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Student Abstracts

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The use of an automated estrous detection monitor during a timed AI program

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The aim of this study was to compare two reproductive programs for first AI based on activity monitors and timed AI and determine cow-based factors that affect estrous expression as measured using an automated estrous detection system. Nine hundred and eighteen lactating Holstein cows from two commercial farms (Farm A, n=466; Farm B, n=452) were enrolled. Animals were pre-synchronized with two injections of PGF followed by an ovsynch protocol. Treatments were 1) TAI: all first inseminations performed by timed AI and 2) ACT: first insemination based upon estrous detection by activity monitors (Heatime, SCR Engineering, Israel) after the pre-synchronization, whereas the remaining non-inseminated cows were enrolled onto the ovsynch protocol. Physical activity information from the activity monitor was continuously recorded on Farm A from a subset of 323 animals; all estrus episodes (n=423) between calving until first AI were included. Estrous expression was quantified using two parameters, 1) peak activity and 2) duration. Peak activity was defined as the maximum activity index during an estrus episode; the threshold activity to be considered an estrus event was set at an index level of 35, approximately 80% increase in activity compared with baseline. The duration of an estrus episode was defined as the amount of time the animal spent with an index level greater than 35. All animals had their body condition score (BCS; scale 1-5), hock score (HS; scale: 1-4), gait score (GS; scale: 1-4) and corpus luteum presence by ovarian ultrasonography recorded twice during pre-synchronization. Continuous data was analysed using ANOVA by Proc GLM, whereas binomial data was analysed by logistic regression using Proc Logistic of SAS. Pregnancy per AI did not differ between treatments (30.7% and 33.1% for ACT and TAI, respectively; $P=0.49$). Pregnancy per AI was similar in cows bred by estrous detection compared with the ovsynch protocol (29.0% vs 33.5%; $P=0.20$). Lameness ($GS>2$; 56.2% healthy vs 38.4% lame; $P<0.001$), swollen hocks ($HS>2$; 58.1% healthy vs 35.4% swollen; $P<0.001$), and non-cyclic animals (52.2% cyclic vs 34.2% non-cyclic; $P<0.01$) had reduced estrous detection. Fertility was affected by lameness and swollen hocks, as lameness (26.4% vs 35.4%; $P<0.01$) and swollen hocks (27.8% vs 34.5%; $P<0.01$) had decreased pregnancy per AI compared with healthy animals. BCS did not affect estrous detection, but low BCS (≤ 2.75) decreased pregnancy per AI (25.5% vs 39.4%; $P<0.01$). Contrary to our initial hypothesis, animals considered lame and those with low BCS did not differ in duration and peak intensity of estrus episodes ($P>0.10$). Estrous duration ($P<0.01$) and intensity ($P=0.04$) were found to be different between cyclic and non-cyclic, where non-cyclic animals had shorter and less intense estrus episodes. Cows that became pregnant from the first postpartum AI had greater intensity episodes at AI (74.5 ± 1.8 vs 70.5 ± 1.5 ; $P=0.03$). Pregnancy success outcome was not affected by estrus duration. In conclusion, pregnancy per AI did not differ between treatments; however, gait score, hock score, BCS and cyclic status highly impacted fertility and the number of animals detected in estrus. Estrous duration and intensity were not affected by lameness or low BCS, but were affected by the cyclic status by 50 DIM. The use of activity monitors for automated estrous detection can be used strategically in a traditional reproductive program for first AI after calving; however, do not seem to be sensitive enough to automatically decipher animals with lameness or low BCS. Alternative programs warrant further research for cows at risk for estrous detection failure. **Key words:** activity monitors, dairy cows, health, reproduction programs

Relationship of concentrations of cortisol in hair with health, plasma metabolites and reproductive parameters in dairy cows

