Lactation Biology II

486 Using infrared thermography for detecting intramammary infections under practical and *E. coli* O55:B5 endotoxin challenge conditions in dairy ewes. A. Castro-Costa[1], G. Caja*[1], A. A. K. Salama[1], M. Rovai[1], C. Flores[1], and J. Aguiló[2], *[1]Ruminant Research Group (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, Bellaterra, Spain. [2]Group of Biomedical Applications (GBA), Department of Microelectronics and Electronic Systems, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

A total of 83 lactating dairy ewes from 2 breeds (Manchega, n = 48; Lacaune, n = 35) were used in 2 experiments for assessing on the use of infrared thermography (IRT) for detecting intramammary infections (IMI). In Exp. 1, ewes were milked twice-daily and IRT pictures taken with an IRI 4010 camera (Irysis, Northampton, UK; accuracy, ± 0.15°C) before and after milking at 46 and 56 DIM. Mean udder skin temperatures (UST) were measured from IRT pictures and IMI detected by bacterial culture of milk samples. A total of 85.5% udder halves were classified as healthy and 14.5% as IMI. No UST differences were detected by udder health (healthy vs. IMI; $P = 0.484$) nor side (left vs. right; $P = 0.879$), but UST varied by effect of breed ($P = 0.003$) and by milking moment ($P = 0.014$) and milking turn ($P < 0.001$). The UST increased linearly with ambient temperature (r = 0.88). In Exp. 2, 3 groups of 3 Lacaune ewes, milked once-daily in late lactation (155 DIM), were used for evaluating the response to an *E. coli* O55:B5 endotoxin challenge (0.083 µg/kg BW). Treatments were: 1) control (C00, both halves untreated), 2) half udder (T10 and C01, one udder half treated and the other untreated, respectively), and 3) treated (T11, both halves treated). Vaginal temperature, UST, milk yield and milk composition changes were monitored at different time intervals during 3 d. Local and systemic signs of IMI, as well as milk changes (flakes, CMT, SCC and composition) were observed from h 6 ($P < 0.05$ to 0.001). For all treatments, UST increased after challenge, peaking at h 6 in T11 ($P < 0.001$) and decreasing thereafter without treatment effects. No differences were detected in fat and protein milk contents, but lactose content and SCC in milk were different between treated vs. untreated udder halves ($P < 0.05$) throughout the challenge. Ambient temperature and UST correlated (r = 0.60). In conclusion, despite the accuracy of the camera and the SEM obtained for UST (±0.05 to ± 0.24°C), we were unable to discriminate between healthy and IMI udder halves (subclinically or clinically infected) in dairy ewes.

Key Words: endotoxin, infrared thermography, mastitis

487 Effect of corn grain and soybean meal with different processing methods on milk protein expression profiles in lactating dairy cow. S. S. Li*[1,2], J. S. Shen[1,2], D. X. Ren[1], and J. X. Liu[1,2], *[1]Institute of Dairy Science, College of Animal Sciences, [2]MOE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou 310058, China.*

A proteomics approach was used to investigate the effects of feeding corn grain and soybean meal with different processing methods on milk protein expression profiles in lactating dairy cows. Twelve multiparous Holstein dairy cows were used in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement. The main factors were corn [finely ground (FGC) and steam-flaked corn (SFC)] and soybean meal [solvent-extracted (SSBM) and heat-treated soybean meal (HSBM)], which were used to formulate 4 diets with a same basal ingredient: 27% FGC and 9% SSBM; 27% SFC and 9% SSBM; 27% FGC and 9% HSBM; and 27% SFC and 9% HSBM. Each period lasted for 21 d. Milk samples were collected on d 17, 18, 19 of each period, and changes in milk proteins were determined by 2-dimensional electrophoresis (2-DE) and Image-Master 2D Platinum 6.0 software. A total of 13 spots were detected with variations in protein spots abundance according to statistical analysis. These spots were identified by matrix-assisted laser desorption/ionization time of flight/ time of flight (MALDI TOF/TOF) mass spectrometry. The milk protein profiles on the gels were similar in cows fed FGC or SFC. However, abundance of α_{S2}-casein fragments were higher in the cows on HSBM than on SSBM, while fragments of β-casein, κ-casein, β-lactoglobulin, α-lactalbumin and zinc-α-2-glycoprotein were downregulated. The relative decrease in β-casein was validated by Western blot, and was in agreement with the mass spectrometry data. These results suggested that feeding diets differing in nitrogen supply principally change the synthesis and secretion of milk proteins in the mammary gland of lactating cows.

