Graduate Student Competition: ADSA Graduate Paper Competition - Production Division - MS Students

226 Toll-like receptors expression in the gastro-intestinal tract of dairy calves. N. Malmuthuge*[1], M. Li[1], P. Fries[2], P. Griebel[2], and L. L. Guan[1], [1]*University of Alberta, Edmonton, Alberta, Canada,* [2]*Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatchewan, Saskatoon, Canada.*

Toll-like receptors (TLRs) are a family of pattern recognition receptors which sense pathogen associated molecular patterns to initiate innate immune responses and maintain intestinal homeostasis. However, the understanding of baseline TLRs expression within specific regions of the gastro-intestinal tract (GIT) of dairy calves and possible age-related expression changes are not well understood. In this study, TLRs expression patterns were investigated throughout the GIT of newborn and weaned dairy calves. Total RNA was extracted from rumen, jejunum, ileum, cecum and colon tissues of 3 weeks old (n = 8) and 6 mo old Holstein male calves (n = 8). Expression of 10 TLRs (TLR1-TLR10) was evaluated relative to β-actin expression in each region using quantitative real time PCR. Gene expression (ΔC_T) data were analyzed using MIXED procedure of SAS and statistical model include gut location and age as fixed effects. Analysis of TLRs data revealed TLR10 expression was significantly ($P < 0.01$) higher in ileum than other GIT regions of 3 week-old calves. TLRs 2, 4 and 10 ($P < 0.01$) were differentially expressed among GIT regions of 6 mo old calves and TLR10 expression was again highest in the ileum. Future studies are required to understand the biological significance of TLR10 in the bovine ileum, which is an important site for B cell development. Expression of most TLRs was lower in rumen than other GIT regions, irrespective of age, supporting the importance of intestinal epithelium in enteric immune responses. Moreover, expression of many TLRs, except TLRs 3, 5 and 6, was significantly downregulated after weaning. The observed downregulation in TLRs expression in weaned calves may reflect host mechanisms to control inflammatory responses to commensal microflora and pathogens as well as development of other immune mechanisms to regulate TLRs signaling. In conclusion, our study indicates that bovine TLRs expression varies throughout the GIT and is age-dependent. These findings are critical for understanding innate immune responses to both commensal microflora and enteric pathogens in dairy calves.

227 Soybean meal substitution by a microbial protein source in dairy cattle diets. J. A. Sabbia*[1], K. F. Kalscheur[1], A. Garcia[1], A. Gehman[2], and J. M. Tricarico[2], [1]*South Dakota State University, Brookings,* [2]*Alltech Inc., Brookings, SD.*