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The use of hair cortisol has been used to measure chronic stress in dairy cows, it offers the advantage of being non-invasive, fast and able to indicate levels of cortisol over long periods of time. The aim of this study was to determine the associations between hair cortisol with clinical and sub-clinical health disorders, and reproductive success in dairy cows. Furthermore, we aimed to determine the association between, blood markers associated with metabolic status and acute inflammation. In Experiment 1, cows (n = 64) were hair sampled every 3 wks from the tail switch from calving (d 0) until d 126 for hair cortisol analysis and blood samples were collected every 3 wks from d 0 until d 42 for BHBA and glucose analysis. In Experiment 2, cows (n = 54) were chosen retrospectively by diagnosis of sub-clinical endometritis (Endo) or as healthy (Control) using a cytobrush and ultrasonography at 30 ± 3 DIM. At the same time, animals were hair sampled for cortisol analysis, and blood sampled for haptoglobin and ceruloplasmin analysis. Health records were recorded by farm personnel throughout both experimental periods. Animals with clinical disease presented higher cortisol concentrations than clinically healthy animals in Experiment 1 (Geometric Mean [95% CI]) (8.8 [7.8, 9.9] vs 10.7 [9.6, 12.0] pg/mg); however, animals diagnosed with subclinical disease in Experiment 2 did not differ in hair cortisol concentrations (11.5 [9.7, 13.7] vs 11.3 [9.6, 13.3] pg/mg for Control and Endo groups, respectively). In Experiment 1, there was an effect of sample day, where d 21 had higher cortisol concentrations than d 42, d 84, and d 126, but not from d 0, for both parities. Within both experiments, a parity effect was present where multiparous animals consistently had higher cortisol concentrations than primiparous animals. Multiparous cows that became pregnant by 100 days post-partum had lower concentrations of hair cortisol at 42 and 84 DIM. Lastly, other biomarkers associated with metabolic status and acute inflammation such as glucose, BHBA, haptoglobin and ceruloplasmin were not strongly correlated with measurements of cortisol in hair. Overall hair cortisol measurements appear to be associated with clinical disorders and have a direct association with pregnancy outcomes; however, concentrations of hair cortisol may not be suited to differentiate situations of stress with lower magnitudes, such subclinical disease. **Key words:** dairy cow, chronic stress, hair cortisol, health

Automated and visual measures of walking activity and behavior frequency during estrus and its sources of variation in nulliparous dairy cows

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Holstein heifers (n = 57) were monitored using accelerometers and video observations with the objective of better understanding the behavioral expression of estrus, variation within and between heifers, and possible sources of variation. IceTags recorded walking activity from seven to 13 mo of age. Activity peaks (n = 282) were obtained from a rolling sum of steps within 24 h periods and validated to be estrus by ovarian ultrasonography. Behavior around activity peak of one estrus for each of 12 heifers was described in detail from video recordings. Baseline (B) behavior was monitored in a corresponding interval one wk before. Estrus (E) and B total steps and steps/h, estrus relative increase in activity, duration, and interval between episodes were analyzed by descriptive statistics and Spearman rank correlations. Effects of category of baseline walking activity, estrus order (pubertal vs. subsequent episodes), season, hour of estrus onset, and number of heifers simultaneously in estrus were evaluated with proc MIXED. Behavioral changes from B to E were evaluated by Signed Rank test. Estrus total steps varied greatly (4743 ± 1740 ; range: 837 to 10,070), as well as relative increase in activity ($290 \pm 160\%$; range: 30 to 1,190%). Duration of estrus was 14 ± 4 h, ranging from 4 to 26 h. Interval between episodes was the trait that varied the least. The pubertal estrus was shorter and had smaller relative increase in activity than the subsequent episodes ($P < 0.05$). Estrus steps were greater for heifers of high baseline activity ($P < 0.01$). Episodes occurring in the winter and starting between 1600 h and 0300 h had the greatest relative increase in activity ($P < 0.05$). The number of heifers simultaneously in estrus did not influence estrus expression ($P > 0.05$). Display of primary and secondary estrous behaviors increased significantly. The greatest change from B to E was observed for chin rest, sniff, back mount, crossover, accept chin rest, and follow, but variation was large. Overall, estrus was apparent in behavioral changes with large variation within and between heifers. Estrus order, season, onset hour, and baseline walking activity are important factors affecting estrus activity. Therefore, estrus detection tools should account for potential sources of variation. The reported visual and automated measures of estrus expression reveal possibility for improved on-farm estrus detection technologies and potential selection for estrus expression. **Key words:** heat, heifer, individual variation, walking activity