Key Words: corn grain, soybean meal, milk proteome

488 Dietary anion-cation difference and day length affect milk calcium content. A. Boudon[1], M. Johan[1], A. Narcy[2], and C. Hurtaud*[1], *[1]INRA-Agrocampus Ouest UMR 1348 PEGASE, Saint-Gilles, France, [2]INRA URA, Nouzilly, France.*

Milk and dairy products are an important source of calcium for humans but recent studies in France have shown a clear decrease in milk calcium content during May and June and with grass diets compared with corn silage diets. The aim of this study was to identify the reasons of this seasonal drop of milk calcium content by testing the effect of 2 levels of dietary anion-cation differences (DCAD; 0 mEq/kg DM for D0 and 400 mEq/kg for D400) and 2 d lengths (8 h of light/d for short days and 16 h/d for long days) on calcium balances of dairy cows. The DCAD treatments were conceive to mimic diets based either on maize silage or on herbage. The cows were only lightened by solarium lights providing UVA and UVB. The trial was carried out according to a Latin square design using 8 dairy cows averaging 103 ± 44 DIM with 4 periods of 14 d. Data were analyzed accordingly using Mixed procedure. The significance threshold was set at $P \leq 0.05$. There was no significant interaction between day length and DCAD level. With D400 compared with D0, blood pH increased and plasma ionized calcium content decreased, while the plasma total calcium content was not different between treatments. However, milk calcium content increased, in relation with a decrease of the amount of calcium excreted in urine. DCAD had no significant effect on protein and casein contents and D400 tended to decrease milk yield. This illustrates that the udder did not decrease Ca uptake from the blood at high DCAD even though high DACA decreased the availability of Ca by decreasing the proportion of blood ionized Ca. Milk calcium and casein contents were significantly lower with long compared with short days, whereas day length had no effect on milk yield. This work highlights that long and sunny days can explain a part of the seasonal decrease of milk calcium content in summer and refutes the hypothesis that low milk calcium contents at grazing could be due to the high DCAD of herbage.

Key Words: dairy cow, milk, calcium

489 Calf sex influences whole-lactation milk and component production in Holstein cows. A. J. Carpenter*[1], K. Hinde[2], J. S. Clay[3], and B. J. Bradford[1], *[1]Kansas State University, Manhattan, [2]Harvard*

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Research in some animals has indicated that sex of progeny may influence milk production and composition in the ensuing lactation, and that this effect is influenced by parity. To investigate whether this phenomenon is observed in dairy cattle, data from 1995 to 1999 were obtained from Dairy Record Management Systems and analyzed by modeling the fixed effects of calf sex, parity, use of BST, and their interactions, and the random effect of year. Only Holsteins with lactations that began with a single heifer or bull calf were included in analysis. The lactation was considered BST positive if BST use was reported in any of the first 5 test days. Response variables analyzed were 305-d milk (305MILK), fat (305FAT), protein (305PROT), and peak milk (PEAK) production, and average somatic cell score (SCS). Over 2.5 million lactation records were used to assess all response variables except for PEAK (~1 million lactations). Cows beginning a lactation with the birth of a heifer had 1.01 ± 0.10% greater 305MILK, 1.02 ± 0.12% greater 305FAT, 0.82 ± 0.12% greater 305PROT, and 0.68 ± 0.15% greater PEAK, but 0.92 ± 0.17% lower SCS than cows with bulls (all $P < 0.001$). Significant sex by BST interactions were detected for all yield variables. For BST negative lactations, heifers resulted in greater yields ($P < 0.001$), but among BST positive lactations, there was no difference in 305MILK ($P = 0.09$), 305FAT ($P = 0.13$), 305PROT ($P = 0.41$), or PEAK ($P = 0.48$) as a function of calf sex, reflecting a ≥ 78% decrease in the marginal advantage of heifers. Sex effects were similar when data were analyzed without lactations involving dystocia, and were generally evident across all parities. Although the magnitudes of the differences are small, the highly significant effects of sex and the fact that BST administration negated these effects suggest that this analysis has revealed a real biological phenomenon. Based on these observations, we suggest that larger-magnitude sex bias in milk secretion observed in previous studies was likely due to postnatal signals (e.g., greater demand) rather than prenatal programming.