The objective of this study was to examine the effect of a source of microbial protein (DEMP, Alltech, Nicholasville, KY) on milk production, DMI, rumen and blood parameters on high-producing dairy cows as replacement for soybean meal (44% CP). Sixteen Holstein cows with 93 + 37 DIM were used in a 4 × 4 Latin square design. Diets contained 40% corn silage, 20% alfalfa hay, and 40% concentrate mix. Cows were fed one of 4 treatments: 0, 1.14, 2.28, or 3.41% of the diet DM with DEMP. DMI showed a cubic effect (25.9, 27.1, 25.9, 26.6 kg/day; $P = 0.04$), as DEMP in the diets increased. Milk production (41.1 kg/d) was not affected by treatment, whereas energy-corrected milk tended to respond quadratically (39.5, 41.5, 41.8, 41.0 kg/d; $P = 0.09$). Milk fat percentage (3.53, 3.66, 3.62, 3.53%; $P = 0.06$) and yield (1.35, 1.45, 1.49, 1.42 kg/d; $P = 0.07$) also tended to show a quadratic response. A similar quadratic response was shown for total solids percentage (12.3, 12.5, 12.5, 12.3%; $P = 0.02$) and yield (4.87, 5.11, 5.12, 5.03 kg/d; $P = 0.08$). Milk urea nitrogen also responded in a quadratic manner (13.4, 13.7, 13.5, 12.9 mg/dl; $P = 0.05$). There was no effect on milk protein and lactose percentage or yield, nor was there an effect on feed efficiency. NEFA concentration in plasma was similar between treatments, whereas plasma glucose (63.7 to 68.5 mg/dl; $P = 0.06$) and BHBA (11.5 to 13.1 mg/dl; $P = 0.04$) increased linearly. Inclusion of DEMP had no effect on most ruminal VFA concentration, except isovalerate which decreased linearly ($P < 0.001$). Ruminal pH was unaffected by DEMP inclusion, but ammonia concentration tended to decrease linearly from 14.1 to 11.7 mg/dl ($P = 0.09$). Ruminal N fractionation showed a cubic response in total free amino acids (12.1, 10.4, 14.1, 11.2 mg/L; $P = 0.02$). There was a quadratic response for peptides that weighed between 3 and 10 kDa (9.1, 14.9, 12.3, 12.0%; $P = 0.05$) and for those less than 3 kDa (30.8, 28.3, 27.7, 29.4%; $P = 0.03$) when expressed as percentage of total nitrogen. In conclusion, soybean meal substitution with DEMP can improve milk and total solids production in high-producing dairy cows.

Key words: microbial protein, nitrogen fractionation, milk production

228 Effect of timing of initiation of Resynch and presynchronization with GnRH on fertility of resynchronized inseminations in lactating dairy cows. G. Lopes Jr.*, J. O. Giordano, A. Valenza, M. M. Herlihy, J. N. Guenther, M. C. Wiltbank, and P. M. Fricke, *Department of Dairy Science, University of Wisconsin-Madison, Madison.*

Lactating Holstein cows (n = 1,512) were randomized to a 2x2 factorial design resulting in 4 Resynch treatments: 1) Ovsynch (GnRH–7 d–PGF–56 h–GnRH–16 h–TAI) initiated d 32 ± 3 d after AI (GPG32); 2) presynchronization with 100 μg GnRH 25 ± 3 d after AI and Ovsynch initiated 32 ± 3 d after AI at nonpregnancy diagnosis (GGPG32); 3) Ovsynch initiated 39 ± 3 d after AI (GPG39); 4) presynchronization with 100 μg GnRH 32 ± 3 d after AI at nonpregnancy diagnosis and Ovsynch initiated 39 ± 3 d after AI (GGPG39). Overall, 296 cows were inseminated to estrus between enrollment (25 ± 3 d after AI) and initiation of Resynch treatments, and 1,216 cows (GPG32 = 315, GGPG32 = 360, GPG39 = 248 and GGPG39 = 293) received TAI. Blood samples were collected from all cows at the first GnRH injection of Resynch (G1), and ovarian structures were evaluated and blood samples were collected at G1, PGF, and TAI of the Resynch protocols in a subgroup of cows (GPG32 = 105, GGPG32 = 119, GPG39 = 93 and GGPG39 = 113). Based on logistical regression analysis, pregnancies per AI (P/AI) 32 d after AI was not affected by parity and did not differ among treatments [GPG32, 33.3% (105/315); GGPG32, 36.1% (130/360); GPG39, 32.7% (81/248); GGPG39, 39.3% (115/293)]. When analyzed as main effects, presynchronization with GnRH increased ($P = 0.05$) P/AI [33.0% (186/563) vs. 37.7% (245/653) for GPG vs. GGPG], whereas timing of initiation of Resynch did not [34.7% (235/675) vs. 35.9% (196/541) for Day 32 vs. Day 39]. Presynchronization with GnRH increased ($P = 0.007$) the proportion of cows with high P4 (>0.5 ng/mL) at G1 [72.6% (403/556) vs. 79.3% (519/653) for GPG vs. GGPG], and cows with high P4 at G1 had greater ($P = 0.006$) P/AI than cows with low P4 [37.5% (346/922) vs. 28.6% (82/287)]. Ovulation to G1 decreased ($P = 0.039$) luteal regression after PGF [87.5% (121/139) vs. 78.5% (143/183)], and high P4 at G1 decreased ($P < 0.001$) ovulation to G1 [68.4% (67/98) vs. 40.9% (136/332)]. We conclude that presynchronization with GnRH

7 d before initiation of Resynch increased fertility of resynchronized dairy cows whereas timing of initiation of Resynch did not.

