

# Animal Health: Lactating cows

**T1 Health and productive responses of dairy cows treated with reduced doses of recombinant bovine somatotropin during the periparturient period.** Paula R. B. Silva<sup>\*1</sup>, Henrique F. Soares<sup>1</sup>, Gabriel D. Bombardelli<sup>1</sup>, Wagner D. Braz<sup>1</sup>, Daniela N. Liboreiro<sup>1</sup>, and Ricardo C. Chebel<sup>1,2</sup>, <sup>1</sup>University of Minnesota, St Paul, MN, <sup>2</sup>University of Florida, Gainesville, FL.

The objectives were to evaluate the effects of treatment of dairy cows with recombinant bovine somatotropin (rbST) during the periparturient period on health and production. Holstein (HO, n = 302) and Jersey (JS, n = 522) cows were enrolled in the experiment 21 d before calving. Cows were assigned randomly to the control and rbST (125 mg of rbST every 7 d from -21 to 21 d relative to calving) treatments. At -21, 0, 30, and 60 d relative to calving, BCS was recorded. Occurrence of retained placenta (RP) was recorded and cows were examined for diagnosis of metritis 4, 7, 10, and 14 d postpartum (DIM). Acute metritis (metritis and fever and/or anorexia) was recorded in the JS herd. Blood was sampled 7 and 14 d postpartum to determine ketosis incidence. Cows were milked thrice daily. In the HO herd, milk yield and composition were determined weekly until 21 d postpartum; thereafter, monthly milk yield was recorded. In the JS herd, milk yield and composition were recorded monthly. Dichotomous data were analyzed by logistic regression and continuous data were analyzed by ANOVA. Incidence of RP tended to be affected by treatment (HO-control = 14.4%, rbST = 6.1%; JS-control = 1.5%, rbST = 1.2%;  $P = 0.10$ ). Incidence of metritis was reduced by rbST treatment (HO-control = 26.2%, rbST = 16.8%; JS-control = 19.9%, rbST = 13.3%;  $P < 0.01$ ). Treatment tended to affect the incidence of ketosis (HO-control = 8.2%, rbST = 10.0%; JS-control = 8.4%, rbST = 13.1%;  $P = 0.09$ ). Postpartum BCS was ( $P < 0.05$ ) lower for rbST treated cows (HO-control =  $3.08 \pm 0.03$ , rbST =  $2.99 \pm 0.03$ ; JS-control =  $2.83 \pm 0.03$ , rbST =  $2.80 \pm 0.03$ ). Yield of energy corrected milk (ECM) in the first 3 weeks postpartum in the HO herd was greater for rbST cows (control =  $33.0 \pm 1.3$ , rbST =  $36.3 \pm 1.3$  kg/d;  $P = 0.05$ ) but yield of ECM in the first month postpartum was not affected by treatment in the JS herd (control =  $26.6 \pm 0.6$ , rbST =  $27.7 \pm 0.6$  kg/d;  $P = 0.15$ ). Milk yield in the first month postpartum was ( $P = 0.05$ ) increased by rbST treatment (HO-control =  $35.0 \pm 0.9$ , rbST =  $37.9 \pm 0.8$  kg/d; JS-control =  $26.9 \pm 0.8$ , rbST =  $28.1 \pm 0.8$  kg/d). Treatment of periparturient cows with small doses of rbST improved postpartum health and increased milk yield.

**Key Words:** transition cow, recombinant bovine somatotropin, health

**T2 Reduction in hepatic functionality can delay resumption of ovarian activity postpartum in dairy cows.** Paula Montagner<sup>\*1,2</sup>, Rubens A. Pereira<sup>1,2</sup>, Ana Rita T. Krause<sup>1,2</sup>, Marina M. Weschenfelder<sup>1,2</sup>, Elizabeth Schwegler<sup>1,2</sup>, Fernanda M. Gonçalves<sup>1,2</sup>, Carolina B. Jacometo<sup>1,2</sup>, Cássio C. Brauner<sup>1,2</sup>, and Marcio N. Corrêa<sup>1,2</sup>, <sup>1</sup>Federal University of Pelotas, Pelotas, RS, Brazil, <sup>2</sup>Center for Research, Teaching and Extension in Animal Science (NUPEEC), Pelotas, RS, Brazil.

After calving, the liver undergoes a metabolic overload due to high energetic and nutritional demand caused by milk synthesis and these challenges can induce inflammation and impair liver function. Several studies demonstrate that inflammation around calving is responsible for decreased productive efficiency and fertility. For this a composite index based on multiple variables associated with inflammation, the liver functionality index (LFI), can be promising for aid in the diagno-

sis of diseases and reproductive problems on dairy farms. The LFI is determined utilizing 3 biomarkers of hepatic function: albumin, total cholesterol and bilirubin, measured on 3 and 28 d after calving. The aim of this study was to evaluate the resumption of postpartum ovarian activity of dairy cows with low LFI. Twenty cows Holstein were evaluated from d 21 prepartum until 30 d postpartum. The cows were divided into 2 groups: Low LFI (LLFI:  $\leq -7$  to  $-12$ ; n: 10) and High LFI (HLFI:  $> -7$  to  $-4$ ; n: 10). Serum concentration of progesterone (P4) was analyzed on d 16, 23, 30, 37 and 44 postpartum to predict the resumption of ovarian activity. The cows which had P4 concentration higher than 1ng/mL in 2 consecutive assays were considered ovulatory, while cows with P4 concentration below 1ng/ml during same period were considered anovulatory. The statistical analyses were performed using SAS 9.0 software. The statistical model CATMOD (Categorical Data Analysis Procedures) was used for analysis of categorical data of ovulation.  $P < 0.05$  was considered significant. The proportion cows that resumed ovarian activity to 44 d postpartum was lower (29%; 3/10;  $P < 0.05$ ) in the LLFI, compared with HLFI group (86%, 9/10). In conclusion, dairy cows with reduced hepatic functionality after calving have delayed resumption of ovarian activity, which demonstrates that the LFI can be a useful index to predict problems in resumption of ovulation in dairy cows.

**Key Words:** liver functionality index, ovarian activity, progesterone

**T3 Organic trace minerals during the transition period. 4. Corium gene expression profiling reveals a beneficial effect of supplementing Zn, Mn, and Cu from Availa Mins and Co from CoPro on hoof health of peripartur dairy cows.** J. S. Osorio<sup>\*1</sup>, E. F. Garrett<sup>1</sup>, M. M. Elhanafy<sup>1</sup>, E. Trevisi<sup>2</sup>, J. K. Drackley<sup>1</sup>, M. T. Socha<sup>3</sup>, and J. J. Loores<sup>1</sup>, <sup>1</sup>University of Illinois at Urbana-Champaign, Urbana, IL, <sup>2</sup>Università Cattolica del Sacro Cuore, Piacenza, Italy, <sup>3</sup>Zinpro Corporation, Eden Prairie, MN.

Lameness remains a major health problem and cause of death, in dairy cows. The negative effects of the transition period may make cows prone to become lame. Positive effects of supplementation of organic trace minerals (AAC) on hoof health have been reported. A hoof biopsy method to study the transcriptome of corium tissue has been published (Osorio et al., J. Dairy Sci. 95:6388–6396). Objective was to evaluate corium mRNA expression of genes related to claw conformation, oxidative stress, chemotaxis, inflammation, and transcription regulation in peripartur cows supplemented with AAC or inorganic (INO) trace minerals. Twenty Holstein dairy cows received a common prepartal (1.5 Mcal/kg DM, 15% CP) and postpartal (1.76 Mcal/kg DM, 18% CP) diet. Both diets were partially supplemented with an INO mix of Zn, Mn, and Cu to supply 35, 45, and 6 ppm, respectively, of the total diet DM. Cows were assigned to treatments in a randomized complete block design, receiving an oral bolus daily with a mix of INO (n = 10) or AAC (n = 9) containing Zn, Mn, Cu, and Co to achieve 75, 65, 11, and 1 ppm, respectively, in total diet DM. Treatments began on -30 d and continued until 30 d postpartum. Inorganic trace minerals were provided in sulfate form and AAC were supplied via Availa Zn, Availa Mn, Availa Cu, and CoPro (Zinpro Corp., Eden Prairie, MN). Hoof biopsies were harvested at 30 d postpartum from the sole of the rear right lateral claw in the right rear limb. Data on 31 genes were analyzed using the MIXED procedure of SAS. Expression of keratin 5 (*KRT5*) was lower ( $P < 0.01$ ) and biotinidase (*BTD*) was greater ( $P < 0.01$ ) in AAC cows, suggesting that AAC cows had a lower requirement of keratins for hoof

tissue repair, while having additional biotin for claw conformation. In contrast, a concomitantly greater ( $P < 0.06$ ) expression of toll-like receptor 2 (*TLR2*), tumor necrosis factor (*TNF*), and interleukin 1  $\beta$  (*IL1B*) in INO cows suggested a greater inflammatory status in hoof tissue. Overall, data suggest that periparturient AAC supplementation ameliorates the negative effect of transition period on hoof health.

**Key Words:** hoof biopsy, trace mineral, transition cow

**T4 Effects of postpartum uterine diseases on milk yield, milk components, and culling in dairy cows under certified organic management.** J. M. Piñeiro<sup>\*1</sup>, M. G. Maquivar<sup>2</sup>, A. A. Barragan<sup>1</sup>, J. S. Velez<sup>3</sup>, H. Bothe<sup>3</sup>, and G. M. Schuenemann<sup>1</sup>, <sup>1</sup>The Ohio State University, Columbus, OH, <sup>2</sup>Washington State University, Pullman, WA, <sup>3</sup>Aurora Organic Farms, Boulder, CO.

The objective was to assess the effect of postpartum uterine diseases on milk yield (kg), milk components (SCC and percent fat and protein), and culling. Cows ( $n = 3,227$ ) from 2 dairy herds were screened for retained placenta (RP;  $> 24$  h after parturition), metritis (within 20 d in milk [DIM]), and purulent vaginal discharge (PVD) at  $26 \pm 3$  DIM. Milk yield and components from the DHIA test-days and cows culled from farm records up to 300 DIM were collected. Weekly, a list of cows by DIM was obtained using on-farm computer records and screened for RP (presence of fetal membranes outside the vulva), metritis (fetid brown-red watery vaginal discharge and fever), and PVD (gloved hand technique). PVD was defined as any cow presenting a score of 2 or 3 (0–3 scale; mucopurulent or worse vaginal discharge) at the time of exam. Parity (lactations 1, 2 and  $\geq 3$ ) of cows was considered for milk yield, milk components, and culling. The statistical analyses were performed using MIXED (milk yield and components) and GLIMMIX (culling) procedures of SAS. Cows with metritis, RP or PVD had an additive effect on milk yield, milk components, and culling. Regardless of parity, lactating cows diagnosed with uterine diseases (all combined) had significantly reduced milk yield (by 2–3.9 kg/cow/d) for at least one of the first 4 DHIA test-days ( $P < 0.05$ ), but was not different at later tests. For the first 2 DHIA test-days, lactating cows diagnosed with uterine disease (all combined) had significantly higher SCC ( $232 \times 10^3$  cells/mL) and fat content (3.7%) compared with cows without uterine diseases ( $164 \times 10^3$  cells/mL and 3.5%, respectively;  $P < 0.05$ ). Milk protein content (%) was not different between cows with or without uterine diseases. Cows with uterine diseases had higher ( $P < 0.05$ ) culling within 60 DIM and significantly lower ( $P < 0.05$ ) pregnancy hazard up to 300 DIM compared with cows without uterine diseases, regardless of parity. Uterine diseases decreased milk yield and changed milk components (SCC and fat) early in lactation; and these diseases were a substantial risk factor within 60 DIM for culling.

**Key Words:** organic, dairy cattle, uterine disease

**T5 Cows diagnosed with metritis showed blood alterations related to innate immunity and carbohydrate and lipid metabolism during early dry off period.** Guanshi Zhang, Dagnachew W. Hailemariam, Elda Dervishi, Qilan Deng, Tran H. Lam, Seyed A. Goldansaz, Suzanna M. Dunn, and Burim N. Ametaj<sup>\*</sup>, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to search for screening biomarkers of metritis in transition dairy cows. Blood samples were collected from the coccygeal vein once per week before morning feeding from 100 multiparous Holstein dairy cows during –8, –4, disease diagnosis, and

+4 and +8 wk relative to parturition. Six healthy cows (CON) and 6 cows that showed clinical signs of disease were selected for intensive serum analyses. Concentrations of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF); haptoglobin (Hp), serum amyloid A (SAA), and lipopolysaccharide binding protein (LBP); and nonesterified fatty acids (NEFA) and  $\beta$ -hydroxybutyrate (BHBA) were measured in serum by ELISA or colorimetric methods. Health status, feed intake, and milk yield were monitored for each cow during the whole experimental period. Data were processed statistically by MIXED procedure of SAS 9.2. Results showed that cows affected by metritis had greater concentrations of lactate ( $2,901$  vs  $2,251 \pm 183$   $\mu\text{mol/L}$ ,  $P = 0.04$ ), IL-6 ( $111$  vs  $27 \pm 13$  pg/mL,  $P = 0.01$ ), TNF ( $0.72$  vs  $0.19 \pm 0.11$  ng/mL,  $P = 0.01$ ), and SAA ( $13,441$  vs  $8,517 \pm 1,517$   $\mu\text{g/mL}$ ,  $P = 0.03$ ) in the serum vs CON. Most interestingly, elevated concentrations of all 4 variables were observed at –8 (lactate:  $3,902$  vs  $2,455$   $\mu\text{mol/L}$ ,  $P = 0.03$ ; IL-6:  $87$  vs  $19$  pg/mL,  $P = 0.05$ ; TNF:  $0.77$  vs  $0.34$  ng/mL,  $P = 0.09$ ; SAA:  $17,960$  vs  $8,448$   $\mu\text{g/mL}$ ,  $P = 0.02$ ) and –4 (lactate:  $3,311$  vs  $2,162$   $\mu\text{mol/L}$ ,  $P = 0.03$ ; IL-6:  $166$  vs  $48$  pg/mL,  $P = 0.02$ ; TNF:  $1.02$  vs  $0.27$  ng/mL,  $P = 0.03$ ; SAA:  $6,003$  vs  $3,461$   $\mu\text{g/mL}$ ,  $P = 0.03$ ) wk before the occurrence of metritis compared with those of CON cows. The disease also lowered the overall milk production ( $38.18$  vs  $42.16 \pm 1.48$  kg/d,  $P = 0.01$ ) and feed intake ( $36.87$  vs  $39.81 \pm 1.30$  kg/d) as well as milk fat ( $3.56$  vs  $3.88$  g/kg,  $P = 0.10$ ) and fat:protein ratio ( $1.10$  vs  $1.38$ ,  $P = 0.05$ ) and was associated with greater SCC ( $44,700$  vs  $29,250 \pm 4,880$  cells/mL,  $P = 0.05$ ). In conclusion, metritis affected serum concentrations of several variables related to innate immunity and carbohydrate metabolism that might serve to monitor health status of transition dairy cows. More research is warranted to validate these data.

**Key Words:** dairy cow, metritis, innate immunity

**T6 Activation of innate immunity ahead of occurrence of ketosis.** Guanshi Zhang, Dagnachew W. Hailemariam, Elda Dervishi, Qilan Deng, Tran H. Lam, Seyed A. Goldansaz, Suzanna M. Dunn, and Burim N. Ametaj<sup>\*</sup>, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to evaluate alterations in blood variables related to innate immunity, carbohydrate and lipid metabolism in transition dairy cows with ketosis. Multiparous Holstein dairy cows ( $n = 100$ ) were involved in the study. Five blood samples were collected from the coccygeal vein during the –8 to +8 wk around parturition, once per week before the morning feeding. Serum samples collected at –8, –4, time of diagnosis of disease, +4 and +8 wk relative to parturition from 6 healthy control cows (CON) and 6 cows with ketosis were selected for analyses. Samples were analyzed for  $\beta$ -hydroxybutyrate (BHBA), lactate, nonesterified fatty acids (NEFA), interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), haptoglobin (Hp), and serum amyloid A (SAA). Health status, feed intake, and milk yield data were monitored for each cow during the whole experimental period. Data were processed statistically by MIXED procedure of SAS 9.2. Results revealed that cows with ketosis had greater concentrations of serum BHBA ( $1,014$  vs  $504 \pm 140$   $\mu\text{mol/L}$ ,  $P = 0.04$ ), lactate ( $4,236$  vs  $2,240 \pm 351$   $\mu\text{mol/L}$ ,  $P < 0.01$ ), IL-6 ( $255$  vs  $27 \pm 55$  pg/mL,  $P = 0.03$ ), TNF ( $0.47$  vs  $0.19 \pm 0.07$  ng/mL,  $P = 0.03$ ), and SAA ( $24,107$  vs  $8,550 \pm 3,457$   $\mu\text{g/mL}$ ,  $P = 0.01$ ) in comparison with the CON animals. Enhanced serum concentrations of BHBA ( $483$  vs  $312 \pm 19$   $\mu\text{mol/L}$  at –4 wk,  $P = 0.02$ ), lactate ( $5,795$  vs  $2,455 \pm 349$   $\mu\text{mol/L}$  at –8 wk,  $P = 0.01$ ; and  $4,478$  vs  $2,162 \pm 185$   $\mu\text{mol/L}$  at –4 wk,  $P = 0.01$ ), IL-6 ( $183$  vs  $19 \pm 6$  pg/mL at –8 wk,  $P < 0.01$ ; and  $330$  vs  $48 \pm 18$  pg/mL at –4 wk,  $P < 0.01$ ) and TNF ( $0.64$  vs  $0.27 \pm 0.05$  ng/mL at –4 wk,  $P = 0.03$ ) at –4 or –8 wks before parturition were identified in cows with ketosis compared with the CON group.

Cows with ketosis also had overall lower feed intake ( $35.70$  vs  $39.81 \pm 1.74$  kg/d,  $P = 0.03$ ) and milk production ( $32.25$  vs  $42.16 \pm 2.53$  kg/d,  $P < 0.05$ ) vs CON animals. Overall results indicate that cows affected by ketosis displayed an activated innate immunity and altered carbohydrate and lipid metabolism several weeks before diagnosis of disease. More research is warranted to better understand the agent(s) that contribute(s) to ketosis in transition dairy cows and to validate utilization of these blood variables to predict disease state in cows.

