quadratic fixed effects of AGP, with random effects of treatment (study). Quadratic effects with \( P > 0.20 \) were removed. Nominal logistics were used to determine the association of AGP concentration with disease incidence using fixed effects of BHB \( (P = 0.02) \) and negatively associated with insulin \( (P = 0.04) \), and d −3 AGP was positively associated with d 21 FFA \( (P = 0.04) \). On d 3 and 7, AGP was positively associated with BHB on d 7 \( (P < 0.01) \). Haptoglobin and AGP were positively associated on d 3 \( (P < 0.001) \), d 7 \( (P < 0.001) \), and quadratically associated on d 14 \( (P = 0.02) \). On d −12, AGP was associated with postpartum incidence of retained placenta (RP; \( P = 0.05 \)) and tended to be associated with milk fever \( (P = 0.06) \). Plasma AGP on d 3, d 7, and d 14 was highly associated with postpartum incidence of displaced abomasum (DA), RP, and metritis \( (P < 0.001) \). Receiver operating characteristics for the association between AGP and postpartum DA incidence on d 3 \( (P = 0.02) \), d 7 \( (P < 0.001) \) and d 14 \( (P = 0.05) \) were excellent, with area under the curve of 0.99, 1.0, and 0.93, respectively. Plasma AGP on d 1 \( (P = 0.06) \) and d 3 \( (P = 0.09) \) tended to be associated with retention to 280 d in milk. Despite significant associations between AGP and postpartum disease, its utility for disease forecasting was less promising. Nevertheless, limited associations between AGP and metabolic biomarkers suggest it may serve as a unique predictive variable.

**Key Words:** acute-phase protein, biomarker