Key words: resynchronization, GnRH, progesterone

229 Somatic cell count and management benchmarks in Minnesota dairy herds. R. F. Leuer* and J. K. Reneau, *University of Minnesota, St. Paul.*

Dairy Herd Improvement Association (DHIA) tests provide a large amount of information about herd milk production and milk quality. Many guidelines have been given about farm SCC performance and its relationship to mastitis and milk quality, however associations are lacking. The objective of this study was to investigate the relationship between herd SCC level and performance rank for mastitis and milk quality benchmarking. Minnesota DHIA monthly average herd records were collected from January 2007 to November 2010. Herd tests without SCC information were removed and only herds with an average of 10 tests per year were included. Herds were divided into 4 categories based on average herd SCC over the collection period. Low herds (L) with less than 200,000 SCC (n = 325), medium low (ML) herds between 200,000 and 300,000 SCC (n = 547), medium high herds (MH) herds between 300,000 and 400,000 SCC (n = 470), and high herds (H) above 400,000 SCC (n = 438). Cows > 200,000 SCC were considered infected. Monthly records (n = 66,296) were analyzed using PROC GLM with significant differences determined at $P < 0.05$ using Tukey's multiple comparisons test. The 4 categories were all significantly different in average SCC (L = 157,000, ML = 251,000, MH = 350,000, H = 513,000), average of total cows on test day (L = 116, ML = 141, MH = 110, H = 86), percent infected (L = 16.4, ML = 24.7, MH = 32.9, H = 43.4), percent of current cows with new infections (L = 8.1, ML = 10.5, MH = 12.5, H = 14.2), percent of fresh cows with chronic infections (L = 6, ML = 11.2, MH = 17.6, H = 27.4), percent of current cows with chronic infections (L = 8.3, ML = 14.2, MH = 20.4, H = 29.2), percent infected <30 d in milk (L = 1.7, ML = 2.3, MH = 2.8, H = 3.3), percent infected between 30 and 220 d in milk (L = 7.7, ML = 11.7, MH = 15.4, H = 20), percent infected >220 d in milk (L = 7, ML = 10.7, MH = 14.7, H = 20.1), percent of herd >220 d in milk (L = 35.4, ML = 36.7, MH = 38.5, H = 40.5), rolling herd average (RHA) milk production (L = 10,351, ML = 9,944, MH = 9,201, H = 8,440 kg), RHA protein production (L = 315, ML = 304, MH = 284, H = 263 kg), and RHA fat production (L = 386, ML = 371, MH = 348, H = 325 kg). The 4 categories demonstrated differences that contribute to herd SCC.

Key words: benchmarking, DHIA, SCC

230 Effect of dietary trans fatty acids on selected inflammatory mediators in early lactating dairy cows. J. S. Watts*, D. L. Sevier, J. K. Kinch, S. M. Clark, M. A. McGuire, and P. Rezamand, *Department of Animal and Veterinary Science, University of Idaho, Moscow.*