**Key Words:** dairy cow, ketosis, blood variable

**T7 Effects of repeated oral administration of lipopolysaccharide and lipoteichoic acid either alone or in combination with subcutaneous exposure on metabolite responses in periparturient dairy cows.** Emily F. Eckel\*, Dagnachew W. Hailemariam, Grzegorz Zwierzchowski, Guanshi Zhang, Suzanna M. Dunn, and Burim N. Ametaj, *University of Alberta, Edmonton, AB, Canada.*

Recent investigations implicate bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA) in the pathogenesis of multiple diseases currently affecting transition dairy cows. The objective of this investigation was to evaluate the effect of repeated oral administration of LPS and LTA alone or with subcutaneous injections on carbohydrate and lipid metabolism which are associated with the immune response to LPS and LTA. Two hundred dairy cows were randomly assigned to one of 4 treatments ( $n = 50$  per group) which they received on d -28, -25, -21, -18, and -14 before the expected calving date: 1) 2 mL oral sterile saline (CON), 2) Flat doses of oral LPS and LTA (TRT1), 3) Flat doses oral LPS and LTA plus subcutaneous (sc) injection on d -18 and -14 (TRT2), 4) Oral increasing doses of LPS at  $6.5 \mu\text{g}$  (d -28 and -25),  $32.5 \mu\text{g}$  (d -21 and -18) and  $65 \mu\text{g}$  (d -14), all with flat doses of LTA (TRT3). Flat doses of LPS and LTA were 100 and  $120 \mu\text{g}/\text{cow}$ , respectively, in 2 mL sterile saline. Blood samples from the coccygeal vein were collected during d -28, -14, 0, +7, +14, and +28 and analyzed for insulin, glucose, cholesterol, nonesterified fatty acid (NEFA), lactate, and  $\beta$ -hydroxybutyric acid (BHBA). Preliminary statistical analysis showed a significant effect of treatment on serum insulin ( $P = 0.02$ ). In general, TRT2 had lower serum insulin concentrations than TRT1 and TRT3 while TRT1 had higher concentrations than CTR. A tendency for treatment to affect serum cholesterol ( $P = 0.10$ ) was observed with TRT3 lowering cholesterol compared CTR and TRT1, but not TRT2. Results also showed a tendency for TRT2 to increase NEFA ( $P = 0.06$ ) compared with all other treatments. No effect of treatment was observed for serum glucose ( $P = 0.11$ ), lactate ( $P = 0.13$ ), or BHBA ( $P = 0.21$ ). Overall, preliminary results of this study suggest that repeated oral administration of LPS and LTA alone or in combination with sc injections were associated with minimal changes to lipid and carbohydrate metabolism.

**Key Words:** transition dairy cow, vaccine, serum metabolite

**T8 Effect of repeated oral administration of lipopolysaccharide and lipoteichoic acid either alone or in combination with subcutaneous exposure on humoral immunity in periparturient dairy cows.** Emily F. Eckel\*, Dagnachew W. Hailemariam, Grzegorz Zwierzchowski, Guanshi Zhang, Suzanna M. Dunn, and Burim N. Ametaj, *University of Alberta, Edmonton, AB, Canada.*

Transition dairy cows experience a high incidence of metabolic and infectious diseases immediately after calving. Recent research implicates bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA) in their pathogenesis. Minimal knowledge exists of the periparturient dairy cow immune response to oral endotoxin exposure while other routes have

been extensively explored. The objective of this study was to evaluate the humoral immune response of periparturient dairy cows to repeated oral administration of LPS and LTA alone or in combination with subcutaneous injections. Two hundred dairy cows were randomly assigned to one of 4 treatments ( $n = 50$  per group; administration d -28, -25, -21, -18, and -14 before expected calving date): 1) 2 mL oral sterile saline (CON), 2) Flat doses of oral LPS and LTA (TRT1), 3) Flat doses of oral LPS and LTA plus subcutaneous (sc) injection d -18 and -14 (TRT2), 4) Oral increasing doses of LPS at  $6.5 \mu\text{g}$  (d -28 and -25),  $32.5 \mu\text{g}$  (d -21 and -18) and  $65 \mu\text{g}$  (d -14), all with flat doses of LTA (TRT3). Flat doses of LPS and LTA were 100 and  $120 \mu\text{g}/\text{cow}$ , respectively, in 2 mL sterile saline. Blood samples collected from the coccygeal vein at d -28, -7, +7, and +28 were analyzed for lipopolysaccharide binding protein (LBP), interleukin 1 (IL-1), transforming growth factor- $\beta$  (TGF- $\beta$ ), immunoglobulin M (IgM), and immunoglobulin G (IgG). Statistical results showed there was no effect of treatment on serum concentrations of LBP ( $P = 0.82$ ), IL-1 ( $P = 0.50$ ), TGF- $\beta$  ( $P = 0.75$ ), IgG ( $P = 0.25$ ), or IgM ( $P = 0.40$ ). Overall, preliminary results suggest that repeated oral administration of LPS and LTA or in combination with sc injections did not modulate humoral immunity in periparturient dairy cows.

**Key Words:** transition dairy cow, vaccine, humoral immunity

**T9 Blood alterations indicate subclinical mastitis diagnosed postpartum might start during early dry-off period.** Guanshi Zhang, Dagnachew W. Hailemariam, Elda Dervishi, Qilan Deng, Seyed A. Goldansaz, Suzanna M. Dunn, and Burim N. Ametaj\*, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to identify alterations in innate immunity reactants, carbohydrate and lipid metabolites in the blood of transition dairy cows with subclinical mastitis (SM). Multiparous Holstein cows ( $n = 100$ ) were involved in the study and the experimental period lasted 16 wk starting at -8 wk before until +8 wk postpartum. Health status, feed intake, and milk yield and composition were monitored for each cow during the whole experimental period. Blood samples were collected from the coccygeal vein once/week before the morning feeding. Six healthy cows (CON) and 6 cows with SM were selected for intensive blood analyses. Serum concentrations of lactate, nonesterified fatty acids (NEFA),  $\beta$ -hydroxybutyrate (BHBA), interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), haptoglobin (Hp), and serum amyloid A (SAA) were determined. Feed intake, milk production and composition also were determined. Data were processed using the MIXED procedure of SAS 9.2. Results indicated that concentrations of lactate ( $3,043$  vs  $2,243 \pm 149 \mu\text{mol/L}$ ,  $P < 0.01$ ), TNF ( $0.52$  vs  $0.19 \pm 0.04$  ng/mL,  $P < 0.01$ ) and SAA ( $23,915$  vs  $8,514 \pm 2,518 \mu\text{g/mL}$ ,  $P < 0.01$ ) were greater in cows with SM than CTR cows. Moreover, serum lactate ( $3,478$  vs  $2,455 \mu\text{mol/L}$  at -8 wk,  $P = 0.03$ ;  $3,467$  vs  $2,162 \mu\text{mol/L}$  at -4 wk,  $P = 0.04$ ) and TNF ( $1.29$  vs  $0.27$  ng/mL at -4 wk,  $P < 0.01$ ) in cows with SM were different from CTR cows starting at about -8 and -4 wks before diagnosis of disease. Overall feed intake ( $36.18$  vs  $39.81 \pm 1.55$  kg/d,  $P = 0.01$ ) and milk production ( $33.76$  vs  $42.16 \pm 2.52$  kg/d,  $P = 0.04$ ) was lower in SM-affected cows. Additionally, milk fat ( $3.32$  vs  $5.08$  g/kg,  $P = 0.02$ ) and fat:protein ratio ( $1.11$  vs  $1.69$ ,  $P < 0.01$ ) were lower, whereas SCC ( $1,867,000$  vs  $28,330$  cells/mL,  $P = 0.02$ ) and milk urea N ( $18.70$  vs  $15.39$  mg/dL,  $P = 0.03$ ) were greater in SM cows at diagnosis of disease vs CON. In conclusion cows affected by SM showed enhanced concentrations of indicators of innate immunity and metabolites related to carbohydrate metabolism weeks before diagnosis of SM. More research is warranted to validate these



data and better understand the reasons for activation of innate immune responses to SM in transition dairy cows.

**Key Words:** dairy cow, subclinical mastitis, innate immunity

**T10 The effect of lipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA) on whole blood oxidative response as assessed by luminol-amplified chemiluminescence in dairy cows.** Y. Qu<sup>\*1</sup>, S. Kahl<sup>2</sup>, T. H. Elsasser<sup>2</sup>, E. E. Connor<sup>2</sup>, and K. M. Moyes<sup>1</sup>, <sup>1</sup>University of Maryland, College Park, MD, <sup>2</sup>Agricultural Research Service, US Department of Agriculture, Beltsville, MD.

The differences between lipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA) on whole blood oxidative response using luminol-amplified chemiluminescence (CL) are currently unknown in cattle. Luminol-dependent CL measures the amount of reactive oxygen species released from leukocytes after stimulation with LPS and/or PMA. The objective of this study was to compare in vitro the effect of PMA and LPS on oxidative response in whole blood of dairy cows during lactation as a rapid means of assaying the oxidative response of blood leukocytes. Jugular blood (20 mL) was collected from 6 healthy multiparous Holstein dairy cows in mid-lactation (>90 DIM) using EDTA Vacutainer tubes. For each cow, 500 µL of blood was incubated at final concentrations of either 0, 200, 800 or 1,600 ng/mL of PMA or LPS for 15 min at 37°C using a heating block. After incubation, oxidative response of whole blood was measured using a chemiluminometer. Data were analyzed by ANOVA using the PROC MIXED procedure of SAS. Overall, whole blood incubated with PMA resulted in higher ( $P < 0.001$ ) CL values (800 ng/mL; 2635 relative units) than LPS (800 ng/mL; 777 relative units). In PMA, a significant dose response relationship was observed where incubation with 200, 800 or 1,600 ng/mL resulted in progressively higher CL values than 0 ng/mL. In addition, incubation with PMA resulted in a higher CL values when compared with LPS. In conclusion, although both LPS and PMA both generated an oxidative response measurable by CL, PMA elicited a CL response greater than that of LPS. The data suggest that PMA stimulation of cells in whole blood may serve as a rapid test of oxidative burst responsiveness to assess a vital aspect of immune function in dairy cows.

**Key Words:** chemiluminescence, cow, endotoxin

**T11 Efficacy of a novel antimicrobial post-milking teat dip on rate of new intramammary infections with an experimental bacterial challenge against contagious mastitis organisms.** David M. Galton<sup>1</sup> and Leo L. Timms<sup>\*2</sup>, <sup>1</sup>Cornell University, Ithaca, NY, <sup>2</sup>Iowa State University, Ames, IA.

Study objective was to determine efficacy of a novel antimicrobial solution containing citrate ion, methylene blue, parabens and emollients (ZuraLac<sup>SD</sup>, Zurex PharmAgra) used as a post-milking teat dip against a positive and negative control in reducing the incidence of new quarter intramammary infections (QIMI) during a period of experimental exposure of teats to contagious mastitis organisms, *Streptococcus agalactiae* and *Staphylococcus aureus*. 120 Holstein cows were used in an 8-wk trial with 40 cows/ treatment. Cows were 66–122 DIM and free of IMI. Three treatments were experimental dip - ZuraLac<sup>SD</sup> containing 10.0% trisodium citrate with 5.0% emollients used as a post-milking teat dip; positive control - Theratec<sup>R</sup> (GEA Farm Technologies Inc.) containing 0.5% iodine with a 3% triple emollient system used as a post-milking teat dip; and a negative control where no post-milking teat dipping occurred. All teats were dipped immediately after machine removal at morning milking with broth suspensions of *Streptococcus agalactiae*

(ATCC 55194) and *Staphylococcus aureus* (ATCC 12600). Teats were post dipped with appropriate teat dip between 2 and 5 min after teats dipped with culture broth. Duplicate quarter samples were taken aseptically weekly (and when clinical mastitis occurred) to determine quarter bacteriological status (3rd sample when results differed). Infection data were analyzed using Student *t*-statistics based on percent eligible quarters becoming infected with respective mastitis pathogens. Novel germicide post milking teat dip (3.3% quarter IMI) significantly ( $P < 0.01\%$ ) reduced the number of new infections of *Streptococcus agalactiae* and *Staphylococcus aureus* compared with both the negative control (53.7% QIMI; 95.3% reduction) and positive control (13.1% QIMI; 80.9% reduction). Incidence of clinical mastitis with a bacteriological positive identification of one of the challenge organisms was 40, 4, and 0 for negative control, positive control and novel germicide dips, respectively. Novel germicide dip was significantly better than positive and negative controls.

**Key Words:** postmilking teat dipping, intramammary infection, germicide

**T12 Effects of a 6-week duodenal supplementation of quercetin on metabolic stress and liver health in periparturient dairy cows.** Ann-Kathrin Stoldt<sup>1</sup>, Manfred Mielenz<sup>1</sup>, Alexander Starke<sup>2</sup>, Siegfried Wolfram<sup>3</sup>, and Cornelia C. Metges<sup>\*1</sup>, <sup>1</sup>Institute of Nutritional Physiology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, <sup>2</sup>Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany, <sup>3</sup>Institute of Animal Nutrition and Physiology, Christian-Albrechts University of Kiel, Kiel, Germany.

The periparturient period poses metabolic challenges for dairy cows resulting in negative energy balance often followed by fatty liver disease, a typical periparturient metabolic disorder, and further health problems. Quercetin (Q), a polyphenolic compound found in plants, has hepatoprotective and antioxidative potential, and can reduce hepatic lipid accumulation in rodents. In ruminants, knowledge on metabolic effects of Q is scarce. Thus, we investigated whether Q affects lipid metabolism, oxidative stress defense, and has hepatoprotective effects in periparturient dairy cows. Because Q is degraded in rumen, 5 cows were given 100 mg Q dihydrate per kg BW daily in 0.9% NaCl solution into a duodenal fistula while control (CTR;  $n = 5$ ) cows received NaCl only, starting 3 wk antepartum (ap) to 3 wk postpartum (pp). Twice-weekly blood samples were collected and liver was biopsied twice ap and once pp. Selected hepatic transcript abundances were determined by quantitative real-time PCR. Effects of Q were analyzed using repeated-measure ANOVA (SAS PROC MIXED). Duodenal supplementation of Q resulted in higher ( $P < 0.05$ ) plasma flavonoid levels in Q than in CTR cows. In Q cows pp plasma values of aspartate aminotransferase (AST) were lower ( $P < 0.05$ ) whereas glutamate dehydrogenase (GLDH) and BHBA levels tended to be lower ( $P = 0.1$ ). Liver fat content tended ( $P = 0.1$ ) to be lower in Q cows pp, although groups did not differ ( $P = 0.7$ ) in fat mobilization indicated by plasma NEFA. We could not show group differences of hepatic mRNA abundance of genes related to lipid metabolism and oxidative stress defense (fatty acid synthase, carnitine palmityltransferase 1A, peroxisome proliferator-activated receptors  $\alpha$  and  $\gamma$ , paraoxonase, superoxide dismutase, catalase and glutathione peroxidase). In conclusion, when Q is systemically available during the periparturient period there is potential that metabolic stress and liver damage in dairy cows can be reduced. However, these results should be verified in a larger number of cows and mechanisms of action of Q in cows need to be clarified. Eventually a rumen-protected form of Q has to be developed.

**Key Words:** transition dairy cow, quercetin, liver health

**T13 Effects of manipulated insulin and glucose plasma concentrations on glucagon secretion during intramammary LPS challenge in dairy cows.** Mousa Zarrin<sup>\*1,2</sup>, Olga Wellnitz<sup>1</sup>, and Rupert Bruckmaier<sup>1</sup>, <sup>1</sup>*Veterinary Physiology Vetsuisse Faculty, University of Bern, Bern, Switzerland*, <sup>2</sup>*Department of Animal Science, Yasouj University, Yasouj, Iran.*

Insulin and glucagon are glucoregulatory hormones that contribute to glucose homeostasis which is also critical during immune reaction. Intramammary LPS challenge causes an immune reaction, which is accompanied by metabolic and endocrine changes. The objective of the present study was to investigate effects of intramammary LPS challenge concomitantly with elevated insulin concentrations on glucagon concentration during simultaneous hypoglycemia or euglycemia in dairy cows. Animals were randomly assigned to 3 treatment groups: an intravenous insulin infusion (HypoG, n = 5) to decrease plasma glucose concentration (2.5 mmol/L), a hyperinsulinemic euglycemic clamp to maintain plasma glucose at pre-infusion level to study effects of insulin at simultaneously normal glucose concentration (EuG, n = 6), and a 0.9% saline solution infusion (Control, n = 8). Glucose was measured in blood at 5-min intervals to allow adjustments of glucose infusion rate. Plasma insulin and glucagon concentrations were analyzed hourly. Area under the curve was evaluated by ANOVA with treatment as fixed effect. Data are presented as means ± SEM. The mean of insulin infusion rate before LPS challenge (48 h), was 0.6 mU/kg BW /min in HypoG and EuG. In EuG the glucose infusion rate was 2.20 ± 0.04 mmol/kg/min to keep plasma glucose concentrations at a pre-infusion level and it unchanged during the LPS challenge. In response to LPS challenge, plasma insulin and glucose increased. Plasma glucose concentration increased in EuG (4.4 ± 0.1 mmol/L) compared with concentrations before the LPS challenge (3.8 ± 0.2 mmol/L; *P* < 0.01), and HypoG (2.6 ± 0.1 mmol/L; *P* < 0.01). Intramammary LPS challenge caused an increase of plasma glucagon in HypoG and control compared with basal level, 48 h infusions period, and EuG, but the increase of glucagon was more pronounced in control (219.7 ± 17.3 pg/mL; *P* < 0.001). In conclusion, intramammary LPS challenge induces increases of glucose, insulin, and glucagon concentrations. The results show that glucagon concentrations dramatically increased in the absence of insulin infusion. This is in agreement with previous reports that suggested a general inhibitory effect of insulin on glucagon secretion.