Automated measures of lying and standing patterns during estrus and its sources of variation in nulliparous dairy cows

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Lying and standing behavior of nulliparous Holstein cows (n=57) was studied from d-7 to +2 relative to estrus (269 episodes) using leg-mounted accelerometers (IceTag). Objectives were to assess effects of estrus on lying (LY) and standing (ST) behavior, variation and its sources, and provide insight into opportunities for using these measurements on automated estrus detection. Heifers (7 to 13 mo old) were housed in a free stall barn. Data exported from accelerometers were used to calculate frequency of ST bouts, total daily duration of ST, and mean bout duration and duration of the longest bout on each day for ST and LY. Results are presented as LSMeans±SEM for d-7, d-2, d-1, d 0 (estrus), d+1, and d+2. Estrus episodes were identified from activity peaks (increased number of steps) and validated by ovarian ultrasonography. Data was analyzed by repeated measures ANOVA with proc MIXED of SAS. Effects of estrus order (pubertal vs. later episodes), season, time of estrus onset, category of baseline activity, and number of heifers in simultaneously in estrus were tested for each variable. Overall, frequency of ST bouts/d was reduced and mean duration of ST bouts, duration of the day's longest ST bout, and total duration of ST were greater on d 0. The longest ST bout of a day increased from d-7 (232±4.7 min) to d 0 (487.8±15.7 min). Coefficients of variation for ST variables ranged from 21.5 to 97.8%. The pubertal estrus had lower mean duration of ST bouts, longest ST bout duration, and total daily duration of ST. The longest ST bout was greater for heifers with high baseline walking activity and cold season episodes. Episodes with morning onset had longer ST bouts only at d 0, while those with afternoon and night onset had the increase in ST bout duration spread over d-1 and 0. Therefore, there was a smaller increase in ST variables on d 0 relative to other d. The number of heifers simultaneously in estrus did not have a significant effect on the studied variables. Daily LY and ST patterns were markedly influenced by estrus and were correlated to walking activity. Bout frequency and duration on days following estrus were similar to d-7, except the longest LY bout of d+1, which was longer for non-pubertal episodes. Incorporating measures of standing and lying patterns and correcting for sources of variation could improve automated estrus detection systems and estrus detection sensors. **Key words:** activity, dairy cow, heat, lying behavior

Social housing reduces food neophobia in dairy calves.

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Animals are often reluctant to consume novel feeds. Research suggests that social housing can reduce fearfulness in animals. The aim of this study was to test the prediction that social housing reduces food neophobia in dairy calves. Beginning immediately at birth, Holstein bull calves were either reared individually (n=18) or in a complex social group with other calves and cows (n=18). In food neophobia tests calves were exposed to two identical buckets, one empty and the other filled with a novel food (chopped hay in Trial 1 and chopped carrots in Trial 2). Calves were tested for 30 min/d for 3 consecutive days starting at 70 d of age. Regardless of the type of food, socially housed calves consumed more of the novel feed compared to individually housed calves. In Trial 1, intake of hay as fed averaged 35 ± 6 g/d versus 18 ± 6 g/d for socially versus individually housed calves. In Trial 2, intake of chopped carrots as fed averaged 27 ± 6 g/d versus 6 ± 6 g/d, for socially versus individually housed calves, respectively. Social rearing decreased the latency to eat the novel feed. Calves housed in a complex social group began eating the hay after $1:23 \pm 1:13$ versus $3:58 \pm 1:10$ min:s for individually housed calves. Latency to begin eating the chopped carrots averaged $3:09 \pm 1:17$ versus $6:38 \pm 1:13$ min:s for socially versus individually housed calves. These results indicate that housing dairy calves in a complex social group reduces food neophobia. More generally, this study contributes to a series of studies showing that calves raised in more complex social environments may be better able to transition to other changes their environment, relative to calves raised in individual pens and hutches. **Key words:** dairy calves, food neophobia, pair housing