Trans fatty acids (tFA) contribute to inflammation. The mechanism responsible is not well understood; however, it is thought to involve integration of tFA into cell membranes of immune cells, affecting membrane fluidity and cell signaling. The objective of this study was to investigate the effects of a ration supplemented with tFA on the fatty acid (FA) profile of peripheral blood mononuclear cells (PBMC) and the gene expression of inflammatory markers in early lactating dairy cows. tFA (Virtus Nutrition; Corcoran, Ca) was fed at 0, 1.5, and 3% of dry matter replacing (1:1 wt:wt) saturated fatty acids (sFA). Multiparous lactating Holstein cows 7 d in milk (n = 12) were randomly assigned to a treatment sequence in a 3x3 Latin square design. Each period lasted 14 d. Heparinized blood was collected on d0 (pretreatment) and on d 10 and 14 of each period. Plasma was collected and solid phase extraction was used to isolate plasma phospholipids. Additionally, PBMCs were isolated for FA analysis by gas chromatography and gene expression analysis by RT-PCR using bovine RPS9 as the endogenous control. Data were analyzed using the MIXED procedure of SAS with significance at $P < 0.05$. As dietary tFA increased, the percentage of 18:1 trans isomers increased with linear increases in the t9 and t12 isomers in plasma phospholipids ($P < 0.002$). In PBMCs, both t10 and t12 isomers of 18:1 increased linearly ($P < 0.02$). Dietary tFA had no detectable effect on mRNA expression of pro-inflammatory TNF-α or IL-6, although pretreatment expression of IL-6 and ICAM-1 differed among groups. Expression of IL-1β and ICAM-1 decreased linearly with increasing tFA and a treatment x day interaction in expression of ICAM-1 was detected. Overall, dietary tFA linearly increased the tFA present in plasma phospholipids and PBMCs; however, dietary tFA decreased PBMC expression of some of the pro-inflammatory markers in early lactating dairy cows.

Key words: trans fatty acids, inflammation, transition cow

231 Effects of physical preparation of diets and level of modified wet distillers grains with solubles on production and rumen measurements of lactating dairy cows. J. C. Ploetz*[1], W. C. Hornback[1], D. E. Beever[2], P. H. Doane[3], M. J. Cecava[3], M. R. Murphy[1], and J. K. Drackley[1], *[1]University of Illinois, Urbana, [2]Keenan Systems, Borris, Ireland, [3]Archer Daniels Midland Company, Decatur, IL.*

Our objective was to determine if methods for preparing TMR (Keenan MechFiber [KMF] technology vs. vertical auger [VA] mixer) would alter physical form of the TMR and enable increased utilization of modified wet distillers grains with solubles (MWDGS). Holstein cows (n = 24 with 12 ruminally cannulated; 144 DIM ± 31 d at start) were used in a split-plot design with mixer type as the whole plot and MWDGS levels as subplots in a 3 × 3 Latin square arrangement with 35-d periods. Inclusion rates of MWDGS were 10, 20, and 30% of diet DM, primarily replacing corn, SBM and whole cottonseed. Feed DMI tended to be less for KMF ($P = 0.06$) but was unaffected by MWDGS level ($P = 0.39$). However, a mixer x MWDGS quadratic interaction ($P = 0.14$) was observed; DMI increased as MWDGS increased when mixed using VA but not when using KMF. Milk production did not differ ($P = 0.75$) by level of MWDGS or interaction of MWDGS x mixer ($P = 0.18$). Milk protein content tended ($P = 0.09$) to decrease linearly with increasing MWDGS. Milk protein yield at 30% MWDGS inclusion decreased for KMF but increased for VA (interaction of mixer x linear effect of MWDGS, $P = 0.05$). Milk fat percentage declined with increasing MWDGS ($P = 0.003$) but the interaction between mixer wagon and MWDGS ($P = 0.006$) showed that decreases were larger with VA mixing. Cows fed the diet containing 30% MWDGS mixed with KMF averaged 3.45% (1.24 kg/d) milk fat whereas cows fed the same diet mixed with VA averaged 2.81% (1.10 kg/d) fat. Particle size of the TMR did not explain differences in milk fat. Feed conversion efficiency (FCE; energy-corrected milk/DMI) decreased linearly ($P = 0.007$) with MWDGS, but FCE tended to be maintained when higher MWDGS diets were mixed using KMF vs. VA (mixer, $P = 0.12$; mixer x MWDGS quadratic, $P = 0.13$). Ruminal pH tended ($P = 0.13$) to be greater for KMF than for VA; ruminal pH ($P = 0.05$) and ammonia concentration ($P < 0.001$) decreased linearly as MWDGS increased. Using the KMF mixer wagon resulted in better FCE with higher amounts of MWDGS primarily because milk fat content and yield were not depressed.