**Key Words:** glucagon, insulin, glucose

**T14 Total immunoglobulin concentration in colostrum produced by dairy cows in Costa Rica.** J. A. Elizondo-Salazar<sup>\*1</sup>, D. Benavides-Varela<sup>2</sup>, and A. Vargas-Ramírez<sup>1</sup>, <sup>1</sup>*Estación Experimental Alfredo Vólio Mata. Facultad de Ciencias Agroalimentarias, Universidad de Costa Rica, San José, Costa Rica*, <sup>2</sup>*Alimentos del Norte S.A-DIPCR, Costa Rica.*

The objective of this study was to determine total immunoglobulin (Ig) concentration in colostrum produced by dairy cows and establish the effect that breed and parity can have on Ig concentration. The data presented correspond to determinations of total Ig concentration determined by a colostrometer in 537 colostrum samples obtained in 50 dairy farms in the provinces of San José, Alajuela, Heredia, and Cartago. Cow breeds were classified into Holstein, Jersey, Holstein × Jersey, and other. Farm size ranged from 10 to 300 milking cows. To determine breed and parity number effect on colostrum Ig concentration, MIXED procedure was used, where dam was considered as a random effect. For the purposes of this study, good quality colostrum was considered when Ig concentration was ≥50 mg/mL. Immunoglobulin concentration ranged from 10 to 140 mg/mL with an average of 85 mg/mL. Of all the

samples analyzed, 13.2% had an inadequate concentration of Ig. When considering breed effect on Ig concentration, no significant differences were found (*P* > 0.05). Parity number significantly (*P* < 0.05) influenced Ig concentration and it was found that Ig concentration increased with parity number. Based on the current study, Ig concentration was adequate for calf feeding in 87% of colostrum samples.

**Table 1 (Abstr. T14).** Effect of dam breed on total Ig concentration in 537 colostrum samples from 50 dairy farms in Costa Rica

Dam breed	Number	Ig (mg/mL)	SEM
Holstein	270	88.8	1.9
Holstein × Jersey	64	85.9	2.5
Jersey	146	85.5	2.6
Other	57	85.6	4.2

**Key Words:** passive immunity, colostrometer, immunoglobulin

**T15 Risk factors associated with milk fever occurrence in Costa Rican dairy cattle.** Alejandro Saborío-Montero<sup>\*2</sup>, Bernardo Vargas-Leitón<sup>1</sup>, Juan José Romero-Zúñiga<sup>1</sup>, and Jorge M. I. Sánchez<sup>2</sup>, <sup>1</sup>*Programa de Investigación en Medicina Poblacional, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica*, <sup>2</sup>*Centro de Investigaciones en Nutrición Animal y Escuela de Zootecnia, Universidad de Costa Rica, San José, Costa Rica.*

The aim of this study was to determine risk factors associated with milk fever (MF) occurrence in dairy cows in Costa Rica. A total of 69,870 cows from 127 dairy herds were included in the study. Data were collected in the VAMPP (Veterinary Automated Management and Production Control Program) software by the Population Medicine Research Program of the Veterinary Medicine School, National University of Costa Rica; from 1985 to 2014. To determine the risk factors, 2 logistic regression mixed models were evaluated. The first model used breed (B), month of calving (M), quinquennium of calving (Y) ecological life zones (Z) and calving number (N) as fixed effects. The second model excluded first lactation animals and cows without productive information, had the same fixed effects of the first model plus: previous MF case (C), previous lactation length (L), previous dry period length (D), previous corrected 305d milk yield (P), and calving interval length (I) as fixed effects. Both models used animal (a) and herd (h) as random effects. Of the 235,971 recorded lactations, 4,312 (1.83%) reported MF event. The significantly associated (*P* < 0.05) risk factors for MF occurrence, ranked by their highest odds ratio (OR) are listed on Table 1. The findings of this study are the first data reported for a population study on risk factors for MF in Costa Rica. Some of these results might be used to improve preventive management practices at the farms to reduce the incidence of this metabolic disease.

*Contd.*

**Table 1 (Abstr. T15).** Risk factors associated with milk fever (MF) occurrence ranked by odds ratio (OR) in Costa Rican dairy cattle<sup>1</sup>

Risk factor	Riskiest category	Reference category	OR	95% CI
Calving number	6th or more calving	1st calving	52.40	40.94–67.08
Breed	Jersey	Brown Swiss	2.88	1.66–4.98
Previous MF case	Previous case reported	No previous case reported	2.39	2.17–2.63
Quinquennium of calving	1990-1994	2005-2009	2.33	2.00–2.78
Month of calving	July	December	1.37	1.16–1.61
Previous 305-d milk yield	1,000 kg over $\mu$	$\mu$ for 305-d milk yield	1.17	1.14–1.21
Previous lactation length	30 d over $\mu$	$\mu$ for lactation length	1.06	1.03–1.09

<sup>1</sup> $\mu$  = population mean; 6,021 kg for previous 305-d milk yield, 316 d for previous lactation length.

**Key Words:** milk fever, risk factor, dairy cattle

**T16 Serum calcium concentration during the peripartum period in a Jersey herd grazing tropical pastures and supplemented with a low calcium grain mixture.** Jorge M. I. Sánchez\* and Alejandro Saborío-Montero, *Centro de Investigaciones en Nutrición Animal y Escuela de Zootecnia, Universidad de Costa Rica, San José, Costa Rica.*

The aim of this study was to analyze the serum Ca concentration in a Jersey herd during the peripartum period in a tropical production system, to compare Ca status in young ( $\leq 2$ nd parity) and adult ( $\geq 3$ rd parity) cows. The study was conducted on a dairy farm located in Cartago, Costa Rica, during a 6-mo period (January–June, 2014) and comprised 161 cows (62 young cows; 99 adult cows). During the close-up period cows grazed lush kikuyu grass (*Kikuyuocloa clandestina*) (14.8% DM, 23.4% CP, 0.35% Ca, 0.31% Mg and 3.5% K) and were supplemented with 4 kg/cow/d of a low Ca grain mixture (88% DM, 14% CP, 0.2% Ca, 0.42% Mg and 1.38% K) and 1 kg of hay (82.3% DM, 5.1% CP, 0.4% Ca, 0.35% Mg and 1.8% K)/d. After calving, cows were fed 1 kg of grain mixture (87.5% DM, 18.6% CP, 0.90% Ca, 0.42% Mg and 1.38% K)/2.5 to 3.0 kg of milk. A total of 752 blood samples were taken from 4d before calving until 5d postpartum and were analyzed for total calcium. Data were analyzed as a repeated measures with the fixed effect of parity class and cow nested within parity was the subject of the repeated measure of time. Statistical analysis showed significant effect for category, not so for day and day x category interaction. Means across the 2 categories were compared by Student's *t*-test (Table 1). Serum Ca concentrations in young cows were usually greater than concentrations in adult cows. Starting at 1 d before calving through 1 d post calving, serum Ca in adult cows was depressed reaching its nadir at 1 d in milk. Results show that adult cows were subclinically hypocalcemic (serum Ca <8.0 mg/dl) from d 2 before calving until 5 d in milk, requiring feeding practices designed to improve Ca metabolism in the periparturient adult cows.

*Contd.*

**Table 1 (Abstr. T16).** Serum Ca concentration (mg/dl) from 4 d before to 5 d after calving for young (1 and 2 parities) and adult (3 or more parities) Jersey cows grazing lush tropical pastures

Category	Day relative to calving									
	-4	-3	-2	-1	0	1	2	3	4	5
Young	8.41 <sup>a</sup>	8.18 <sup>a</sup>	8.19 <sup>a</sup>	8.37 <sup>a</sup>	7.57 <sup>a</sup>	7.85 <sup>a</sup>	7.90 <sup>a</sup>	8.15 <sup>a</sup>	7.97 <sup>a</sup>	8.09 <sup>a</sup>
Adult	8.66 <sup>a</sup>	8.09 <sup>a</sup>	7.74 <sup>a</sup>	7.49 <sup>b</sup>	6.98 <sup>b</sup>	6.72 <sup>b</sup>	7.42 <sup>a</sup>	7.67 <sup>a</sup>	7.68 <sup>a</sup>	7.86 <sup>a</sup>
<i>P</i> -value	0.781	0.901	0.280	0.046	0.027	0.009	0.127	0.092	0.445	0.438

<sup>ab</sup>Means in the same column with different superscripts are different ( $P < 0.05$ ).

**Key Words:** grazing cow, hypocalcemia, milk fever

**T17 Bacterial diversity and pathogen load in recovered dairy cows bedding materials following an aerobic composting of dairy manure.** Maral Rahmani\*, Hooman Derakhshani, Hein M. Tun, Jacqueline Donogh, Shadi Sepehri, and Ehsan Khafipour, *University of Manitoba, Winnipeg, MB, Canada.*

The FAN bedding recovery system consists of a solid separator and an in-vessel aerobic composting system, allowing the treatment of dairy manure into separate liquid and solid streams, with the solid stream later used within the barn as dairy cows bedding. The objective was to evaluate the effect of composting on the microbiota profile and pathogen load of recovered bedding material (RBM). Manure (MAN), liquid stream (LS), solid stream undigested (SSU) and solid stream digested (SSD) samples were collected on a weekly basis for a 2-mo period during summer and winter seasons ( $n = 36$ /category). Genomic DNA was extracted and subjected to bacterial 16S rRNA gene sequencing for community analysis and qPCR for absolute quantification of generic *Escherichia coli*, *E. coli* O157:H7, *Staphylococcus aureus*, *Mycobacterium avium* ssp. *paratuberculosis* (MAP); *Arcanobacterium pyogenes*, and *Klebsiella pneumoniae*. On average, 29,982 high-quality sequences were generated per sample. The  $\alpha$ -diversity of SSD microbial communities was found to be lower ( $P < 0.001$ ) than other treatment groups, with the MAN samples showing the most diverse microbiota followed by LS and SSU. The proportion of phylum Firmicutes (members of the family Bacillaceae) was greater ( $P < 0.05$ ) in SSD group, and the relative abundances of several members of the phyla Proteobacteria (family Moraxellaceae and genus *Acinetobacter*) and Bacteroidetes (family Sphingobacteriaceae) were found to be significantly ( $P < 0.05$ ) higher in SSU compared with other groups. The proportions of several members of the phylum Proteobacteria were also found to be higher in SSU compared with LS, suggesting that, in the absence of composting process, separation of the solid and liquid part of the RBM may increase the proportion of the opportunistic microorganism in the solid fraction. *E. coli* O157:H7, MAP and *S. aureus* were present only in a limited number of samples and only 2 SSD samples found to be MAP, O157:H7 or *S. aureus* positive. Aerobic composting had limited effect on the population of *E. coli*, *A. pyogenes*, and *K. pneumoniae*.

**Key Words:** aerobic composting, microbial community, pathogen load

**T18 Massive shotgun metagenomic sequencing reveals the potential mode of action of *Saccharomyces cerevisiae* fermentation product (SCFP) on rumen microbiome during subacute ruminal acidosis (SARA) in dairy cows.** H. M. Tun<sup>\*1</sup>, S. Li<sup>1</sup>, I. Yoon<sup>2</sup>, M. Scott<sup>2</sup>, J. C. Plaizier<sup>1</sup>, and E. Khafipour<sup>1</sup>, <sup>1</sup>*Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada,* <sup>2</sup>*Diamond V, Cedar Rapids, IA.*



The effects of *Saccharomyces cerevisiae* fermentation product on rumen microbiome composition and function were studied in 8 rumen-cannulated lactating cows in a crossover study with two 5-wk periods. Each period consisted of a 4-wk normal feeding and a 1-wk grain-based SARA challenge. A 3-wk washout period separated the experimental periods. During each period, 4 cows received 14 g/d of SCFP (Original XPC, Diamond V) mixed with 126 g/d ground corn and the other 4 received 140 g/d ground corn as control. Rumen fluid was collected during wk 4 and 5 of each period. Genomic DNA was extracted and subjected to shotgun metagenomic sequencing using a MiSeq Illumina platform. Host genomic sequences were removed before analysis. Taxonomies were annotated against Greengenes database and functional genes were annotated against SEED subsystems in the MG-RAST pipeline. Both compositional and functional differences among treatments were analyzed by the linear discriminant analysis effect size. In total, 103.4 GB sequences were obtained from 32 shotgun metagenomic samples with an average of 10 millions sequences per sample. The SCFP supplement altered the  $\beta$ -diversity of rumen functional metagenome under both control and SARA conditions ( $P < 0.05$ ). The SCFP supplement restored several cellulolytic populations, including *Fibrobacteres*, *Paenibacillaceae* and *Spirochaetaceae*, as well as *Burkholderiales* that were suppressed during SARA ( $P < 0.05$ ). The SCFP supplement also tended to increase the population of *Eubacteriaceae*, *Coriobacteriaceae* and unclassified *Clostridiales* observed during SARA ( $P < 0.05$ ). Several enzymatic pathways were downregulated during SARA. The SCFP supplement increased the abundance of formate dehydrogenase and methylmalonyl-CoA mutase (MCM) that were suppressed during SARA ( $P < 0.05$ ). The MCM is a vitamin B12-dependent enzyme constantly found in methanol-utilizing bacteria, such as members of *Burkholderiales*. Data reveals potential mechanisms through which SCFP supplement contributes to the resilience of the rumen microbiome, especially during SARA.

**Key Words:** shotgun metagenomic, *Saccharomyces cerevisiae* fermentation product, subacute ruminal acidosis

**T19 Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on the predicted functional profiles of rumen microbiome in lactating dairy cows with subacute ruminal acidosis (SARA).** S. C. Li<sup>\*1</sup>, H. M. Tun<sup>1</sup>, P. Azevedo<sup>1</sup>, I. Yoon<sup>2</sup>, M. Scott<sup>2</sup>, J. C. Plazier<sup>1</sup>, and E. Khafipour<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, <sup>2</sup>Diamond V, Cedar Rapids, IA.

The effects of *Saccharomyces cerevisiae* fermentation product (SCFP) on the rumen bacterial function were studied in 8 rumen-cannulated lactating cows in a crossover study with 2 5-wk periods. Each period consisted of 4 wk of normal feeding and a 1-wk grain-based SARA challenge. A 3-wk washout period separated the experimental periods. During each period, 4 cows received 14 g/d of SCFP (Original XPC, Diamond V) mixed with 126 g/d ground corn and the other 4 received 140 g/d ground corn as control. Rumen fluid was collected during wk 4 and 5 of each period. DNA was extracted and V1-V3 region of 16S rRNA gene was amplified and subjected to pyrosequencing. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to impute metagenomic information based on 16S rRNA sequencing data. The SARA challenge and SCFP supplementation did not affect the  $\alpha$ -diversity of the predicted functional gene families of rumen bacterial communities. However, the  $\beta$ -diversity was affected by SARA ( $P < 0.01$ ), but not by SCFP. A total of 9 out of 36 level-2, and 39 out of 254 level-3 KEGG Orthology groups were found to be affected by SARA challenge ( $P < 0.01$ ). At the KEGG level-2, pathways upregulated during SARA included cell motility, membrane

transport, signal transduction and transcription, while pathways involved in the metabolism of terpenoids and polyketides, biosynthesis of other secondary metabolites, amino acid metabolism, signaling molecules and interaction, transport and catabolism were downregulated. However, SCFP supplementation and interaction between SARA challenge and SCFP supplementation were not significant. The relevance and accuracy of PICRUSt application to predict the function of microbiome in the rumen need further validation using shotgun sequencing approach.

**Key Words:** dairy cow, SARA, *Saccharomyces cerevisiae* fermentation product

**T20 Effect of milk yield genotype on response to repeated lipopolysaccharide (LPS) administration to lactating Holstein cows.** Georgina Cousillas<sup>\*1</sup>, Wanda J. Weber<sup>1</sup>, Stanislaw Kahl<sup>2</sup>, Bruce Walcheck<sup>1</sup>, Ricardo Chebel<sup>1</sup>, David Kerr<sup>3</sup>, Theodore H. Elsasser<sup>2</sup>, and Brian A. Crooker<sup>1</sup>, <sup>1</sup>University of Minnesota, St. Paul, MN, <sup>2</sup>USDA-ARS, Beltsville, MD, <sup>3</sup>University of Vermont, Burlington, VT.

Cows (n = 12/genotype) from unselected (stable milk yield since 1964, UH) and contemporary (CH) Holsteins that differed by more than 4,500 kg milk/305 d were fed the same diet ad lib and housed together for more than 4 mo before being blocked (2/genotype) by DIM and randomly assigned within genotype to receive saline or 0.25  $\mu$ g LPS (*Escherichia coli* 055:B5) per kg BW. Cows were synchronized to be at d 8 of their estrous cycle for the first challenge (C1) at 70–84 d in milk. Jugular catheters were implanted 24 h before C1. Blood samples were collected at -1, -0.5, 0, 1, 2, 3, 4, 6, 8, 12, and 24 h relative to treatment administration and plasma harvested. Body temperatures (BT) were determined at these times and at 5 and 7 h. Liver biopsies and blood for flow cytometry and hemogram assays were obtained at 0, 4, and 24 h. A second identical challenge (C2) and sampling was conducted 4 d later. Data were analyzed by repeated measures using PROC MIXED (SAS). Means differed when  $P < 0.05$ . Pre-challenge glucose and IGF-1 were greater ( $P < 0.01$ ) and BT was less ( $P < 0.01$ ) in UH than CH. Glucose response to LPS was greater ( $P < 0.01$ ) in UH than CH, but IGF-1 and BT response was similar in both genotypes. TNF $\alpha$  and cortisol response to LPS was greater during C1 than C2 ( $P < 0.02$ ). TNF $\alpha$  response to LPS was greater ( $P < 0.05$ ) in UH than CH in C1 but similar in C2. Cortisol response to LPS increased in both genotypes but returned to baseline earlier in CH than UH ( $P < 0.05$ ). LPS decreased white blood cell count ( $P < 0.01$ ) but response did not differ between genotypes or challenge. Neutrophil oxidative burst was greater ( $P < 0.05$ ) and phagocytic capacity tended ( $P = 0.07$ ) to be greater in UH than CH. CD11b expression increased ( $P < 0.05$ ) in response to LPS at 4h, was less in CH than UH at 24h and did not differ between C1 and C2. L-selectin decreased in response to LPS at 4hr but did not differ between challenge or genotype. Results indicate that genotype affects bovine response to LPS and this effect differs among the response variables assessed.