Effects of elevated subcutaneous fat stores on serum phospholipids fatty acid profile in periparturient dairy cows

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Periparturient dairy cows with increased subcutaneous lipid stores release greater concentrations of nonesterified fatty acids (NEFA) into circulation around parturition. Phospholipids (PL) are essential for maintaining cellular plasma membrane integrity, lipoprotein synthesis and intercellular signaling. Large quantities of circulating NEFA alter circulating PL fatty acid (FA) profile. The objective of this study was to determine the effect of elevated lipid mobilization during the periparturient period on serum PL profile. Thirty-four cows were blocked by parity; treatment group received a dry cow ration with an additional 10 kg of corn / head per day starting -28 d relative to parturition. The control group received the dry cow ration (no additional corn) with 400 mg of monensin / head per day. Immediately postpartum, treatment cows were fasted for approximately 8 h. Serum samples were collected on -28, -7, +1, +6, +15, and +21 d for FA analysis of specific lipid fractions. Data were analyzed as repeated measures analysis of variance using mixed model procedures in SAS (9.3) and significance was declared at $P \leq 0.05$. Within the serum PL fraction, C18:3n6, C20:4, C20:5, total n3, and n6:n3 ratio varied significantly by a treatment \times parity \times time interaction. Total n6:n3 was greater for treatment prepartum animals and multiparous cows postpartum. Circulating concentrations for C20:4 were greater in multiparous cows prepartum as compared to primiparous cows before sharply declining after parturition to similar concentrations observed in primiparous cows. In contrast, control cows had greater concentrations of C20:5 during the prepartal period and primiparous cows had greater concentrations as compared to multiparous cows following parturition. In summary, increased subcutaneous fat stores altered FA profile of serum PL fraction in periparturient dairy cows. **Keywords:** lipid mobilization, fatty acid profile, phospholipids

The effect of dietary saturated and unsaturated fatty acid on gene expression and fatty acid composition of serum, adipose and liver lipid fractions in pre-ruminant calves

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Dietary saturated fatty acids (SFA) and unsaturated fatty acids (UFA) affect the adipogenic, lipogenic and immune system activities, and fatty acids (FA) composition of various tissues including serum. Dietary UFA however, can be rehydrogenated by rumen microbes. The objective of this study was to study the effect of dietary SFA and UFA on polymorphonuclear leukocyte cells (PMN) and peripheral blood mononuclear cells (PBMC) gene expression and FA composition of serum, adipose and liver lipid fractions in pre-ruminant calves. Twelve Holstein male calves were assigned to two treatments, 6 calves each, in a completely randomized design. Starting 3 d of age, calves on treatment 1 were fed SFA consisting of 120 mL palm oil/d, and treatment 2 were fed UFA consisting of 80 mL flaxseed oil and 40 mL conjugated linoleic acid/d. After 50 d, all animals were euthanized, and samples were obtained. Total RNA were isolated from PBMC and PMN cells. Peripheral blood mononuclear cells were analyzed for gene expression of IL-6, IL-1 β , TNF- α , and ICAM; PMN cells were analyzed for Casp1, IL-8R and L-selectin. Plasma, liver and adipose tissues lipids were processed by gas-chromatography following lipid extraction, fractionation and methylation. Data were analyzed using the Proc TTEST of SAS with significance declared at $P \leq 0.05$. L-selectin was decreased in calves treated with UFA ($P = 0.04$). In addition, UFA caused total polyunsaturated FA (PUFA) to increase in the phospholipids fraction of adipose tissue, whereas PUFA content of non-esterified FA and neutral lipid fractions were increased in both serum and adipose. Feeding dietary UFA increases storage, in adipose tissue, and serum circulation of PUFA while decreasing L-selectin. This may inhibit the migration of PMN cells from the blood to the tissues, affecting inflammatory responses. **Key words:** Gene expression, fatty acid composition, calves

Improving ensiling characteristics of lower-quality forages using combination homofermentative Lactic Acid-producing Bacteria and Fibrolytic enzymes.