Key words: TMR mixer, distillers grains, milk fat

232 Modifying the double-Ovsynch protocol to include human chorionic gonadotropin to synchronize estrus in lactating dairy cows. J. A. Binversie*, K. E. Pfeiffer, and J. E. Larson, *Mississippi State University, Mississippi State.*

The objectives of this study were to determine whether conception, ovulation or presynchronization rates were altered, or follicle and CL characteristics were altered after modifying the double-Ovsynch (DO) protocol to include human chorionic gonadotropin (hCG) compared with the DO protocol. Holstein (n = 146) and Jersey (n = 37) cows were blocked by parity and randomly assigned to 1 of 2 treatments. Cows received either an injection of 100 μg of GnRH (n = 91) or 2000 IU of hCG (n = 92) at the initiation of the Pre-Ovsynch (PO) portion of the DO protocol (PO: GnRH/hCG-7d-$PGF_{2\alpha}$-3d-GnRH). After 7 d, cows started the Breeding-Ovsynch (BO) portion of the DO protocol (BO: GnRH-7d-$PGF_{2\alpha}$-56h-GnRH-16h-TAI with sex-sorted semen). Transrectal ultrasonography and blood samples were used to assess ovarian structures, ovulation, pregnancy diagnosis (at d 32 post TAI), and circulating concentrations of progesterone (P4). Conception rates were similar for cows treated with GnRH or hCG (32.2 and 25.0%; $P > 0.1$). Ovulation rates at the onset of PO were increased in cows treated with hCG compared with GnRH (77.2 and 62.2%; $P < 0.05$). Concentrations of P4 7 d post hCG/GnRH treatment for cows that ovulated were greater in cows treated with hCG compared with those treated with GnRH (LSMeans ± SEM; 5.1 ± 0.3 and 3.8 ± 0.4 ng/ml; $P < 0.05$). The size of the largest follicle 7 d post hCG/GnRH treatment for cows that had ovulated was smaller in cows treated with hCG compared with cows treated with GnRH (12.4 ± 0.5 and 13.8 ± 0.6 mm; $P < 0.05$). Luteal regression (P4 < 1.0 ng/ml) from the injection of $PGF_{2\alpha}$ of PO did not differ between GnRH and hCG treated cows (67.0 and 60.9%; $P > 0.1$). Although more cows ovulated to hCG, a greater proportion of these cows tended to fail to have undergone luteolysis by 3 d post $PGF_{2\alpha}$ compared with cows that had ovulated to GnRH (29.6 and 16.1%; $P = 0.09$). Therefore, the overall percentage of cows which were synchronized to the PO did not differ between GnRH and hCG treated cows (61.5 and 52.2%; $P > 0.1$). In conclusion, no improvement was achieved by replacing the first injection of GnRH in the DO protocol with hCG.

Key words: double-Ovsynch, hCG, presynchronization

233 Fibroblast growth factor 9 influences steroidogenesis and gene expression in ovarian granulosa and theca cells of cattle. N. B. Schreiber* and L. J. Spicer, *Oklahoma State University, Stillwater.*