**Key Words:** innate immunity, Holstein genotype, lipopolysaccharide

**T21 Use of chitosan microparticles to prevent metritis in lactating dairy cows.** Rodolfo Daetz<sup>\*2</sup>, Federico Cunha<sup>2</sup>, Yosuke Maeda<sup>3</sup>, Carlos A. Risco<sup>2</sup>, Kwang C. Jeong<sup>1,4</sup>, Jose Eduardo P. Santos<sup>1</sup>, and Klibis N. Galvao<sup>2</sup>, <sup>1</sup>Department of Animal Sciences, University of Florida, Gainesville, FL, <sup>2</sup>Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, <sup>3</sup>School of Veterinary Medicine, Kitasato University, Towada, Japan, <sup>4</sup>Emerging Pathogens Institute, University of Florida, Gainesville, FL.

The objective was to determine the efficacy of chitosan microparticles (CM) in preventing metritis in dairy cows. Holstein cows ( $n = 101$ ) from a 4,500-cow commercial herd that had risk factors for metritis (dystocia, twins, stillbirth, retained placenta) were randomly assigned to one of 2 treatments 1 d postpartum (DPP): CM ( $n = 51$ ) = intrauterine (i.u.) infusion of 8 g of CM dissolved in 40 mL of sterile water for 5 d; Control ( $n = 50$ ) = i.u. infusion of 40 mL of sterile saline solution for 5 d. Metritis prevalence was analyzed by logistic regression using the LOGISTIC procedure of SAS using a one-side test in accordance with sample size calculation for reduction in metritis prevalence using CM. Continuous outcomes were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS. Models included the effects of treatment, parity, specific risk factor, body condition score at enrollment and interaction between treatment and other covariates. The effect of time and interaction between treatment and time was also included in repeated measures analyses. Treatment with CM resulted in decreased incidence of metritis at 7 DPP compared with Control (45.1 vs. 64.0%, respectively;  $P = 0.03$ ); however, there were only numerical differences at 4 (11.8 vs. 18%, respectively;  $P = 0.23$ ), 10 (60.1 vs. 72%, respectively;  $P = 0.12$ ), and 14 (62.7 vs. 72.0%, respectively;  $P = 0.16$ ). Treatment with CM resulted in decreased NEFA plasma concentrations at 10 DPP ( $464.2 \pm 57.2$  vs.  $639.5 \pm 57.2$   $\mu\text{Eq/L}$ ;  $P = 0.04$ ); however, there were no differences at 4 ( $813.8 \pm 56.7$  vs.  $780.4 \pm 56.7$   $\mu\text{Eq/L}$ ;  $P = 0.67$ ), 7 ( $669.9 \pm 56.7$  vs.  $692.9 \pm 56.7$   $\mu\text{Eq/L}$ ;  $P = 0.77$ ), and 14 ( $527.6 \pm 57.7$  vs.  $420.7 \pm 57.7$   $\mu\text{Eq/L}$ ;  $P = 0.18$ ). The uterine discharge pH was lower in Control than in CM cows ( $6.84 \pm 0.03$  vs.  $6.93 \pm 0.03$ ;  $P = 0.02$ ). BHBA ( $647.4 \pm 30.0$  vs.  $589.3 \pm 30.0$   $\mu\text{mol/L}$ ;  $P = 0.36$ ), temperature ( $39.2 \pm 0.04$  vs.  $39.1 \pm 0.04^\circ\text{C}$ ;  $P = 0.62$ ) and milk production ( $29.3 \pm 1.0$  vs.  $28.8 \pm 1.0$  L/d;  $P = 0.69$ ) were not different between CM and Control groups. In conclusion, CM may be a viable alternative for treatment of metritis; however, the duration of treatment may have to be extended to maintain differences in the incidence of metritis.

**Key Words:** chitosan microparticle, metritis, dairy cow

**T22 The role of Bacteroidetes and Bacteroides species in the development of metritis and fever in dairy cows.** Soo Jin Jeon<sup>\*2</sup>, Achilles Vieira-Neto<sup>1</sup>, and Klíbs N. Galvão<sup>2</sup>, <sup>1</sup>Department of Animal Sciences, University of Florida, Gainesville, FL, <sup>2</sup>Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.

Objective was to evaluate if uterine microbiota was associated with the presence of fever in metritic cows. Uterine swabs were collected at  $6 \pm 2$  d postpartum (DPP) from 92 cows. Uterine swabs from 11 metritic cows that had a fever (rectal temperature  $\geq 39.5^\circ\text{C}$ ) at the time of metritis diagnosis (MF), 12 metritic cows that did not have a fever at the time of metritis diagnosis (MNF), and 11 cows that remained healthy (Healthy) were used for metagenomic sequencing of the 16S rRNA gene using the Illumina MiSeq platform. Data were analyzed by ANOVA using the GLM procedure of SAS. Rectal temperature was  $38.97 \pm 0.12$ ,  $38.96 \pm 0.08$ , and  $39.89^\circ\text{C} \pm 0.11$  for Healthy, MNF, and MF, respectively. At the phylum level, Bacteroidetes were found to have lower ( $P < 0.01$ ) relative abundance in Healthy than in MNF and MF (27.3 vs. 51.1 vs. 53.3%), whereas there was no difference ( $P > 0.25$ ) in Fusobacteria (23.9 vs. 28.6 vs. 24.6%), Actinobacteria (1.2 vs. 0.5 vs. 1.1%), and Firmicutes (18.6 vs. 12.4 vs. 15.6). Proteobacteria in Healthy was similar ( $P = 0.13$ ) to MNF but greater ( $P = 0.05$ ) than MF (16.6 vs. 6.5 vs. 2.9%). Tenericutes was lower ( $P < 0.04$ ) in Healthy than in MNF and MF (10.6 vs. 0.7 vs. 2.0%). At the species level, principal coordinates analysis showed that MNF were clustered together with MF, whereas Healthy were mostly separated from metritic

cows. Relative abundance of *Bacteroides heparinolyticus* in Healthy was lower ( $P = 0.01$ ) than in MNF and tended ( $P = 0.06$ ) to be lower than in MF (7.0 vs. 20.3 vs. 16.8%). *Bacteroides pyogenes* was lower ( $P \leq 0.05$ ) in Healthy and MNF than in MF (4.0 vs. 3.8 vs. 10.0%), but similar between Healthy and MNF. There was no difference in the relative abundance of *Fusobacterium necrophorum* ( $P = 0.68$ ) or other 10 most prevalent bacteria ( $P > 0.15$ ). In conclusion, Bacteroidetes is more abundant, whereas Firmicutes and Tenericutes are less abundant in metritic cows. Furthermore, *B. heparinolyticus* is an important species for the development of metritis, whereas *B. pyogenes* seem to be involved in the development of fever in metritic cows.

**Key Words:** Bacteroidetes, Bacteroides species, metritis

**T23 Incidence of health treatments among pure Holsteins of 8 high-performance dairies in Minnesota.** M. R. Donnelly<sup>\*1</sup>, A. R. Hazel<sup>1</sup>, B. J. Heins<sup>2</sup>, and L. B. Hansen<sup>1</sup>, <sup>1</sup>University of Minnesota, St. Paul, MN, <sup>2</sup>West-Central Research and Outreach Center, Morris, MN.

Health treatments of pure Holstein cows ( $n = 3,936$ ) were evaluated in 8 high-performance Minnesota dairies. Cows calved from March 2008 to February 2013, and 16 types of health treatments were defined uniformly across herds. For analysis, treatments were grouped into 5 categories: mastitis, reproduction (cystic ovary, retained placenta, and metritis), hoof, metabolic (milk fever, displaced abomasum, ketosis, and digestive), and miscellaneous (respiratory, injury, and other). Excluded from analysis were California Mastitis Test/culture, hoof trimming, palpation, reproductive aid, and abortion. Parity was coded as 1 ( $n = 2,285$ ), 2 ( $n = 2,529$ ), or 3 to 5 ( $n = 3,842$ ), and later lactations were deleted. Observations were recorded for entire lactations and coded as treated (1) or not treated (0) during a lactation. Independent variables for statistical analysis of all 5 treatment categories were the fixed effects of herd, year-season nested within herd, parity, and interaction of herd and parity. Cow was considered a repeated measure. For all 5 treatment categories, herd and year-season nested within herd were highly significant ( $P < 0.01$ ). Parity was significant ( $P < 0.05$ ) for all 5 categories of health treatments, except parity was not significant ( $P = 0.71$ ) for miscellaneous. Interaction of herd and parity was significant ( $P < 0.05$ ) for all categories. Least squares means increased with advancing parity for all 5 treatment categories except for the miscellaneous category. Treatment rates for parities 1, 2, and 3 to 5, respectively, for each health treatment category were 0.25, 0.34, and 0.42 (mastitis); 0.12, 0.13, and 0.16 (reproduction); 0.32, 0.39, and 0.40 (hoof); 0.07, 0.13, and 0.18 (metabolic); 0.14, 0.13, and 0.14 (miscellaneous). Across herds and parities, the largest treatment rate was for the hoof category followed by mastitis, and the other 3 treatment categories had similar treatment rates.

**Key Words:** health, treatment, mastitis

**T24 Rumination behavior alert indexes for detecting health disorders during early lactation.** Sushil Paudyal<sup>1</sup>, Fiona Maunsell<sup>2</sup>, Carlos Risco<sup>2</sup>, Arthur Donovan<sup>2</sup>, Albert De Vries<sup>3</sup>, John Richeson<sup>1</sup>, and Pablo Pinedo<sup>\*4,5</sup>, <sup>1</sup>West Texas A&M University, Canyon, TX, <sup>2</sup>Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, <sup>3</sup>Department of Animal Sciences, University of Florida, Gainesville, FL, <sup>4</sup>Texas A&M AgriLife Research, Amarillo, TX, <sup>5</sup>Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University System, College Station, TX.



Monitoring of health disorders during early lactation is a key component in the management of dairy systems. Electronic systems that allow for monitoring of rumination are now available. The objective was to evaluate the association between changes in rumination time (RT) and early stages of disease within the prepartum and early lactation. Two weeks before the due date, 198 cows at the University of Florida (UF) Dairy Unit were affixed with a neck collar containing rumination loggers (Hr-Tag rumination monitoring system, SCR Engineers Ltd., Netanya, Israel), providing rumination time (RT) for each 2-h interval. Occurrence of health disorders [mastitis (MAS), metritis (MET), clinical hypocalcemia (HYC), digestive disorders (DIG), lameness (LAM), and ketosis (KET),] were assessed until 60 DIM by UF veterinarians and farm personnel. Two indices were developed to explore the association between RT and disease: i) Cow Index (CI) = 24-h RT on the day of diagnosis (d0) minus average RT on d -3 to -5 relative to d0 (0to3-5RT), divided by the -3 to -5 d RT average; ii) Mates Index (MI) = (0to3-5RT - pen mates 0to3-5RT)/pen mates d0 RT. Subsequently an alert value (ACI and AMI) was determined for both indices when the value was lower than -0.1. Alert indices were evaluated by ROC curve analyses. Average CI in healthy cows ranged from 0.0491 to 0.0495 while CI in sick cows were -0.165, -0.029, -0.513, -0.022, -0.098, and -0.081 for MAS, MET, HYC, DIG, LAM, and KET, respectively. Average MI in healthy cows ranged from 0.0001 to 0.001 while MI in sick cows were -0.183, -0.101, -0.424, -0.101, -0.148, and -0.147 for MAS, MET, HYC, DIG, LAM, and KET, respectively. Sensitivity/specificity (%) of ACI were 56/72, 39/73, 100/72, 44/72, 67/72, and 61/72 for MAS, MET, HYC, DIG, LAM, and KET, respectively. Sensitivity/specificity (%) of AMI were 63/71, 42/72, 100/72, 48/72, 55/72, and 67/72 for MAS, MET, HYC, DIG, LAM, and KET, respectively. Area under the curve for our proposed cut-off value ranged from 0.56 (KET) to 0.87 (HYC) for CI and from 0.51 (MET) to 0.87 (HYC) for MI. Consistent negative changes in rumination activity, both within cow (CI) and compared with cohorts (MI), were observed on the day of diagnosis for each postpartum disease.

**Key Words:** rumination, disease, alert index

**T25 OmniGen-AF alters rectal temperature (RT) and leukocyte profiles in dairy cows exposed to heat stress (HS) following acute activation of the stress axis.** Nicole C. Burdick Sanchez<sup>1</sup>, Jeffery A. Carroll<sup>1</sup>, Paul R. Broadway<sup>1</sup>, Matthew L. McBride<sup>2</sup>, Xavier A. Ortiz<sup>2</sup>, Jayne L. Collier<sup>2</sup>, James D. Chapman<sup>3</sup>, Derek McLean<sup>3</sup>, and Robert J. Collier<sup>2</sup>, <sup>1</sup>USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, <sup>2</sup>University of Arizona, Department of Animal Science, Tucson, AZ, <sup>3</sup>Phibro Animal Health Corp., Quincy, IL.

Differences in the response of OmniGen-AF (OG) supplemented dairy cows to a corticotropin releasing hormone (CRH) and vasopressin (VP) or an adrenocorticotrophic hormone (ACTH) challenge when housed at different temperature-humidity indices (THI) were studied. Holstein cows (n = 12; 162 ± 1 DIM) were balanced by milk yield, BW and DIM into 1 of 2 trts: 1) OminGen-AF, supplemented with OG at 4.54 g/kg BW for 70d; or 2) Control (CON), no supplement. Cows were moved to individual tie stalls in 1 of 2 temperature controlled chambers on d51 and fitted with indwelling RT devices and jugular catheters on d52. Initially THI was cycling at thermoneutrality (TN; THI < 72 for 24 h/d) for 7d, followed by HS (THI > 72 for 12h/d) for 10d. Cows were challenged with CRH (0.3 µg/kg) and VP (1 µg/kg) at 1000h on d4 of TN and d1 of HS, and with ACTH (0.1 IU/kg) at 1000h on d5 of TN and d2 of HS. Blood samples were collected from -2 to 8h at 30-min intervals relative to each challenge and analyzed for leukocyte profiles. There was a THI × time interaction ( $P \leq 0.01$ ) for RT such that RT was greater during

HS than TN (2 to 9h, and at 11h for CRHVP and 4 to 9h and 11 to 13h for ACTH). Also, RT was greater ( $P \leq 0.02$ ) in OG than CON cows regardless of challenge. Total white blood cells (WBC) and neutrophils (NT) increased ( $P < 0.01$ ) in response to CRHVP and ACTH. There was a trt × THI interaction ( $P < 0.01$ ) for WBC during both challenges. Specifically, WBC were decreased in CON during HS compared with TN in response to CRHVP, while WBC were greatest in CON during TN and least in OG during HS in response to ACTH. There was a trt × THI interaction ( $P = 0.02$ ) for NT in response to CRHVP challenge; NT decreased in CON cows during HS compared with TN. Also, NT were decreased during HS ( $P < 0.01$ ) compared with TN and decreased in OG compared with CON ( $P < 0.01$ ) following ACTH. Lymphocytes were decreased during HS compared with TN ( $P < 0.01$ ) and in OG compared with CON cows ( $P < 0.01$ ) regardless of challenge. These data suggest supplementing cows with OG can reduce the negative effects of HS on leukocyte profiles following activation of the stress axis.

**Key Words:** heat stress, immune, OmniGen-AF

**T26 Altered microbiomes in bovine digital dermatitis lesions, and the gut as a pathogen reservoir.** Martin Zinicola<sup>\*1</sup>, Fabio Lima<sup>1</sup>, Svetlana Lima<sup>1</sup>, Vinicius Machado<sup>1</sup>, Charles Guard<sup>1</sup>, Dörte Döpfer<sup>2</sup>, and Rodrigo Bicalho<sup>1</sup>, <sup>1</sup>Cornell University, Ithaca, NY, <sup>2</sup>University of Wisconsin, Madison, WI.

Bovine digital dermatitis (DD) is the most relevant infectious disease associated with lameness affecting cattle worldwide. Notwithstanding decades of research, the pathogenesis of this disease and the potential reservoir of pathogenic microbes involved in DD remain unclear. Here, we characterize the microbiome of healthy skin (HS) and lesions from dairy cows affected with different stages of DD and we also identified DD-causing *Treponema* spp. in rumen and fecal samples. A total of 140 biopsy samples (BS) (51 HS and 89 DD lesions) were collected from Holstein dairy cows housed in 3 different dairy farms. BS were trimmed in 2 different layers, resulting in 280 samples. Rumen fluid (n = 8) and fecal (n = 14) samples were also collected. DNA was extracted and the microbiome was determined by shotgun and 16S metagenomic techniques using Illumina MiSeq platform. Discriminant analysis revealed that microbiomes of HS, active and inactive DD lesions were completely distinct. The differences in microbiomes between the superficial and deep strata were found to be minor. *Treponema* spp. were found in greater ( $P < 0.05$ ) relative abundance in active DD lesions when compared with HS and inactive DD lesions and these *Treponema* spp. were nearly ubiquitously present in rumen and fecal microbiomes. *Candidatus amoebophilus asiaticus*, a bacterium not previously reported in DD lesions, was encountered in high ( $P < 0.05$ ) relative abundance in active and inactive lesions but not in HS. In conclusion, our data support the concept that DD is a polymicrobial disease, with active DD lesions having a markedly distinct microbiome dominated by *T. denticola*, *T. maltophilum*, *T. medium*, *T. putidum*, *T. phagedenis* and *T. paraluiscauniculi*. Furthermore, these *Treponema* species are nearly ubiquitously found in rumen and fecal microbiomes, suggesting that the gut is an important reservoir of microbes involved in DD pathogenesis. Further investigation into the potential role of the gut microbiome as a reservoir for pathogens leading to DD development and of prophylactic measures to control the potential environmental shedding of these pathogens is needed.