Key Words: lactation, sex effect, prenatal

490 The effect of induced involution on DNA methylation upstream of milk protein genes, α-lactalbumin and β-lactoglobulin. S. Pryor*, J. Dobson, and K. Singh, *AgResearch, Hamilton, New Zealand.*

Bovine mammary gland involution following termination of milking results in a decline in milk protein gene expression, including expression of major milk proteins α-lactalbumin (α-LA) and β-lactoglobulin (β-LG). Increased DNA methylation has previously been observed around a STAT5 binding site within the 10-kb region upstream of the α_{S1}-casein gene with cessation of milking. The aim of the present study was to determine if termination of milking has an effect on DNA methylation within the 10-kb region upstream of the α-LA and β-LG genes. Mammary alveolar tissue was obtained from non-pregnant cows in mid-lactation slaughtered at 6 h (lactating) or 7 and 28 d (n = 5/group) after the last milking. Following 7 d non-milking, quantitative RT-PCR analysis showed the level of α-LA and β-LG mRNA decreased 140-fold ($P < 0.001$) and 17-fold ($P < 0.01$), respectively, compared with lactating controls. After 28 d non-milking, α-LA and β-LG mRNA levels were downregulated 10-fold and 5-fold ($P < 0.05$) respectively, relative to lactation. Quantitative Sequenom MassARRAY analysis of DNA from mammary alveolar tissue revealed a similar level of methylation at analyzed sites within the 10 kb region upstream of the α-LA gene in lactating cows and those not milked for 7 or 28 d. In contrast, DNA methylation was increased around a putative STAT5 binding site directly upstream of the first β-LG exon with cessation of milking. In lactating cows, methylation of 35% was detected at a CpG dinucleotide located within this putative STAT5 binding site, which increased to 45% and 50% ($P < 0.05$) following 7 and 28 d non-milking, respectively. These results indicate that increased methylation within a putative STAT5 binding site upstream of the β-LG gene may be associated with the decline in β-LG expression occurring with termination of milking. However, despite the decreased level of α-LA mRNA with non-milking, methylation of analyzed sites upstream of the α-LA gene did not change, suggesting that the role DNA methylation plays in regulation of gene expression with involution may differ for each milk protein.

Key Words: lactation, epigenetics

491 Udder cistern size affects lactation persistency and ability to adapt to once-daily milking in dairy cows. A. Molenaar*[1], G. Caja[2], S. Leath[1], H. Henderson[1], C. Cameron[1], M. Challies[1], K. Taukiri[1], T. Chikazhe[3], S. Kaumoana[3], B. Lannou[1], A. Dorleac[1], A. Guy[1], C. Gavin1,4, and K. Singh[1], *[1]AgResearch Ruakura, Hamilton, New Zealand, [2]Group of Ruminant Research (G2R), Universitat Autonoma de Barcelona, Bellaterra, Spain, [3]AgResearch, Tokanui Dairy Research Farm, Te Awamutu, New Zealand, [4]Faculty of Science and Engineering, University of Waikato, Ruakura, New Zealand.*