Ovarian cysts result in the loss of millions of dollars to the dairy industry annually because of increased number of days open, reduced milk production, and increased culling rate. Fibroblast growth factor 9 (FGF9) is downregulated in cystic follicles versus normal dominant follicles in cattle. Therefore, experiments were conducted to evaluate the role of FGF9 in hormone-stimulated steroidogenesis, proliferation and gene expression in bovine ovarian granulosa and theca cells from antral follicles of cattle. Quantitative PCR was used to measure gene expression of side-chain cleavage enzyme (CYP11A1), aromatase (CYP19A1), 17-hydroxylase (CYP17A1), LH receptor (LHCGR) and/or FSH receptor (FSHR). Small (1–5 mm) follicle granulosa cells (SMGC), large (8–22 mm) follicle theca cells (LGTC) and large follicle granulosa cells (LGGC) were grown in vitro and treated for 48 h with 0, 3, 10, or 30 ng/mL of recombinant human FGF9 to evaluate the effects on steroid production, cell proliferation, and gene expression. In SMGC, FGF9 (30 ng/mL) decreased ($P < 0.05$) estradiol and progesterone production by 80% and 56.4%, respectively, after cells were stimulated with 30 ng/mL of FSH and IGF1. In LGTC, FGF9 (30 ng/mL) decreased ($P < 0.05$) progesterone and androstenedione production by 71% and 79%, respectively, after cells were stimulated with 30 ng/mL of LH and IGF1. In contrast, IGF1-induced SMGC and LGTC proliferation was further stimulated ($P < 0.05$) 1.8- and 1.5-fold by FGF9, respectively. FGF9 decreased ($P < 0.05$) CYP11A1 and FSHR mRNA abundance and had no effect on CYP19A1 mRNA abundance in SMGC and LGGC treated concomitantly with FSH and IGF1. FGF9 decreased ($P < 0.05$) abundance of CYP11A1, CYP17A1, and LHCGR mRNA by 97%, 77%, and 97%, respectively, in LGTC treated concomitantly with LH and IGF1. In conclusion, FGF9 regulates ovarian function in cattle by stimulating cell proliferation and inhibiting steroidogenesis of both granulosa and theca cells. FGF9 inhibition of steroid production is likely via attenuation of both gonadotropin receptor and steroidogenic enzyme gene expression.

Key words: cystic follicles, granulosa cells, theca cells

234 Relationships among temperature, moisture, bacterial counts, and animal hygiene in compost bedded pack barns. R. A. Black*, J. L. Taraba, G. B. Day, F. A. Damasceno, M. C. Newman, K. A. Akers, and J. M. Bewley, *University of Kentucky, Lexington.*

The objective of this study was to assess the relationships among temperature, moisture, bacterial counts, and animal hygiene for composted material collected from compost bedded pack (CBP) barns. Compost samples were collected from 54 CBP barns in Kentucky from October 2010 to February 2011. A composite sample was collected from 9 evenly distributed sampling areas throughout each barn for analysis of nutrient composition and bacterial counts. Compost moisture was measured using an oven at 75°C. Compost temperatures (CT) were measured 10.2 cm below the pack surface. Subjective hygiene scores were collected by the same observer for 50.4 ± 16.1 cows per herd using a 4 point scoring system described by Cook (2007, 1-clean, 4-dirty). Producers reported their most recent SCC. The MEANS procedure of SAS® (Cary, NC) was used to calculate mean (±SD) SCC (238,162.2 ± 81,701.5 cells per ml, n = 37), hygiene score (2.22 ± 0.46, n = 43), moisture (54.9 ± 12.5%, n = 51), CT (30.5 ± 11.4°C, n = 52), coliform (6.09 ± 0.63 $\log_{10}$ cfu/g, n = 54), *Escherichia coli* (5.73 ± 0.68 $\log_{10}$ cfu/g), streptococcal species (7.00 ± 0.68 $\log_{10}$ cfu/g, n = 54), staphylococcal species (7.60 ± 0.49 $\log_{10}$ cfu/g, n = 53), and bacillus species (7.30 ± 0.56 $\log_{10}$ cfu/g, n = 54). Moisture was highly correlated with ambient temperature ($r = -0.73$, $P < 0.01$). Moisture was negatively correlated with CT ($r = -0.38$, $P < 0.01$) and positively correlated with hygiene score ($r = 0.68$, $P < 0.01$). Hygiene score and CT were also negatively correlated ($r = -0.42$, $P < 0.01$). *Escherichia coli* count was moderately correlated with CT, moisture, SCC, and hygiene score ($r = 0.62$, $P < 0.01$; $r = -0.41$, $P < 0.01$; $r = 0.42$, $P < 0.01$; and $r = -0.39$, $P < 0.02$). No significant correlations between coliform, staphylococcal species, streptococcal species, and bacillus species counts and CT ($P > 0.10$) were observed. These results suggest that high CT and low moisture are important for maintaining a dry resting surface for cows and may contribute to pack bacterial counts.