**Key Words:** bovine digital dermatitis, dairy cow, microbiome

## **T27 Characterization of the leukocyte transcriptome in cows challenged with *Mycobacterium bovis* and healthy controls.**

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*Mycobacterium bovis* (*M. bovis*) is a pathogenic bacteria that generates approximately \$3 billion worldwide production loss for the cattle industry, in terms of animal value and productivity. To help understand the response to this pathogen, the objective of this study was to characterize the transcriptome of cows infected with *M. bovis* relative to healthy controls. Cows positive for *M. bovis* were identified using the single intradermal comparative tuberculin test, and in vitro ELISA-based interferon-gamma. The transcriptome of peripheral blood leukocytes from Holstein cows detected positive was compared with healthy control cows ( $n = 8/\text{group}$ ) using individual RNA-Seq libraries. Single-end reads were mapped to the *Bos taurus* reference genome (UCSC\_bosTau7) using Tophat v2.0.12. In total, 8,309 isoform transcripts pertaining to 8,127 genes were identified and 2,620 isoform transcripts pertaining to 2,608 genes were identified as differentially expressed (false discovery rate-adjusted  $P$ -value  $< 0.05$ ) using Cufflinks v2.2.1. Among the top 25 differentially abundant transcripts using log fold change, 76% were overexpressed in the infected group relative to control cows including polo-like kinase 3 (PLK3), atypical chemokine receptor 3 (CXCR7), and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor delta (NFKBID). PLK3 participates in the tuberculosis response pathway, activating the nuclear factor- $\kappa$ B, which in turn stimulates transcription of the inducible nitric oxide synthase as well as pro-inflammatory cytokines. CXCR7 is an innate immune gene reported in leukocytes infected with *M. bovis*. Also, NFKBID may regulate the expression of cytokines by regulating nuclear factor- $\kappa$ B activity. Functional analysis of the differentially expressed genes using DAVID identified 14 enriched functional category clusters (enrichment score  $> 3$ ) including leukocyte mediated immunity, leukocyte activation and proliferation, cellular apoptosis, together with cytokines and chemokines production. The majority of the genes in these clusters were overexpressed in the infected group relative to control cows. These results offer insights on leukocyte transcriptome changes in response to *M. bovis* infection.

**Key Words:** transcriptome, tuberculosis, production

**T28 Evaluation of antimicrobial activity of chitosan microparticles in different matrices from dairy cows.** Zhengxin Ma<sup>\*1,2</sup>, Lin Teng<sup>1,2</sup>, Donghyeon Kim<sup>1</sup>, Kliks N. Galvão<sup>3</sup>, Corwin D. Nelson<sup>1</sup>, Adegbola T. Adesogan<sup>1</sup>, and K. Casey Jeong<sup>1,2</sup>, <sup>1</sup>*Department of Animal Sciences, University of Florida, Gainesville, FL*, <sup>2</sup>*Emerging Pathogens Institute, University of Florida, Gainesville, FL*, <sup>3</sup>*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.*

The objective of this study was to evaluate antimicrobial activity of chitosan microparticles (CM) in different matrices from dairy cows. CM was prepared by cross-linking of chitosan solution to enhance antimicrobial activity. Chitosan is biocompatible, natural antimicrobial biopolymer and considered Generally Recognized As Safe in many countries. Although CM shows strong antimicrobial activity in vitro, antimicrobial activity of CM in different animal matrices is not understood. We assayed antimicrobial activity of CM in cow uterine fluids ( $n = 3$ ), milk samples ( $n = 5$ ) and rumen fluids ( $n = 4$ ) that were collected from metritic, subclinical mastitic, and fistulated dairy cows, respectively. To evaluate antimicrobial activity in the rumen fluids, we inoculated  $5 \times 10^4$  cfu/mL *E. coli* O157:H7 into the fluids, but we used

endogenous microorganisms for milk and uterine samples. Concentrations of CM ranging from 0.1% to 0.8% were tested in these matrices. Efficacy of antimicrobial activity of CM was measured by direct plating of treatments to measure the concentration of pathogens. Milk and uterine fluid samples were plated on LB agar, but the rumen fluids were plated on CT-SMAC agar after 0, 2, 4 and 6 h incubation to select *E. coli* O157:H7. The experiment in each fluid was conducted as a completely randomized design independently and in triplicate. Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Treatment with CM effectively reduced the concentration of pathogens in all of the tested samples, even though the antimicrobial activity varied depending on matrices and CM concentrations. In milk, naturally occurring pathogens were completely eliminated within 4 h with 0.1% CM ( $P < 0.001$ ). Regarding the cow uterine fluid, although the growth of naturally occurring pathogens was inhibited at 0.1% ( $P < 0.001$ ), higher concentration (0.6%) of CM was required to eradicate pathogens ( $P < 0.001$ ). Finally, 0.2% CM reduced *E. coli* O157:H7 by 2 log cfu/mL in spiked rumen samples ( $P < 0.001$ ). These data demonstrate that a natural antimicrobial agent, CM, retains antimicrobial activity in different matrices from dairy cows.

**Key Words:** chitosan microparticle, antimicrobial activity

**T29 Targeted oxylipid analyses of milk obtained from periparturient dairy cows.** Jeffery C. Gandy\*, Vengai Mavangira, and Lorraine M. Sordillo, *Michigan State University, East Lansing, MI.*

Dysfunctional mammary gland inflammatory responses around the time of parturition contribute to immunopathology associated with mastitis. Oxylipids are bioactive lipid mediators that restore tissue homeostasis following injury by orchestrating the initiation and resolution of inflammation. The objective of this study was to develop a method to detect milk oxylipid concentrations and profile changes with respect to lactation stage. Milk samples were obtained from 11 multiparous cows at the time of calving and again at approximately 80 and 220 d in milk. Analytes in milk were obtained using solid-phase column extraction and then measured using liquid chromatography with tandem mass spectrophotometry (LC/MS/MS). The amount of oxylipids in each sample were quantified relative to internal standard abundance and calibrated against standard curves. Using this newly developed extraction and analytical methodology, a total of 31 of the 63 total oxylipids species targeted for analyses could be detected in milk samples within the limits of detection. Linoleic acid (2,419  $\mu\text{M}$ ) was the predominant polyunsaturated fatty acid detected in milk samples regardless of lactation state followed by arachidonic (743  $\mu\text{M}$ ), docosahexaenoic (89.9  $\mu\text{M}$ ), and eicosapentaenoic (82.14  $\mu\text{M}$ ) acids. The linoleic acid-derived oxylipids were the most abundant found in milk with significant increases ( $P < 0.05$ ) in several hydroxyl products including 9,10-dihydroxy-octadecenoic acid during late (109.6 nM) when compared with milk samples obtained in early lactation (0.01 nM). Lipoxin A4 is an arachidonic acid-derived oxylipids with known anti-inflammatory functions that was higher ( $P < 0.05$ ) in milk samples obtained at the time of calving (0.01 nM) when compared with late lactation samples (0.002 nM). These are the first observations of how oxylipids profiles change with respect to lactation stage and in response to changing concentration of fatty acid substrates in milk. A better understanding of how individual milk oxylipids affect mammary gland immunity may provide new insight of how to better control dysfunctional inflammatory responses during disease.

**Key Words:** oxylipid, eicosanoid, inflammation

**T30 Prevalence and antimicrobial resistance of mastitis pathogens in cattle dairy in a region of Colombia.** Maria del P. Sanchez\*, Norma P. Gutierrez, and Ivan J. Posada, *Universidad Cooperativa de Colombia Sede Ibagué, Ibagué, Tolima, Colombia.*

Bovine mastitis is the most common and major economic problem in the dairy industry worldwide, with a wide variety of microorganisms involved. Identification of mastitis pathogens is important for the selection of appropriate antimicrobial therapies. Antimicrobial agents are used to treat these infections caused by bacteria in particular but in recent years some bacteria have demonstrated full or partial resistance to different antibiotics. This phenomenon called antimicrobial resistance is a rising concern in both public and animal health and in this case, dairy science. The objective of the research was to determine the prevalence and antimicrobial resistance of pathogens involved in bovine mastitis in a region of Colombia. 1392 quarters of 348 cows from Anaime Colombia region were tested, using the California mastitis test (CMT). The positive samples to CMT were cultured for bacteriological isolation and were tested for antibiotic susceptibility and resistance by disk diffusion method and performed according to CLSI guidelines in Mueller-Hinton agar. Analysis of the results was made through descriptive statistics and prevalence ratio. 190 (54,6%) cows were considered mastitis free. One hundred fifty-eight cows (45,4%) were positive for CMT and bacteriological culture. Clinical mastitis in 20 (5,74%) cows characterized by milk abnormalities and subclinical mastitis in 138 (39,65%) were found. Coagulase-negative staphylococci (CoNS) was the main pathogenic agent with 44,79% of the total isolations, followed by *Staphylococcus aureus* (CoNP) 30,73% and *Streptococcus* spp. 22,39%, both *Klebsiella pneumoniae* and *E. coli* with 1,1%. CoNS and *S. aureus* exhibited the highest degree of resistance to penicillin G, *S. aureus* and *Streptococcus* spp. showed a high resistance to streptomycin and erythromycin respectively. The most prevalent organism was CoNS considered currently a worldwide emerging mastitis and it was mainly evidenced as a cause of subclinical mastitis with high degree of resistance to penicillin.

**Key Words:** clinical mastitis, subclinical mastitis, coagulase-negative staphylococci

**T31 Variation in clinical mastitis detection frequency and etiology among milkers.** Paulo Cesar Duque-Madrid<sup>1,3</sup>, Cedric Blanc<sup>2</sup>, and Alfonso Lago<sup>\*3</sup>, <sup>1</sup>*Universidad de Caldas, Manizales, Colombia*, <sup>2</sup>*GTV Dairies, Tipton, CA*, <sup>3</sup>*DairyExperts Inc., Tulare, CA.*

Early detection of clinical mastitis cases is considered key for the success of therapy and to maintain milk quality. Our objectives were to describe variation in 1) clinical mastitis detection frequency among milkers; and, 2) etiology of clinical mastitis among low and high detection milkers. Clinical mastitis records were from 712 cases from one California Central Valley large dairy herd where cows were milked twice a day in 2 separate parlors. Milking procedures included forestripping with observation of milk appearance on the parlor floor. When abnormal milk was observed a milk sample was collected, labeled with the milker ID, and cultured on-farm following the Minnesota Easy Culture system. Culture results were classified as no-growths, gram-negatives, gram-positives, and, mixed culture when gram-negatives and gram-positives were isolated. The total number of cases detected among 10 milkers ranged from 21 to 113 and the number of working hours from 817 to 987. Milkers 1 to 5 detected 2 to 7 cases per 100 h and were classified as low detection milkers. Conversely, milkers 6 to 10 detected 9 to 12 cases per 100 h and were classified as high detection milkers. The percentage

of samples from which bacteria were isolated ranged from 38% to 71% among milkers. However, milker's clinical mastitis detection rate did not affect the percentage of samples with bacterial growth ( $P = 0.93$ ) - it was 58% for the 2 clinical mastitis detection rate groups. Gram-positives were the bacteria most commonly isolated from both groups with 32% of the samples; gram-negative bacteria represented 21% and 16% of isolates in groups low and high, respectively; and, mixed cultures 5% and 10% in groups low and high, respectively. In conclusion, there was a large variation among milkers in clinical mastitis detection rate and in the percentage of samples with bacterial growth. However, neither the percentage of samples with bacterial growth nor the etiology were influenced by the level of detection. Therefore, it appears that high clinical mastitis detection rates can be achieved without increasing the percentage of cases without bacterial growth.

**Key Words:** clinical mastitis, mastitis detection, milkers

**T32 The effect of concentrate allocation strategy on the metabolic and immune function of high genetic merit dairy cows offered a grass silage based diet.** Mark W. Little<sup>\*1,2</sup>, Niamh O'Connell<sup>2</sup>, Jason Barley<sup>3</sup>, Michael D. Welsh<sup>3</sup>, and Conrad P. Ferris<sup>1</sup>, <sup>1</sup>*Agri-Food and Biosciences Institute, Hillsborough, UK*, <sup>2</sup>*School of Biological Sciences, Queens University Belfast, Belfast, UK*, <sup>3</sup>*Agri-Food and Biosciences Institute, Veterinary Sciences Division, Belfast, UK.*

The impact of concentrate allocation strategy on metabolic and immune function has received little attention. This 140-d study (commencing at calving) examined the effect of either a group fed (GF) or individual cow fed (ICF) concentrate allocation strategy on hematology, biochemistry, inflammatory and immune competence of Holstein Friesian dairy cows ( $n = 72$ ). With GF, cows were offered a total mixed ration comprising grass silage and concentrates (50: 50 DM ratio) plus 0.35 kg chopped straw/cow/day throughout the study. With ICF, cows were offered a basal ration consisting of grass silage, concentrates (6 kg/cow/day) and chopped straw (0.35 kg/cow/day), with this diet designed to meet the cows energy requirements for maintenance plus 24 kg milk/cow/day. With this treatment additional concentrates were offered 'feed-to-yield' via an out-of-parlor feeding system (0.45 kg concentrate/kg milk) based on each individual cow's milk yield during the previous week. Blood samples were obtained from the coccygeal vein or artery of each cow at wk 2, 4, 6, 8, 10, 12, 16 and 20 ( $\pm 3$  d) post-calving. Data were analyzed using a residual maximum likelihood (REML) mixed model analysis using GenStat (Version 16.2). Cows on the GF treatment had a higher mean hemoglobin ( $P = 0.009$ ) and packed cell volume ( $P = 0.018$ ), higher lymphocyte ( $P = 0.020$ ) percentage and lower neutrophil percentage ( $P = 0.018$ ) than cows on the ICF treatment. Cows on the GF treatment had lower serum NEFA concentrations ( $P = 0.028$ ) and tended to have a higher serum albumin concentration ( $P = 0.055$ ) than cows on the ICF treatment. There was no effect of concentrate allocation strategy on serum haptoglobin ( $P = 0.356$ ) or interferon gamma production of whole blood incubated with pokeweed mitogen ( $P = 0.115$ ). Allocating concentrates on a group basis resulted in small physiological improvements to metabolic function, but had no effect on immunological function, compared with offering the same amount of concentrates on an individual cow basis.

**Key Words:** concentrate allocation, metabolic function, immune function



**T33 Evaluation of experimental novel germicide postmilking teat dips and a commercial iodine barrier postmilking teat dip on teat end and teat skin health and integrity.** Rae Sires, Kia Knutson, and Leo L. Timms\*, *Iowa State University, Ames, IA, 50011.*

Study objective was to evaluate prototype novel germicide post milking teat dips versus a control commercial iodine barrier post milking teat dip on overall teat end (TE) and teat skin (TS) health and integrity. Control dip was Bovi-Kote (1% iodine with 10% multiple emollient system (low drip): Boumatic, Inc.) and treatment dips were a novel germicide dip with 10% citrate, parabens, and varying methylene blue (MB) (0.1, 0.25, and 0.4%) and emollient (5, 10, and 11%) concentrations (Zurex Pharma Inc.). Trial used a split pen and split udder design. Twenty-four early- mid lactation Jersey cows in a single pen were used with 12 cows having a blue leg band (BLB group) to designate them as trial group 1 while the other 12 had no leg band (NLB group). Left teats of all 24 cows were post dipped with Bovi-Kote (control) while right teats were were initially dipped with a 0.1% methylene blue dip (1/29) but differed in emollient % (5 v 11%). Dips changed to .25% MB (2/10) to enhance coloration. NLB teats switched to a 0.4% MB with 11% emollients (2/19), while BLB teats switched to a higher emollient (10 v 5%) no drip teat dip (2/20). Trial was 5 weeks in duration (Jan–Feb). TS and TE scoring were performed 1st 4 trial days, then 3×/week using Goldberg and Timms methods (1–5 scoring for both, 0.5 increments for TE scores). Mixed procedure of SAS with repeated measures (quarter within cow as a repeated measure) were used to analyze TS and TE data and GENMOD procedures of SAS with repeated measures was used to analyze % rough teat ends or dry/chapped teat skin, with  $P < 0.05$  considered significant. Results of experimental vs commercial control dip were similar across groups so data were combined. No significant differences in TS score were seen (99+% excellent). TE scores and % rough teats were not significantly different during weeks. One and 4, but were significantly higher [1.6, 13% (Exp) v 2.1, 27% (Con)  $P < 0.01$ ] and compromised in control teats during wks. 2–3, corresponding to temperature change (30°F (–1°C) to –4°F (–20°C)). Experimental dip showed significantly better TE during colder weather and temperature changes.

**Key Words:** postmilking teat dip, germicide, teat health

**T34 Study of the activity of soluble and nanostructured IFN $\gamma$  and metalloproteinases as a new tool for the optimization of the dry-off of dairy cows.** Francesc Fabregas<sup>1</sup>, Olivia Cano<sup>2,3</sup>, Sandra Genís<sup>\*1</sup>, Silvia Parés<sup>1</sup>, Joaquim Seras-Franzoso<sup>2,3</sup>, Alex Bach<sup>1,4</sup>, Antonio Villaverde<sup>2,3</sup>, Elena Garcia-Fruitos<sup>2,3</sup>, and Anna Aris<sup>1</sup>, <sup>1</sup>*Department of Ruminant Production, Institute of Research in Agriculture and Technology, Caldes de Montbui, Spain*, <sup>2</sup>*Department of Genetics and Microbiology, Institute of Biotechnology and Biomedics, Universitat Autònoma de Barcelona, Bellaterra, Spain*, <sup>3</sup>*CIBER de Bioingeniería, Biomateriales y Nanomedicina, Bellaterra, Spain*, <sup>4</sup>*ICREA, Barcelona, Spain*.

The use of bovine metalloproteinases and IFN $\gamma$  to hasten mammary gland involution and the stimulation of immune system response has been proposed to accelerate the dry off period and diminish the risk of intramammary infections. The protein nanoparticles technology has shown to be an economically viable production strategy of recombinant proteins. The objective of this work was to study the activity of bovine metalloproteinase 2 and 9 (MMP-2, MMP-9) and IFN $\gamma$  produced in *Lactococcus lactis* either as nanoparticles (Lu et al. Mol. Biotechnol. 2013 54:170–176) or soluble recombinant proteins. The activity of IFN $\gamma$  was tested using a macrophage activation test along with an assay of induction of nuclear factor kappa- $\beta$  (NF- $\kappa$ B) expression in mammary

epithelial cells. Monocytes were isolated from cow blood, differentiated to macrophages, and treated with IFN $\gamma$  for 24 h at 37°C, 5% CO<sub>2</sub> while primary culture of mammary gland epithelial cells were treated with IFN $\gamma$  for 4 h at 37°C, 5% CO<sub>2</sub>. Cell samples were taken to analyze the expression of NF- $\kappa$ B by qPCR. Activity of MMP-2 and MMP-9 soluble or as nanoparticles was tested by both zymography and ex vivo. For the ex vivo assay, mammary gland explants were incubated with MMP for 7 h at 37°C and 5% CO<sub>2</sub>. Then, apoptotic and proliferation gene expression was assessed by qPCR (PCNA, SPC25, CASP3 and BNIP). The sensitivity of NF- $\kappa$ B assay was greater than the macrophage activity test (detection limit of 19.6  $\mu$ g and 140  $\mu$ g IFN $\gamma$ , respectively;  $P < 0.05$ ). Soluble IFN $\gamma$  upregulated ( $P < 0.05$ ) NF- $\kappa$ B, whereas similar amounts of IFN $\gamma$  nanoparticles had no significant effect. Metalloproteinase activity was detected for MMP-2 and MMP-9 in both forms by zymography. As expected, the soluble form was more active than the nanostructured form ( $P < 0.05$ ). Only soluble MMP9 activity was detected ex vivo, stimulating ( $P < 0.001$ ) the expression of SPC25 and PCNA proliferative genes. In conclusion, the bovine recombinant MMP and IFN $\gamma$  proteins elicited the expected biological response and thus they can be used in further in vivo dry-off studies in dairy cows.