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Silage pH is decreased predominately by lactic acid produced by homofermentative lactic acid-producing bacteria (LAB). Increased plant maturity inhibits organic acid production and rate of pH decline in silage. A combination inoculant of four homofermentative LAB and four fibrolytic enzymes was applied to mature alfalfa and compared to a control (final DM= 42%) and packed into mini-silos (1206 cm³ volume; n = 3) and buckets (21,504 cm³ volume; n = 5) for 59 d. The pH, DM, CP, ammonia, sugar, fiber, VFA and rumen degradation characteristics of the silage were measured. Data were analyzed using PROC NLIN and PROC Mixed of SAS and significance was declared at $P \leq 0.05$. Rate of pH decline was greater ($P < 0.01$) and d 59 pH tended to be lower ($P = 0.07$) in the inoculated silage. Increased rate of pH decline and d 59 pH could be a result of increased lactic acid production in the inoculant, as evidenced by the inoculated silage having a greater amount of lactic acid compared to control (2.37 vs 1.68 ± 0.18 , mmol/g DM). Acetate, propionate, and butyrate were not significantly different between treatments ($P > 0.23$); as these acids are stronger antifungals than lactic acid, this could possibly contribute to yeast and mold counts (\log_{10} CFU/g DM) that were not significantly different ($P = 0.79$). The percent DM, DM loss, ammonia and sugar did not differ between treatments ($P > 0.05$). While NDF, ADF and cellulose were significantly lower in the inoculated silage, the rumen DM disappearance was greater as compared to control (58.9 vs 60.2 ± 0.53 , % DM). The inoculant appeared to increase the rate of pH decline, and rumen DM disappearance, while having little effect on nutrient composition of the treated silage. **Keywords:** silage, inoculants, LAB

Effects of Elevated Lipomobilization on Non-Esterified Fatty Acids and the Gene Expression of Inflammatory Markers in Early Lactating Dairy Cows

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A net negative energy balance in lactating animals during the transition period from pregnancy to lactating can cause massive lipomobilization. This can lead to elevated fatty acids (FA) in the blood, mainly in the nonesterified (NEFA) form. The circulating FA can alter phospholipids (PL) on cell membranes, and other lipid fractions such as neutral lipids (NL), which affects cell function and signaling. Thirty-four cows were blocked by parity, and received two rations from d -28 to d +21 relative to calving date. Treatment ration contained basal diet plus an additional 10 kg of corn/day, and the control group received no additional corn but added 400 mg monensin; treatment cows were fasted for 8 h on d +3. FA profiles were analyzed through Gas Chromatography on serum samples obtained at d -28, -7, and +6. Additionally, gene analysis was performed on genes that are active during pro-inflammatory responses such as Interleukin-8 receptor (IL-8R), Caspase-1 (CASP-1) and L-Selectin (SELL) in polymorphonucleocytes (PMNs). Real-time q-PCR was performed on circulating PMNs obtained at d -28, +3, +12, and +21. Dietary treatment had no detectable effect on mRNA expression of CASP-1, IL-8R, and SELL ($P > 0.05$). However, expression of genes tested was changed over time ($P < 0.001$). Expression of IL-8R and SELL tended to also differ by parity ($P < 0.09$). In the NL serum fraction, C18:1cis, C18:3n3, and n6:n3 ratio showed significant variance by treatment \times time \times parity interaction. The treatment group's n6:n3 ratio increased prepartum whereas the control group stayed the same. Additionally, there was a significant alteration observed in C20:5 where the control was greater than treatment. Overall, elevated circulating NEFA altered FA profiles; however, further research is needed to mechanistically explain the effect of excess adipose stores on PMN responses to both immunologically challenged and un-challenged states. **Keywords:** lipid mobilization, nonesterified fatty acids, pro-inflammatory mediators