Key words: compost bedded pack barn, bacterial analysis, SCC

235 Objective assessment of pain in dairy cattle with clinical mastitis. C. E. Fitzpatrick*[1], N. Chapinal[1,2], C. S. Petersson-Wolfe[3], and K. E. Leslie[1], *[1]University of Guelph, Guelph, Ontario, Canada, [2]University of British Columbia, Vancouver, British Columbia, Canada, [3]Virginia Polytechnic Institute and State University, Blacksburg.*

Clinical mastitis has negative effects on profitability and cow welfare, with significant discomfort and pain. This study was conducted to objectively assess pain in cases of experimentally induced clinical mastitis, involving 24 (12 primiparous and 12 multiparous) lactating Holstein cows enrolled in an LPS endotoxin challenge study. Each animal was challenged in one rear mammary quarter by intramammary infusion with 25 µg of *E. coli* LPS. Subsequently, a subcutaneous injection of either a placebo (n = 12) or NSAID treatment (meloxicam) (n = 12) was randomly allocated and administered using, yet to be identified, double-blind methods (Treatments A and B). The animals were monitored for 2 d before, and 2 d following, the intramammary challenge. Several behavioral, physiological and performance parameters were monitored throughout the study period, including activity, rumination, body temperature, milk weights, DMI, SCC and clinical scores of milk, udder edema, pain sensitivity of the mammary glands, serum amyloid A and haptoglobin. During the first 6 h after inoculation and treatment, cows ruminated 14.6 ± 2.1 min/2 h interval ($P < 0.001$) less compared with the same baseline time period before challenge. Overall, multiparous cows were found to ruminate 6.1 ± 1.6 min/2h ($P = 0.001$) more than primiparous cows. There was no difference in rumination between treatment groups. Using a pain pressure algometer, the difference between the pressures applied to the control quarter was compared with the challenge quarter for each sampling time period before, and after inoculation. There was an effect at hour 6 after inoculation and treatment as compared with the baseline readings. For Treatment A animals, more pressure could be applied on their challenge quarter than their control quarter (1.9 ± 0.9 lbs; $P = 0.0445$). Treatment B animals registered more pressure applied to the control quarter than the challenge quarter (2.5 ± 0.9 lbs.; $P < 0.01$). These results indicate the potential for using continuous measurement of rumination and pain pressure sensitivity for objective assessment of pain due to illness in cases of clinical mastitis.

Key words: mastitis, pain management, behavior

236 Herd reproductive performance with an automated activity monitoring system versus a synchronized breeding program in 3 commercial dairy herds. R. C. Neves*, K. E. Leslie, J. S. Walton, and S. J. LeBlanc, *University of Guelph, Guelph, ON, Canada.*