**Key Words:** dry period, nanoparticle, metalloproteinase

**T35 Metabolic parameters of cows with different status for bovine leukemia.** Irina V. Vinogradova<sup>\*1</sup>, Elena A. Gladyr<sup>1</sup>, Ludmila A. Ivanova<sup>2</sup>, Alexandr S. Kramarenko<sup>1</sup>, Igor V. Gusev<sup>1</sup>, Roman V. Rykov<sup>1</sup>, Michael I. Guljukin<sup>2</sup>, and Natalia A. Zinovieva<sup>1</sup>, <sup>1</sup>*L.K. Ernst Institute of Animal Husbandry, Dubrovitsy, Moscow, Russia*, <sup>2</sup>*Y.R. Kovalenko Institute of Experimental Veterinary Medicine, Moscow, Russia*.

Increased susceptibility of high-productive dairy cows to infectious diseases is associated with metabolic stress which is developed on the background of a negative energy balance. The objective of our study was to determine the differences in metabolic parameters and blood leukocyte profiles in cows infected by bovine leukemia virus (BLV+) compared with non-infected cows (BLV–). The blood samples were collected from 150 cows of the Russian Black-and-White breed. The cows were housed at identical environmental conditions in one free-stall facility and were fed by the same diets. BLV status was determined by immune diffusion reaction and confirmed by enzyme immune assay. The blood serum concentrations of carbohydrate, protein and lipid metabolites were measures and the blood leukocyte profile was evaluated. The experimental data were analyzed using logistic regression analysis of binary data, (<http://www.statistica-help.ru/node/28>) ( $p_1$ ) and linear model ANOVA ( $p_2$ ), where the BLV status and correlation coefficient were taken as a random factor. Student  $t$ -test was performed to evaluate the statistical significance of differences between the groups. The serum concentrations of albumin, creatinine and ALT did not differ significantly between the groups, but the concentrations of the total protein (+6.89 g/L,  $p_1 \leq 0.0001$ ,  $p_2 \leq 0.0141$ ), globulin (+6.95 g/L,  $p_1 \leq 0.0002$ ,  $p_2 \leq 0.0483$ ), urea (+1.02 mM/L,  $p_1 \leq 0.0020$ ,  $p_2 \leq 0.0006$ ) and bilirubin (+2.01 mM/L,  $p_1 \leq 0.0162$ ,  $p_2 \leq 0.0016$ ) were significantly higher among BLV+ cows compared with non-infected cows, but the concentrations of glucose (–0.50 mM/L,  $p_1 \leq 0.0007$ ,  $p_2 \leq 0.0940$ ), triglycerides (–0.04 mM/L,  $p_1 \leq 0.0384$ ,  $p_2 \leq 0.0065$ ), alkaline phosphatase (–63.1 IE/L,  $p_1 \leq 0.0384$ ,  $p_2 \leq 0.0065$ ) and AST enzyme (–1.56 IE/L,  $p_2 \leq 0.0713$ ) were significantly decreased. According to clinical indicators of blood (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW) significant differences between the groups were not found. The data obtained confirm the assessment that bovine leukemia has effect on the biochemical parameters of carbohydrate, lipid, protein metabolism.

The research was supported by the Russian Science Foundation, project number 14–16–00046.

**Key Words:** dairy cattle, bovine leukemia virus, biochemical paramete

**T36 High forage diet alters feeding behavior, health, and milk production in fresh Holstein dairy cows.** Juliana M. Huzzey<sup>\*1,2</sup>, Hesam A. Seifi<sup>1,3</sup>, Muhammad A. Khan<sup>1,4</sup>, Marina A. G. von Keyserlingk<sup>1</sup>, and Daniel M. Weary<sup>1</sup>, <sup>1</sup>*Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada*, <sup>2</sup>*Animal Science Department, California Polytechnic State University, San Luis Obispo, CA*, <sup>3</sup>*Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran*, <sup>4</sup>*AgResearch, Grasslands Research Centre, Palmerston North, New Zealand*.

High-forage diets for close-up dairy cows are gaining popularity and are increasingly perceived to provide health and production advantages. After calving cows are typically transitioned to a high-energy diet but it is not clear how this transition affects their health and behavior. Our objective was to test the effect of maintaining cows on a high-forage diet for 3 wk after calving, versus switching to the conventional high-energy diet. Sixty-eight Holstein dairy cows (21 primiparous and 47 multiparous cows) were all fed the same high-forage prepartum diet. After calving, cows were moved to a postpartum pen for 21 d and randomly assigned to feed bins according to their respective treatments (Straw TMR: STMR vs. Control TMR: CTMR). After 21 d, all cows were moved to a new pen and fed CTMR. An electronic feeding system continuously monitored individual feeding behavior and intake. Rumination time was recorded using data loggers. Blood was sampled twice per week and tested using the  $\beta$ -hydroxybutyrate (BHBA) Precision Xtra kit. Cows were considered subclinically ketotic (SCK) if BHBA was  $\geq 1.0$  mmol/L in the first wk postpartum or  $\geq 1.2$  mmol/L from d 7 to 35 postpartum. Milk yield was recorded daily. Data were analyzed using the Mixed and Genmod procedures of SAS; cow was treated as a random effect and period (wk or d) as the repeated measure. Fresh cows fed STMR had lower DMI, fewer meals, spent more time at the feed bunk and had a slower feeding rate ( $P \leq 0.02$ ). Rumination time of cows fed STMR was higher than cows fed CTMR ( $P = 0.04$ ). Cows fed STMR had higher BHBA concentrations during wk 3 ( $P = 0.03$ ) and were 4.9 times more likely than the CTMR group to develop SCK by wk 3 (odds ratio = 4.9, 95% confidence interval = 0.95 - 25.47,  $P = 0.06$ ). Over the first 35 d of lactation, cows fed CTMR produced more milk than those fed STMR ( $33.3 \pm 1.0$  vs.  $30.7 \pm 1.0$  kg/d;  $P = 0.03$ ). These results indicate that maintaining cows on a high-forage diet after calving can have detrimental effects on energy balance and cow health.

**Key Words:** transition cow, nutrition, welfare

**T37 Prepartum rumination patterns in dairy cows that develop health disorders in the early postpartum period.** Matias L. Stangaferro<sup>\*</sup>, Robert Wijma, Miranda M. Medrano, Mohammed A. Al Abri, and Julio O. Giordano, *Department of Animal Science, Cornell University, Ithaca, NY*.

The objective of this study was to compare prepartum rumination patterns of lactating dairy cows that developed health disorders (HD) versus cows that did not develop HD (NoHD) up to 30 DIM. A total of 559 Holstein cows (222 primiparous and 337 multiparous) were fitted with a rumination and activity monitoring tag (HR Tags, SCR Dairy) from -28 to 30 DIM. Rumination time (RT) was recorded in minutes per 2 h intervals and summarized in 24 h periods. After calving, farm

personnel examined cows daily for signs of clinical disease following farm standard operating procedures. From 1 to 10 DIM, personnel evaluated: appetite, rectal temperature, ketone bodies in urine, rumen fill and movements, retained placenta, vaginal discharge, daily milk weights, and milk conductivity. Milk culture was performed for all mastitis cases. Number of cows suffering HD was displaced abomasum (DA) 17, ketosis (KET) 16, indigestion (IND) 5, metritis (MET) 171, retained placenta (RP) 49, and mastitis (MAST) 36. Rumination time for the 7 d preceding calving was evaluated for cows with HD or NoHD within 30 DIM. Also, data were evaluated for all metabolic and digestive diseases (MDZ = DA, KET, IND) combined and RP, MET, and MAST individually. Data were analyzed by ANOVA with repeated measurements using PROC MIXED of SAS. For all HD combined, RT was less ( $P = 0.02$ ) for cows with HD ( $439.1 \pm 2.8$  min) than for cows with NoHD ( $455.9 \pm 2.0$  min). Rumination time was lowest ( $P < 0.01$ ) on the day of calving ( $391.3 \pm 4.3$  min) than the 6 d preceding calving (mean range =  $457.5 \pm 4.0$  –  $462.6 \pm 4.1$  min) for all cows. Cows with MDZ had reduced RT ( $P < 0.01$ ) than NoHD cows ( $409.6 \pm 7.3$  vs  $455.9 \pm 2.0$  min). Cows with RP had similar RT ( $P = 0.25$ ) than NoHD cows ( $440.2 \pm 5.0$  vs  $455.9 \pm 2.0$  min). Cows with MET tended to have reduced RT ( $P = 0.05$ ) than NoHD cows ( $440.5 \pm 3.1$  vs  $455.9 \pm 2.0$  min). Cows with MAST had similar RT ( $P = 0.82$ ) than NoHD cows ( $452.4 \pm 6.1$  vs  $455.9 \pm 2.0$  min). We conclude that starting 7 d prepartum, rumination patterns are altered in cows that suffer health disorders within 30 DIM. Rumination time is reduced in cows that suffer MDZ (DA, KET, IND) and MET but not in cows with RP and MAST

**Key Words:** rumination, dairy cow, disease

**T38 Plasma phosphatidylcholine and lysophosphatidylcholine profiling of heat-stressed lactating dairy cows.** He Tian<sup>1</sup>, Jianbo Cheng<sup>2</sup>, Yangdong Zhang<sup>1</sup>, Nan Zheng<sup>\*1</sup>, and Jiaqi Wang<sup>1</sup>, <sup>1</sup>*Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China*, <sup>2</sup>*College of Animal Science and Technology, Anhui Agricultural University, Hefei, China*.

Heat stress (HS) is a global challenge for the dairy industry; however, its abnormal physiological alterations still remain elusive. Here, a LC-MS-based study on lysophosphatidylcholine (lysoPC) and phosphatidylcholine (PC) in plasma of HS dairy cows was performed to explore HS-induced metabolic alterations. LysoPCs or PCs exist in 2 forms/isomers, with the fatty acyl groups at positions 1 (sn-1) or 2 (sn-2) on the glycerol backbone. First experiment, the LC-MS-based method of discriminating isomers of lysoPCs or PCs was constructed. The isomeric lysoPCs or PCs can be differentiated by chromatographic separations and relative intensities of their characteristic fragments. Second experiment, the enrolled 2 groups of Holstein cows were in second parity, mid-lactation stage, had similar milk yield (both around 40 kg/day), and fed the same diet. The HS-free group consisted of 22 cows, with plasma samples obtained in spring season, after temperature-humidity index (THI) was around 50–55 for 1 mo. The HS group consisted of another 22 cows, with samples obtained in summer season, after THI gradually increased from 68 to 80 over 1 mo. LC-MS in combination with multivariate analyses on plasma profiling of HS-free and HS groups was conducted to discover HS-induced discriminating metabolites ( $P < 0.05$ ), from which isomeric lysoPCs or PCs were identified by the constructed method in first experiment. The results revealed that concentrations of 14 lysoPCs and 17 PCs were significantly up- or downregulated in HS compared with in HS-free group, indicating HS-induced alterations of lysoPCs and PCs. Of these 31 discriminating metabolites, concentrations of sn-1 lysoPC 14:0, sn-2 lysoPC 15:0, sn-1 lysoPC 18:0, and sn-2 lysoPC 22:4 were upregulated in HS group ( $P < 0.05$ ), concentrations

of sn-1 PC 14:0/18:3, sn-1 PC 14:0/18:2, sn-2 PC 15:0/17:1, sn-1 PC 14:0/20:4, sn-1 PC 18:0/15:1, sn-1 PC 18:0/15:0, and sn-2 PC 15:0/20:4, were downregulated in HS group ( $P < 0.05$ ); whereas, concentrations of their respective isomers remained unchanged, suggesting isomers of these lysoPCs and PCs have different biological functions played in lactating dairy cows under HS states.

**Key Words:** lactating dairy cow, heat stress, phosphatidylcholine and lysophosphatidylcholine profiling

**T39 Assessment of an application to collect calving-related events in dairy herds.** A. A. Barragan\*, J. D. Workman, S. Bas, K. L. Proudfoot, and G. M. Schuenemann, *The Ohio State University, Columbus, OH.*

Calving-related losses (survival, health, and productivity of cows and calves) are known challenges for the dairy industry worldwide, and poor management practices can increase the risk of these losses. It is common to observe large variation within and between herds on the quality and quantity of calving-related records. The objective of the present study was to assess an application (APP) for hand-held smart devices to capture defined calving-related events in dairy herds. Calving events collected by personnel ( $n = 23$ ) from 6 large dairy operations (range: 900–5,000 cows) were recorded. Calving personnel received the same training on calving management and use of the APP before collecting events. Immediately after training, personnel recorded calving events ( $n = 448$ ) using the APP for 7 d. Personnel satisfaction with the APP and knowledge gained from the training were assessed. Calving personnel reported that the information provided during the training was relevant (agree = 14.3% and strongly agree = 85.7%) and of great immediate use (agree = 33.3% and strongly agree = 66.7%). The presented materials and demonstrations substantially increased ( $P < 0.05$ ) the knowledge level of the participants by 23.7 percentage points from pre- to post-test scores. The APP accurately captured (100%) calving events and integrated multiple metrics with personnel performance (accounting for the effect of shift change) such as the dam (e.g., date-time of calving), colostrum (e.g., timing, quality, and quantity) and newborn calf (e.g., presentation, vigor). The follow-up assessment with participants revealed that the APP was easy to use (91.3%) and that they would like to keep using it (100%). Dairywomen, consultants, and veterinarians often trouble-shoot calving related losses within-herd; however, the lack of meaningful records makes it difficult to implement effective corrective measures. It is important to note that the timing and accuracy of data is always dependent on the willingness of the individual recording the information. However, this effective and easy-to-use tool requires minimal personnel training. These findings showed that decision-makers can monitor calving events and losses (magnitude and time) at the farm level accounting for the effect of management.

**Key Words:** dairy cattle, calving, application

**T40 Difuctose anhydride III supplementation promotes passive calcium absorption in the small intestine immediately after calving in dairy cows.** Makoto Teramura\*<sup>1,2</sup>, Syaw Wynn<sup>1</sup>, Maimaiti Reshalaitihan<sup>3</sup>, Wakana Kyuuno<sup>2</sup>, Tadashi Sato<sup>2</sup>, Masayuki Ohtani<sup>2</sup>, Chiho Kawashima<sup>3</sup>, and Masaaki Hanada<sup>3</sup>, <sup>1</sup>*United Graduate School of Agricultural Sciences, Iwate University, Morioka, Iwate, Japan*, <sup>2</sup>*Nippon Beet Sugar Manufacturing Co., Ltd., Obihiro, Hokkaido, Japan*, <sup>3</sup>*Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.*

Difuctose anhydride (DFA) III is an indigestible oligosaccharide. It has been shown to reach the duodenum without being degraded by ruminal bacteria and to promote the intestinal calcium (Ca) absorption via paracellular pathway in cattle, but these studies did not mention DFA III's effectiveness during early postpartum (pp) period. The objective of this study was to determine whether DFA III could promote intestinal paracellular Ca absorption during early pp period. Seventy-four multiparous Holstein cows were separated into the DFA and control groups based on their parity ( $3.7 \pm 0.3$  and  $3.7 \pm 0.3$ , respectively). The DFA group was fed 40 g/d of DFA III from -14 d to 6 d relative to calving and the control group was not fed DFA III. At calving (0 h pp), the serum Ca declined below 9 mg/dL in both groups. However, the serum Ca was significantly greater in the DFA group than in the control group at 6 h, 12 h, 24 h, and 48 h pp ( $P < 0.05$ ) and the time interval for serum Ca recovery to  $\geq 9$  mg/dL during the pp period was shorter in the high-parity cows of the DFA group than in those of the control group. The serum parathyroid hormone (PTH) increased at 0 h pp in both groups and was significantly greater in the control group than in the DFA group at 12 h and 24 h pp ( $P < 0.05$ ). The serum 1,25-dihydroxyvitamin D [ $1,25-(OH)_2D$ ] increased at 0 h and 12 h pp in both groups and was significantly greater in the control group than in the DFA group at 72 h pp ( $P < 0.01$ ). The serum bone-resorption marker cross-linked N-telopeptide of type I collagen (NTX) was not significantly different between the groups during peripartum period, and the serum NTX in all of the cows was lesser at 0 h, 6 h, 12 h, 24 h, 48 h, and 72 h pp than at -21 d, 4 d, and 5 d relative to calving ( $P < 0.05$ ). These results suggest that the DFA group had rapid recovery of serum Ca, although bone resorption was restrained and active intestinal Ca absorption via transcellular pathway was impaired. In conclusion, DFA III promotes intestinal passive Ca absorption via paracellular pathway during early pp period, which is unaffected by aging.

**Key Words:** hypocalcemia, dairy cow, DFA III

**T41 Supplementation with *Bacillus pumilus* 8G-134 enhances expression of T cell markers in dairy cows during early lactation.** Megan Duersteler\*<sup>1</sup>, Mike Brouk<sup>2</sup>, and Elizabeth Galbraith<sup>1</sup>, <sup>1</sup>*DuPont, Waukesha, WI*, <sup>2</sup>*Kansas State University, Manhattan, KS.*

Infectious disease during transition and early lactation periods has the potential to decrease production performance and increase morbidity and mortality in dairy cows. Enhanced adaptive immunity through nutritional supplementation may counter immunosuppression that occurs naturally during the periparturient and early lactation periods. The objective of this study was to evaluate the effect of a direct-fed microbial, *Bacillus pumilus* 8G-134, on systemic immunity in dairy cows through flow cytometric analysis of peripheral blood mononuclear cells. Thirty cows were fed a control diet or a diet including  $5 \times 10^9$  cfu/head/day *B. pumilus* 8G-134 from 3 weeks prepartum to 22 weeks postpartum. Peripheral blood mononuclear cells were collected at 60 d in milk and analyzed by flow cytometry. Leukocytes and lymphocytes were gated based on forward and side scatter, and relevant cell surface markers were examined in each population. *B. pumilus* 8G-134 supplementation increased ( $P < 0.05$ ) expression of T cell surface markers CD4, CD8, CD62L, CD25, and CD45RO, but did not affect proportions of T cell subsets. There was no effect on expression of cell surface markers on innate immune cells (Mono, CD14, CD45, CD172a, and CD62L) or B cells (Bcell, and CD21). Feeding *B. pumilus* 8G-134 induced expression of T cell surface markers that are naturally suppressed during parturition. By targeting the adaptive immune system, *B. pumilus* 8G-134 was able to enhance T cell marker expression without activating a systemic



immune response that would adversely affect energy balance. This may help dairy cows recover faster from periparturient immunosuppression.

**Key Words:** dairy, direct-fed microbial, immunology

**T42 A stochastic estimate of the economic impact of oral calcium supplementation in postparturient dairy cows.** J. A. A. McArt<sup>1</sup> and G. R. Oetzel<sup>\*2</sup>, <sup>1</sup>*Department of Population Medicine and Diagnostic Services, Cornell University, Ithaca, NY*, <sup>2</sup>*Department of Medical Sciences, University of Wisconsin-Madison, Madison, WI*.

Stochastic models were developed to estimate the economic impact in the first 30 d in milk of oral calcium supplementation (Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) to multiparous postparturient dairy cows using 4 different strategies: 1) supplementation of cows with a high previous lactation mature equivalent milk yield, 2) supplementation of lame cows, 3) supplementation of cows that either have a high previous lactation mature equivalent milk yield or are lame, and 4) supplementation of all cows. Data from current literature was used to model input variables associated with the costs and risks related to milk production, postparturient disease, and culling. The mean net herd impact per 1,000 calvings for each of the 4 supplementation strategies was \$4,425, \$5,812, \$8,313, and \$3,065 respectively. Return on investment was 6.5 to 1 for supplementation of lame cows, 1.8 to 1 for high milk yield and lame cows, 1.1 to 1 for high milk yield cows, and 0.3 to 1 for supplementation of all multiparous postpartum cows. A herd's average milk yield at first test had the highest influence on the net impact of oral calcium supplementation to all multiparous cows and accounted for 30% of the variation in net herd financial impact of oral calcium administration. Other variables that explained at least 5% of the variation in financial impact included decreased risk of health events in lame cows, herd prevalence of lameness, and milk price. Whereas supplementation of all postpartum multiparous cows returned a positive net herd impact approximately 80% of the time, if a herd was willing to devote time to mature-equivalent milk yield calculations and locomotion scoring, supplementation of this subpopulation of postpartum cows with oral calcium was estimated to have a positive economic impact in all iterations. Depending on the supplementation strategy chosen and baseline milk yield and immediate postpartum lameness prevalence in a herd, a herd with 1,000 calvings per year can expect to see an average net impact ranging from approximately \$3,000 to \$8,000 after postpartum supplementation of oral calcium in multiparous animals.

**Key Words:** dairy cow, oral calcium, economic impact

**T43 Mastitic cows management practices on California dairies.** Pau Pallarès<sup>\*1</sup>, Arnau Espadmalà<sup>1</sup>, Alfonso Lago<sup>2</sup>, and Noelia Silva-del-Río<sup>1</sup>, <sup>1</sup>*UC Davis School of Veterinary Medicine, VMTRC, Tulare, CA*, <sup>2</sup>*DairyExperts, Tulare, CA*.

Minimizing the risk of antibiotic resistant organisms and antibiotic residues in dairy and dairy beef products is a topic of nationwide interest. However, to design an effective outreach program on judicious use of antibiotics, it is imperative to describe the actual practices on dairies. Thus, our objective was to summarize management and treatment practices for mastitic cows on 15 California dairies as a first step in that outreach effort. Herds ranged in size from 600 to 9,500 cows. Data were collected by 2 bilingual veterinarians during the milking of mastitic cows, based on cow-side observation and responses from dairy employees. Cows identified with mastitis were kept in the same pen (n = 1) or moved to the hospital pen (n = 14). The hospital pen housed mastitic cows (n = 5); mastitic, sick and lame cows (n = 3); or mastitic,

sick, lame and fresh cows (n = 6). Four of the 10 herds housing mastitic and non-mastitic cows in the hospital pen did not clean milking units in between cows. Five dairies were fitted with a milking parlor just for fresh and mastitic cows. Dairies with a single parlor milked the mastitic cow pen last. Mastitic cows were identified during regular milking based on quarter inflammation (n = 2) and quarter inflammation and milk appearance (n = 13). Two dairies used California Mastitis Test (CMT) for confirmation. Cows with abnormal milk were sampled as soon as they were identified (n = 2) or after they were moved to the hospital pen (n = 8). Milk culture results were used for selective treatment of mastitis (n = 4). The first treatment options were intramammary cephapirin [1 d (n = 1), 3 d (n = 2), 4 d (n = 1) or 5 d (n = 1)], ceftiofur [2 d (n = 1), 3 d (n = 4) or 4 d (n = 3)], hetacillin [3 d (n = 1)] or intravenous oxytetracycline and sulfamides [5 d (n = 1)]. If after treatment completion cows showed abnormal milk (n = 13) or positive CMT (n = 2) the antibiotic therapy was continued with the same (n = 10) or a different (n = 5) drug. Milk culture was used on some dairies as a treatment decision tool. Although the antibiotic drug of choice was similar across dairies, the length of treatment varied widely across dairies.

**Key Words:** dairy cattle, mastitis, intramammary treatment

**T44 Concentrations of saturated fatty acids in whole raw milk of dairy cows under different management systems and country of origin: A meta-analytical study.** Grzegorz Zwierchowski\* and Burim Ametaj, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada*.

The objective of this meta-analytical study was to investigate concentrations of milk saturated fatty acids (SFA) including C4, C6, C8, C10, C12, C14, C15, C16, C17, and C18 in whole raw dairy cow milk from different production systems (conventional [CVN] vs organic [ORG]) and several countries of origin. Data from 18 studies and 13 countries were used to create a data set, which was used for statistical analyses. Data are reported in milk fat percent. The average concentrations of C15 and C17 in the raw milk from ORG system were greater (1.49 vs 1.12 and 1.17 vs 0.61%, respectively) than those from CVN farms ( $P < 0.05$ ). However, no differences were found with regards to concentrations of C4 (3.21 vs 3.17%), C14 (10.70 vs 10.13%), and C16 (28.69 vs 26.82%), in the raw milk between ORG and CNV systems. Also no differences were obtained in the concentration of C6, C8, C10, C12, and C18. On the other hand, country of origin affected concentrations of SFA ( $P < 0.05$ ). For example, raw milk from Ireland had lower amounts of C4 compared with milk from Belgium (1.33 vs 6.26%;  $P < 0.05$ ). Additionally, the greatest concentrations of C6, C8, C10, and C14 were reported in Norway (3.11, 1.95, 4.43, and 13.88%, respectively), whereas the lowermost levels of those fatty acids were reported in Germany (0.82%), Switzerland (0.95%), Mexico (0.91%), and United Kingdom (7.13%), respectively ( $P < 0.05$ ). Average C12 levels varied from 1.97% (Mexico) to 4.83% (Netherlands) ( $P < 0.05$ ). Likewise, milk originating from US had lower means of C15 (0.63 vs 2.53%) and C17 (0.33 vs 1.61%) compared with milk from Germany ( $P < 0.05$ ). Additionally milk from Germany had greater C16 levels compared with milk from UK (15.84 vs 41.31%) ( $P < 0.05$ ). Means of C18 varied from 5.49 to 13.12% (China,  $P < 0.05$ ). In conclusion, data from this meta-analytical study indicated that ORG farms were characterized by greater concentrations of some of the SFA compared with CVN system of management. This study also showed high variability in concentrations of SFA in the raw milk with regards to the country of origin.

**Key Words:** whole raw milk, saturated fatty acid, meta-analysis

**T45 Concentrations of unsaturated fatty acids in the whole raw milk of dairy cows under different management systems and country of origin: A meta-analytical study.** Grzegorz Zwi-  
 erzchowski\* and Burim Ametaj, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

The objective of this meta-analytical study was to investigate concentrations of milk unsaturated fatty acids (UFA), including C14:1, C18:1, C18:2, CLA, C18:3, C20:1, C20:2, C20:4, docosapentaenoic (DPA), and eicosapentaenoic acid (EPA) in whole raw bovine milk from different production systems (conventional (CNV) vs organic (ORG)) and countries of origin. Data from 18 studies and 13 countries were used to create a data set, which was used for statistical analyses. Data are reported in milk fat percent. The average concentrations of C18:2 and C18:3 in the raw milk from CNV system were greater (1.94, and 0.60 respectively) than those coming from ORG farms (1.90, and 0.54%, respectively), but difference reached no significance. In addition, there were greater concentrations of C20:4 (0.14%) in the raw milk from CNV vs ORG system (0.11%) ( $P < 0.05$ ). Lower ( $P < 0.05$ ) amounts of C14:1 (0.76%), C20:1 (0.17%), C20:2 (0.03%), and EPA (0.04%) were reported for milk originating from ORG vs CNV (0.97, 0.26, 0.13, and 0.10%, respectively). In addition, no differences with regards to C18:1 ( $P = 0.90$ ), CLA ( $P = 0.31$ ), and DPA ( $P = 0.07$ ), were reported for milk from the ORG vs CNV system. Country of origin had significant effect on concentrations of UFA ( $P < 0.05$ ). The lowermost concentrations of C18:1, C18:2, and C20:4 were reported in Germany ( $P < 0.04\%$ ), whereas the greatest levels of those UFA were reported in Belgium and US ( $P < 0.05$ ). Milk from Poland had lower amounts of C14:1 and C20:1 compared with milk from Germany and Norway, respectively ( $P < 0.05$ ). Average DPA levels varied from 0.01% (Norway) to 0.19% (Switzerland). Moreover, milk from UK had greater means of C18:3 and CLA compared with milk from Norway and Netherlands, respectively ( $P < 0.05$ ). Norwegian milk had greater EPA levels compared with milk from China ( $P < 0.05$ ). In conclusion, data from this meta-analytical study indicated that ORG farms were characterized by greater concentrations of n-3 FA (CLA, DPA, EPA) in the milk compared with milk from the CNV system. This study also showed high variability in concentrations of UFA in raw milk with regards to the country of origin.

**Key Words:** whole raw milk, unsaturated fatty acid, meta-analysis

**T46 Owner and veterinarian involvement on fresh cow health management on California dairies.** Pau Pallarés<sup>1</sup>, Arnau Espadamala<sup>1</sup>, Alfonso Lago<sup>2</sup>, and Noelia Silva-del-Río<sup>1</sup>, *<sup>1</sup>UC Davis School of Veterinary Medicine, VMTRC, Tulare, CA, <sup>2</sup>DairyExperts, Tulare, CA.*

Minimizing the risk of antibiotic resistant organisms and antibiotic residues in dairy and dairy beef products is a topic of nationwide interest. However, to design an effective outreach program on judicious use of antibiotics, it is imperative to describe the actual practices on dairies. Thus, as a first step in that outreach effort, our objective was to evaluate the involvement of owners and veterinarians on fresh cow (FC) evaluations and to identify who FC evaluators requested advice from. Herds ( $n = 15$ ) ranged in size from 600 to 9,500 cows. Cow-side observation and responses from FC evaluators were collected by 2 bilingual veterinarians during FC evaluations. Dairy owner conducted FC evaluations ( $n = 2$ ), supervised FC evaluations ( $n = 4$ ) or delegated FC evaluations ( $n = 9$ ). The dairy veterinarian identified and treated cows once a week ( $n = 2$ ) or every other week ( $n = 2$ ), provided occasional advice on treatments ( $n = 7$ ) or they were not involved on FC evaluations ( $n = 4$ ). Dairies

with minimum veterinarian involvement relied on pharmaceutical sales representatives (PSR) and other consultants for advice on herd health. During FC evaluations a single individual identified sick cows and decided on treatments [dairy owner ( $n = 2$ ), herdsman ( $n = 5$ ), herdsman assistant ( $n = 8$ )]. Evaluators had  $< 1$  yr ( $n = 2$ ), 1–5 yr ( $n = 3$ ),  $> 5$  yr ( $n = 10$ ) of experience. New knowledge was acquired based on formal training [ $< 1$  year ( $n = 4$ ) or 1–5 yr ( $n = 1$ ) ago], communication with other dairies workers ( $n = 7$ ), working with more experienced employees at the dairy ( $n = 7$ ) or through self-teaching ( $n = 3$ ). Based on FC evaluators, treatments were decided by the owner ( $n = 8$ ) or manager ( $n = 7$ ). And, when advice on treatments was needed, the owner ( $n = 6$ ), the veterinarian ( $n = 8$ ), or the PSR ( $n = 1$ ) were consulted. Information on new drugs and treatments came through veterinarians ( $n = 8$ ), and/or PSR ( $n = 13$ ). Veterinarians involvement on FC evaluations can be strengthened. Furthermore, many FC evaluators relied on their peers to learn and discuss work related issues. This information suggested that teaching tools that promote peers networking might be of relevance for fresh cow employees.

**Key Words:** dairy cattle, fresh-cow evaluation, veterinarian

**T47 Fresh cow evaluations and treatments on California dairies.** Arnau Espadamala<sup>1</sup>, Pau Pallarés<sup>1</sup>, Alfonso Lago<sup>2</sup>, and Noelia Silva-del-Río<sup>1</sup>, *<sup>1</sup>UC Davis School of Veterinary Medicine, VMTRC, Tulare, CA, <sup>2</sup>DairyExperts, Tulare, CA.*

Minimizing the risk of antibiotic resistant organisms and antibiotic residues in dairy and dairy beef products is a topic of nationwide interest. To design an effective outreach program on judicious use of antibiotics, it is imperative to describe the actual practices on dairies. Thus, our objective was to summarize management and treatment practices for fresh cows (FC) on 15 California dairies (600 to 9,500 cows) as a first step in that outreach effort. Data were collected by 2 bilingual veterinarians during the FC evaluations, based on cow-side observations and responses from dairy employees. Daily ( $n = 14$ ) or thrice a week ( $n = 1$ ) FC evaluations and treatments administrations lasted [median (range)] 13.8 (1.5 to 45) s/cow. To identify sick cows evaluators relied on thermometer ( $n = 1$ ), stethoscope ( $n = 7$ ) or both ( $n = 3$ ). All dairies visually inspected cows for abnormal uterine discharge. Twelve dairies evaluated 2 to 5 signs of diseases [rumen fill ( $n = 7$ ), eyes-ears ( $n = 7$ ), milk yield / udder fill ( $n = 7$ ), appetite ( $n = 7$ ), feces ( $n = 5$ ), temperature ( $n = 5$ )]. Antibiotic therapy was given systematically after eutotic ( $n = 2$ ), twinning ( $n = 7$ ) and dystotic calvings [all FC ( $n = 5$ ), primiparous FC ( $n = 1$ ), severe cases ( $n = 7$ )]. Cows with retained placenta were treated at 24 ( $n = 8$ ), 48 ( $n = 4$ ) and 72 ( $n = 3$ ) h postpartum with systemic ceftiofur ( $n = 9$ ), penicillin ( $n = 1$ ) or ampicillin ( $n = 3$ ), or intrauterine urea or essential oils ( $n = 2$ ). Cows with foul-smelling vaginal discharge were treated for metritis with NSAIDs ( $n = 3$ ) and systemic antibiotics [ceftiofur ( $n = 12$ ), penicillin ( $n = 2$ ) or ampicillin ( $n = 1$ )], as well as with antiseptic ( $n = 1$ ) or antibiotic ( $n = 2$ ) uterine flushing. Two dairies used antibiotic uterine flushings if systemic antibiotics were ineffective. Non-foul-smelling abnormal vaginal discharge was treated with uterine antiseptic flushings ( $n = 4$ ). On 2 dairies, antibiotics were the treatment of choice for sick cows with an unknown disease. In this study we have observed that some dairies used antibiotics either as prophylactic therapy or to treat sick cows with unspecific diagnose. Sick cows identification, disease classification, and treatments were not consistent across dairies.

**Key Words:** dairy cattle, fresh cow, metritis

**T48 Association among gestation length with health, reproduction, and production in Holstein cows.** Achilles Vieira-Neto\*, Klibis N. Galvao, and Jose E. P. Santos, *University of Florida, Gainesville, FL.*

Objectives were to evaluate the association between gestation length (GL) and incidence of diseases, reproduction, and milk production. The data were screened to eliminate cows with GL longer or shorter than 3 SD from the population mean, resulting in 104 cows excluded from the analyses. Holstein cows ( $n = 6,254$ ) on a farm using only artificial insemination (AI) were evaluated. Responses measured included the incidences of stillbirth, retained placenta (RP), metritis, mastitis, and other diseases within 90 d in milk (DIM). Pregnancy at first AI and interval to pregnancy were evaluated. Milk yield and removal from the herd by death or culling were recorded for the first 300 DIM. Gestation length was categorized as short (S; at least 1 SD below the population mean; group mean = 266, range 256 to 269), normal (N; population mean  $\pm$  1 SD; group mean = 276, range 270 to 282 d), and long (L; > 1 SD above the population mean; group mean = 285, range 283 to 296 d). Data were analyzed by ANOVA, logistic regression, and the Cox's proportional hazard model using the GLIMMIX and PHREG procedures of SAS. Models included the fixed effects of GL category (S, N, L), gender of calf (female, male, twin), parity (1 or >1), season of calving (cool or hot), and all 2-way interactions. Gestation length affected ( $P < 0.01$ ) the incidences of stillbirth (S = 11.4,  $n = 7.3$ , L = 6.7%), RP (S = 32.7,  $n = 10.3$ , L = 9.4%), metritis (S = 51.1,  $n = 36.5$ , L = 33.9%), but not ( $P = 0.85$ ) that of mastitis (S = 4.7,  $n = 4.0$ , L = 5.2%). The rate of removal from the herd by culling or death was faster ( $P < 0.01$ ) for S than N (adjusted hazard ratio [HR] = 1.32; 95% CI = 1.10–1.58) and tended ( $P = 0.10$ ) to be faster for L than N (adjusted HR = 1.12; 95% CI = 0.98–1.29). Pregnancy at first AI did not differ ( $P = 0.94$ ) among groups (S = 33.2,  $n = 34.3$ , L = 33.5%). The rate of pregnancy was greater ( $P < 0.05$ ) for N than L (adjusted HR = 1.11; 95% CI = 1.02–1.21), but it did not differ between N and S or L and S. Daily milk yield was greater ( $P < 0.01$ ) for N than S or L (S = 35.2,  $n = 38.1$ , L =  $35.8 \pm 0.4$  kg/d). Cows with GL within 1 SD of the population mean (range 270 to 282 d) had improved health, reproduction, and production.