The objective of this study was to compare overall herd reproductive performance with an automated activity monitoring system relative to a synchronized breeding program. A pen-level randomized trial was performed over 1 year using 3 commercial herds in Ontario, Canada, in which cows were housed in a primiparous and a multiparous pen on each farm. Pens were randomly assigned to an automated heat detection (AHD) system based on monitoring activity levels (Heatime, SCR Engineers Ltd.) or a timed artificial insemination program (TAI; Ovsynch), and a crossover occurred after 6 mo of the trial to avoid confounding treatment with parity. Insemination based on additional detection of estrus by observation was practiced in all pens. Herds A, B, and C milked 495, 305 and 260 cows on average, respectively. Herd A had 1476 AI, herd B 781 AI, and Herd C 988 AI throughout the study period. Analyses of the 3 herds were conducted using pen as the experimental unit. The proportion of TAI in the TAI pen was 49%, 71% and 55% for herds A, B and C. The proportion of AI in the AHD group after a heat signaled by the AHD system was 64%, 52% and 61% for herds A, B, and C. Conception risks for TAI and AHD were 32% and 32% in herd A, 40% and 44% in herd B and 26% and 29% in herd C. The mean annual 21-d pregnancy rates across the 3 herds were analyzed utilizing least squares means controlling for herd effect. There was no difference ($P = 0.25$) in the overall mean pregnancy rates between TAI program (15.9%) and AHD system (14.6%). Under the conditions in which a substantial minority of AI in both groups was based on visually detected estrus, herd pregnancy rate was not different between a TAI program and an AHD system. Further investigation of variables that may influence herd performance and of cow-level performance is required.

Key words: reproduction management, estrus detection, pregnancy rate

237 Effects of time and storage conditions on Johne's disease milk ELISA test results. C. M. Innes*, D. F. Kelton, D. L. Pearl, and T. F. Duffield, *University of Guelph, Guelph, Ontario, Canada.*

The Ontario Johne's Education and Management Assistance Program (OJEMAP) utilizes an individual cow milk ELISA test for antibody to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) to determine the Johne's disease status of participating herds. Concerns were raised about the age of the samples at the time of testing and the temperature extremes that milk samples could be subjected to while being transported to the Dairy Herd Improvement (DHI) laboratory, and the impact on integrity of test results. This objective of this study was to investigate the impact of storage time and conditions (room temperature, frozen, refrigerated) on Johne's disease milk ELISA test results. Milk ELISA tests were completed using a commercially available kit following standard manufacturer instructions on days one, 4, 7 and 10 post collection. The time between collection and storage of the samples and the differences between storage conditions were compared graphically and statistically using the paired *t*-test, with a level of significance of $P < 0.05$. There were no significant differences in test results for any of the storage conditions for up to 7 d post collection. Based on these results, samples could be stored under various conditions for up to a week with no significant changes in test results.

Key words: Johne's, storage, ELISA

238 The evaluation of bulk tank tests for the surveillance of Johne's disease. C. M. Innes*, D. F. Kelton, D. L. Pearl, and T. F. Duffield, *University of Guelph, Guelph, Ontario, Canada.*

The objective of this study was to evaluate the utility of bulk tank tests to detect the presence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) antibody in dairy herds for the purpose of Johne's disease surveillance. Individual cow milk samples were collected by CanWest Dairy Herd Improvement customer service representatives in herds across Ontario, Canada. These samples along with bronopol preserved bulk tank samples collected by milk transporters from herds participating in the Ontario Johne's Education and Management Assistance Program (OJEMAP), a producer funded Johne's control scheme. The bulk tank ELISA results were expressed as percentage S/P (sample to positive). A S/P ratio of ≥ 30% was considered positive for MAP and a ratio of 20–30% was considered to be suspect for MAP.

The individual cow ELISA results were expressed as an optical density value, with a positive result having an optical density of 0.1 or higher. There were 309 farms tested, with herd size from 15 to 986 milking cows. The relative sensitivity and specificity of the bulk tank ELISA test when a positive herd was defined as 1 or more positive cows was 54.7% and 90.6%, respectively. When 2 or more positive cows defined a positive herd, the relative sensitivity increased to 63.3% while the specificity decreased to 84.2%.

Key words: Johne's, bulk milk, ELISA