**Key Words:** dairy cow, gestation length, health

**T49 Hepatic mRNA expression of genes related to inflammatory and immune responses of dairy cows treated with recombinant bovine somatotropin during the periparturient period.**

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Objectives were to determine the effects recombinant bovine somatotropin (rbST) treatment during the peripartum period on hepatic mRNA expression of genes related to inflammation and immune response. Holstein cows were assigned randomly to receive no treatment (control;  $n = 10$ ), 87.5 mg (rbST87.5;  $n = 12$ ), or 125 mg (rbST125;  $n = 10$ ) of rbST every 7 d from -21 to 21 d relative to calving. Liver biopsies were collected -21, -7, and 7 d relative to calving. Twenty-four genes were assessed by direct molecular counts using NanoString technology. Continuous data were analyzed by ANOVA. Gene expression on d -21 was used as a covariate for analyses of mRNA expression on d -7 and 7. No differences in mRNA expression were observed among treatments on d -21 for any of the genes except SOCS3, which had lower ( $P \leq 0.05$ ) mRNA expression in control cows compared with rbST87.5 cows. On d -7, expression of mRNA for ANGPTL4 and SCARB1 was higher ( $P \leq 0.05$ ) in rbST87.5 and rbST125 than control cows. The rbST87.5

cows had higher ( $P \leq 0.05$ ) mRNA expression for HP, ICAM1, SOCS2 and XBP1 on d -7 than control and rbST125 cows. Control cows had higher ( $P \leq 0.05$ ) mRNA expression for HIF1A than rbST125 cows on d -7. On d 7, control cows had higher ( $P \leq 0.05$ ) mRNA expression for CXCL1, IL1RN, MYD88, NFKB1A, and SOCS3 compared with rbST87.5 and rbST125 cows. Control cows had higher ( $P \leq 0.05$ ) mRNA expression for ICAM1 and XBP1 than rbST125 cows and had higher mRNA expression for HIF1A than rbST87.5 cows. Expression of mRNA for NR3C1 and SOCS2 was lower ( $P \leq 0.05$ ) in control cows than rbST125 and rbST87.5 cows, respectively. Treatment did not affect hepatic expression of CEBPD, JUN, M-CSF1, NFKB1, PPARGC1A, STAT5B, TLR2, TNF, TNFRSF1 and TNFRSF5. Weekly treatment of periparturient cows with rbST regulates liver mRNA expression of genes related to inflammation and immune response during the prepartum and postpartum periods. These rbST-induced changes in gene expression have resulted in improved neutrophil function, antibody production, and reduced incidence of retained placenta and metritis.

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

**T50 Changes in serum triacylglycerols may indicate disease risk for retained placenta and mastitis in multiparous dairy cows.**

Fereshteh Zandkarimi, Massimo Bionaz, Jan S. Stevens, Claudia S. Maier, and Gerd Bobe\*, *Oregon State University, Corvallis, OR.*

Compromised liver function has been proposed to be involved in the etiology of many diseases in early lactation dairy cows. Examining changes in individual serum triacylglycerols (TAG) may identify previously unknown metabolic pathways involved in the etiology of disease in early lactation. We used ultraperformance liquid chromatography (UPLC) in conjunction with high-resolution accurate mass spectrometry to comprehensively and quantitatively profile individual TAG in serum. The objective of this study was to examine whether individual TAG in serum may serve as disease risk indicators of mastitis, retained placenta, or both. Serum samples were collected from 161 multiparous cows 3, 2, and 1 week before calving and at calving. For this nested case-control study, serum samples of 3 groups of cows that either developed after calving retained placenta (RP;  $n = 8$ ), mastitis (MA;  $n = 8$ ), or remained healthy (Healthy;  $n = 9$ ) were selected and serum levels of individual TAG were measured. Using PROC MIXED, overall and individual TAG levels in serum were compared between groups. The mass spectrometry-driven strategy resulted in the identification and comparative quantification of 24 individual saturated and unsaturated TAG between C48:1 and C54:4. Total TAG levels decreased until calving ( $P < 0.0001$ ). Cows that subsequently developed RP had and tended to have lower TAG levels than cows that subsequently developed mastitis ( $P = 0.03$ ) or remained healthy ( $P = 0.12$ ). Similarly, RP cows had lower TAG with 4 double-bonds compared with Healthy cows ( $P = 0.04$ ) and MA cows ( $P = 0.0006$ ), which could serve as potential RP indicator. In contrast, both diseased groups had lower saturated TAG levels compared with Healthy cows (MA:  $P = 0.03$ ; RP:  $P = 0.05$ ). Consequently, the ratio between TAG with 4 double bonds to saturated TAG was higher in MA cows compared with Healthy ( $P = 0.007$ ) and RP cows ( $P = 0.01$ ), which, thus, could be a potential MA indicator. These findings suggest changes in serum TAG levels are an early indicator for the development of retained placenta and mastitis in multiparous dairy cows.

**Key Words:** dairy cow, early disease indicator, triacylglycerols



**T51 Changes in serum nonesterified fatty acids precede retained placenta and mastitis in multiparous dairy cows.**  
Fereshteh Zadnkari, Massimo Bionaz, Jan S. Stevens, Claudia S. Maier, and Gerd Bobe\*, Oregon State University, Corvallis, OR.

We previously documented that elevated serum nonesterified fatty acids (NEFA) before calving are a general disease risk indicator in dairy cows. Examining changes in individual or subclasses of NEFA may improve disease risk detection. Untargeted lipid profiling using ultra performance liquid chromatography-high resolution mass spectrometry (UPLC-MS) is a comprehensive technique that allowed simultaneous analysis of individual NEFA in complex biological samples. The objective of this study was to examine the association between serum levels of individual or subclasses of NEFA and subsequent development of diseases. Serum samples were collected from 161 multiparous cows 3, 2, and 1 week before calving and at calving. For this nested case-control study, serum samples of 3 groups of cows that either developed after calving retained placenta (RP; n = 8), mastitis (MA; n = 8), or remained healthy (Healthy; n = 9) were selected and serum levels of individual NEFA were measured. Using PROC MIXED, serum NEFA levels were overall and individually compared between groups. Levels of 22 individual NEFA between C14:0 and C26:0 were above the limit of detection. Overall levels of NEFA identified by UPLC-MS analysis were in good agreement with chemical analysis of total NEFA concentrations. Cows that developed RP and MA in early lactation had 1 week before calving ( $P = 0.05$ ) and at parturition ( $P = 0.005$ ) elevated overall NEFA levels compared with Healthy cows. Similar directional changes were observed for C14:0, C16:0, C16:1, C18:1, as well as the C18:1 to C18:0 ratio, which could serve as general disease risk indicators. In contrast, cows that subsequently developed diseases had lower elongation and/or very long chain fatty acid ( $\geq C20$ ) desaturation indices compared with Healthy cows. MA cows had greater  $\geq C20$  n6 to  $\geq C20$  n3 and C20:1 to C20:0 ratios and lower  $\geq C20:0$  levels compared with Healthy and RP cows, which could be used as potential MA indicators. These findings suggest changes in very long chain fatty acids levels and their ratios are an early indicator for the development of retained placenta and mastitis in multiparous cows.

**Key Words:** dairy cow, early disease indicator, nonesterified fatty acids

**T52 Distribution of most common coagulase-negative species over parity and lactation in Canadian dairy herds.** Larissa A. Z. Condas<sup>\*1</sup>, Diego B. Nobrega<sup>2,1</sup>, Domonique Carson<sup>1</sup>, Jeroen De Buck<sup>1</sup>, and Herman W. Barkema<sup>1</sup>, <sup>1</sup>University of Calgary, Calgary, AB, Canada, <sup>2</sup>Universidade Estadual de Campinas, Campinas, SP, Brazil.

Coagulase-negative staphylococci (CNS) are the most frequently isolated group of microorganisms from the bovine udder. Research has shown that certain CNS species can be protective, while other species are prone to decrease milk quality and production. Our objective was to describe the distribution of CNS species for the lactation period and parities in Canadian herds, as an initial effort to understand the disease. Cows were sampled randomly across Canada in 2007 and 2008. In total, 1965 cows had udder infection with CNS, being compared with a total of 6067 cows sampled in the study. Chi-squared was used to test different prevalences ( $P < 0.05$ ). The 5 most common CNS species identified in were *S. chromogenes* (49%), *S. simulans* (17%), *S. xyloso* (11%), *S. haemolyticus* (7%) and *S. epidermidis* (4%). Parity and lactation prevalences are presented in Table 1. *S. chromogenes* was predominant in heifers, and its prevalence increased during the lactation. *S. epidermidis* was mostly isolated from older cows and the prevalence did not increase

as lactation progressed. *S. haemolyticus* isolated equally often in all parities; its prevalence increased during lactation. *S. simulans* seems to be more isolated in heifers, and its prevalence did not increase over the lactation. *S. xyloso* fluctuates over parities and its prevalence increased during the lactation. The distribution of these CNS species differed over parities and lactation period. The prevalence of species such as *S. chromogenes*, which maybe less pathogenic, was very high in heifers compared with older cows. Throughout lactation, it seems that most species can persist or reinfect udder. Understanding the distribution of CNS species will possibly lead to selective management practices and may lead to identification of species or genotypes that act as protective udder pathogens.

**Table 1 (Abstr. T52).** Relative frequency (%) of CNS species distribution over parity and over lactation thirds

CNS species	Parity				Lactation thirds			
	1	2	3	$\geq 4$	1	2	3	$>305$ d
<i>S. chromogenes</i>	60	23	24	26	8	8	11	11
<i>S. epidermidis</i>	1	3	3	5	1	1	0.5	1
<i>S. haemolyticus</i>	5	5	6	5	1	1.2	2	2
<i>S. simulans</i>	15	9	10	13	4	2	2	3
<i>S. xyloso</i>	6	9	7	10	1	1	2	3

**Key Words:** coagulase-negative staphylococci, intramammary infection

**T53 Phospholipids are potential early risk indicator for retained placenta and mastitis in multiparous dairy cows.**  
Fereshteh Zandnkari, Massimo Bionaz, Jan S. Stevens, Claudia S. Maier, and Gerd Bobe\*, Oregon State University, Corvallis, OR.

Phospholipids such as phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylinositols (PI), and phosphatidylserines (PS), have been proposed to be involved with compromised liver function in dairy cows. Examining changes in individual phospholipids may identify potential therapeutic targets of diseases in early lactation. We employed an untargeted mass spectrometry-based lipid profiling strategy to analyze simultaneously individual phospholipids in serum. The objective of this study was to examine whether individual phospholipids in serum may serve as disease risk indicators of mastitis, retained placenta, or both. Serum samples were collected from 161 multiparous cows 3, 2, and 1 week before calving and at calving. For this nested case-control study, serum samples of 3 groups of cows that either developed after calving retained placenta (RP; n = 8), mastitis (MA; n = 8), or remained healthy (Healthy; n = 9) were selected and serum levels of individual phospholipids were measured. Using PROC MIXED, serum levels of phospholipid classes and subclasses were compared between groups. Total PC, lysoPC, and lysoPE levels decreased until calving (all  $P < 0.0001$ ). At calving, cows that subsequently developed mastitis had lower lysoPC, lysoPE, and inositol phosphate levels than cows that subsequently developed RP or remained healthy (lyso PC: both  $P = 0.03$ ; lyso PE: both  $P = 0.02$ ; inositol phosphate:  $P = 0.04$  and  $P = 0.03$ ) and thus, could be used as MA indicators. In contrast, RP cows had lower levels of highly unsaturated PS (PS with 4 double bonds) than MA or Healthy cows (both  $P = 0.02$ ), which could serve as RP indicator. These findings suggest changes in phospholipids are an early indicator for the development of retained placenta and mastitis in multiparous cows and may be potential therapeutic targets for diseases in early lactation.

**Key Words:** dairy cow, early disease indicator, phospholipids

**T54 Hoof measurements before and after hoof trimmer intervention on dairy lame cows on California dairies.** Marc Pineda<sup>\*1</sup>, Ibrahim Akin<sup>2</sup>, and Noelia Silva-del-Rio<sup>1</sup>, <sup>1</sup>*Veterinary Medicine Teaching and Research Center, UC Davis, Tulare, CA*, <sup>2</sup>*Adnan Menderes University Veterinary Faculty Department of Surgery, Aydin, Turkey*.

The objective of this study was to describe lame cow hoof measurements before and after hoof trimmer intervention on California dairies. A total of 17 dairies ranging in size from 1,000 to 10,000 were enrolled in the study. Hoof trimmers were dairy workers (n = 11) or outside service providers (n = 6). Researchers collected information from rear hooves from 10 (n = 15) or 9 (n = 2) lame cows per dairy (dorsal wall length, hoof angle, heel height, and abaxial groove length) before and after the hoof trimmer intervention. Descriptive statistics were analyzed with PROC MEANS, PROC UNIVARIATE and PROC CORR of SAS 9.4. Before the hoof trimmer intervention 10.1% of the hooves had a desirable dorsal wall length (>7 to <8.5 cm). After the hoof trimmer intervention, at least 70% (n = 7) or less than 30% (n = 4) of the hooves were within the desirable range. Dorsal wall length of hooves of the same claw differed by >0.5 cm at least 5% of the time (n = 8). Dorsal wall angle was within a desirable range (<sup>3</sup>45° to <50°) on 52.2% and 50.9% of the hooves before and after hoof trimmer intervention respectively. After the hoof trimmer intervention, at least 60% (n = 4) or less than 30% (n = 3) of the hooves were within the desirable range. Dorsal wall angle of hooves of the same claw differed by 3° at least 25% of the time (n = 11). Heel height was within > 3.4 to < 4.4 cm on 20.0 and 36.8% of the hooves before and after hoof trimmer intervention respectively. After the hoof trimmer intervention, 5 herds had at least 50% (up to 65%) of hooves with heel height within > 3.4 to < 4.4 cm. Heel height of hooves of the same claw differed by >0.5 cm at least 25% of the time (n = 9). The abaxial groove length was >4.5 cm on 93.5 and 90.8% of the hooves before and after hoof trimmer intervention respectively. The correlation between heel height and abaxial groove length was significant ( $P < 0.01$ ) but with a low correlation coefficient before ( $r = 0.47$ ) and after ( $r = 0.20$ ). Our data indicates that there is an opportunity to improve hoof angle of lame cows after hoof trimmer intervention on California dairies.

**Key Words:** lameness, hoof trimmer, dairy cow

**T55 Correlation of ACTH test results with hormonal, metabolic and cardiac stress responses during stress challenge in dairy cows.** Lea Fieguth<sup>1</sup>, Lena Locher<sup>1</sup>, Anja Schacht<sup>1</sup>, Akos Kenez<sup>2</sup>, Asako Kinoshita<sup>1</sup>, Ulrich Meyer<sup>3</sup>, Sven Dänicke<sup>3</sup>, and Juergen Rehage<sup>\*1</sup>, <sup>1</sup>*Clinic for Cattle, University of Veterinary Medicine Hannover, Hannover, Germany*, <sup>2</sup>*Department of Physiology, University of Veterinary Medicine Hannover, Hannover, Germany*, <sup>3</sup>*Department of Animal Nutrition, Friedrich-Loeffler-Institute, Braunschweig, Hannover*.

Cows react to stressors with release of cortisol and catecholamines after activation of the hypothalamic-pituitary-adrenal axis (HPA) and

of the hypothalamic-adrenal medullary axis, respectively. Increases in heart rate, hyperglycemia, and hyperlipidemia are typical metabolic responses. The extent of the individual stress response depends on individual experience and stress responsiveness. ACTH-test results are assumed to reflect the individual HPA reactivity. The aim of the study was to compare results of the ACTH test with hormonal, metabolic and cardiac stress responses of cows on claw trimming as a typical acute non-painful stressor. In 18 pluriparous German HF cows claw trimming (CT) was performed in lateral recumbency 40 d postpartum (pp). Blood level of cortisol, glucose, and nonesterified fatty acids (NEFA) and heart rate (HR) and heart rate variability (HRV; standard deviation of R-R intervals: SDNN, root mean square of successive differences: RMSSD) were measured at T1: 30 min before CT in the herd, T2: standing at the surgical table, T3: end of claw trimming, T4: 30 min after CT in the herd. ACTH tests were performed on d 110 pp (80 µg/cow ACTH; repeated blood samples for cortisol measurement over 120 min) from which the area under the cortisol time curve (AUC) were assessed. AUC was correlated (SPEARMAN, SAS package) with cortisol, glucose and NEFA blood concentrations as well as HR and HRV at T1-T4 and with differences between time points during the CT procedure. AUC correlated positively with T3 cortisol ( $r: 0.76, P = 0.002$ ) and  $\text{Diff}_{T4-T1}$  cortisol ( $r: 0.75, P = 0.002$ ), and negatively with T4 NEFA ( $r: -0.64, P = 0.014$ ), T4 HR ( $r: -0.64, P = 0.014$ ), T4 SDNN ( $r: -0.59, P = 0.027$ ),  $\text{Diff}_{T4-T3}$  glucose ( $r: -0.56, P = 0.037$ ),  $\text{Diff}_{T4-T1}$  NEFA ( $r: -.73, P = 0.003$ ),  $\text{Diff}_{T4-T1}$  HR ( $r: -.71, P = 0.005$ ),  $\text{Diff}_{T4-T1}$  SDNN ( $r: -0.65, P = 0.012$ ). High ACTH test reactivity was associated with high cortisol level during but a quick decrease to baseline values in blood NEFA and glucose concentrations and in HR, and HRV after CT, which may indicate a close association between individual stress responsiveness and metabolic and cardiac adaptability to stress challenges in cows.

**Key Words:** ACTH test, stress reactivity, dairy cow