Sixty NZ dairy cows (Holstein and Jersey crossbred; 505 ± 1 kg BW; 4.4 ± 0.3 yr) were allocated into 4 groups (n = 15) according to previous seasons lactation persistency (HP, high; LP, low) and milk yield (HY, high; LY, low) to study the relationship between udder cistern size and milk loss when moved from twice (2×) to once (1×) daily milking in late lactation. Groups had similar parity and half the cows remained at 2× as matched controls. Cows were grazed, feed supplemented, and milked on a rotary parlor with automatic data recording. At 16 to 21 h after last milking, cistern size of rear udder quarters was measured by ultrasonography (sectorial probe, 5 MHz) at 3 scanning sessions done in the rotary, as (1) distended cistern size (DCS) after inducing milk letdown (oxytocin, i.m. 20 IU/cow) on d −15 and +11 of start of 1×; and (2) natural cistern size (NCS) after blocking milk letdown (atosiban, i.v. 5 mg/cow, given before heading to the rotary), followed by DCS, on d +15. Milking was performed after DCS and NCS-DCS to obtain total, cisternal and alveolar milk volumes, respectively. A final scan was done after milking to detect residual milk. Cistern size was assessed using a linear scoring template (0 to 5; accuracy, ± 0.5). Milk yield at d −15 varied by group (HP, 12.6; LP, 11.0; HY, 13.1; LY, 10.5 L/d; SEM, ± 0.3; $P < 0.05$), but DCS only differed ($P < 0.05$) between HP and HY (3.29, 3.59, 3.93 and 3.69; SEM, ± 0.07, respectively). Post milking scans showed that cows left milking with 5 to15% residual milk. Changing to 1× produced 30% milk loss on average, being greater in LP cows ($P < 0.05$) than in other groups (27, 36, 29 and 29%). No DCS differences were observed after changing to 1×. Values of NCS (3.23, 3.14, 3.64 and 3.08; SEM, ± 0.10) were the greatest in HY and cisternal milk was lowest in LY cows (55, 54, 61 and 47% total milk) ($P < 0.05$), the rest being no different. Cisternal milk volume positively correlated with milk yield (2×, $r^2 = 0.48$; 1×, $r^2 = 0.67$). In conclusion, udder cistern size was related to lactational performances of dairy cows and could be used as a tool for dairy management decisions.

Key Words: atosiban, udder cistern, ultrasonography

492 The appearance of blood components in milk during the first hours of endotoxin induced mastitis follows two different chronological patterns. O. Wellnitz[1], C. Zbinden[1], J. Lüttgenau[2], H. Bollwein[2], and RM Bruckmaier*[1], *[1]Veterinary Physiology, Vetsuisse*

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During mastitis considerable changes in milk composition occur. Besides an increase of somatic cells several blood components start to increase in milk within 2 to 4 h after endotoxin challenge. This study aimed to investigate the sequence and pattern of appearance of several blood components in milk during the first hours of an endotoxin induced mastitis. Eight Holstein dairy cows were challenged with 200 µg *E. coli* lipopolysaccharide (LPS) into one quarter. Milk samples (~10mL) were taken at 0, 1, and 2 h after challenge and then every 15 min until 5 h after challenge from the LPS treated and one control quarter. Somatic cell count (SCC; using DeLaval cell counter), Immunoglobulin (Ig)G1 and 2 (by ELISA), and lactate dehydrogenase (LDH), and L-lactate (enzymatically using commercial kits) were analyzed. Differences between LPS treated and control quarters were evaluated using mixed model procedure including cow as repeated subject with a Bonferroni correction and are considered significant if $P < 0.05$. SCC, L-lactate, immunoglobulin (Ig)G1, IgG2, and LDH increased in LPS challenged quarters until the end of the experiment but did not increase in control quarters. Milk IgG1, IgG2, and LDH were already significantly increased at 2 h after challenge. In contrast milk SCC and lactate remained at a basal level until 2 h after challenge. A significant increase was detected at 2.75 h (SCC) and at 2.25 h (L-lactate) after challenge. In conclusion, the blood components IgG1, IgG2 and LDH increase in milk in response to endotoxin challenge before an elevation of SCC and lactate can be detected. The increase of blood components in milk follows 2 different patterns: There is a fast increase within the second hour of challenge for IgG1, IgG2, or LDH, reaching a plateau within 3 h. On the other hand, SCC and L-lactate show a consistent increase not reaching a plateau within 5 h.

Key Words: mastitis, milk, blood-milk barrier

493 Milk production during the colostral period is not related to the later production level in dairy cows. E. C. Kessler, R. M. Bruckmaier, and J. J. Gross*, *Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland.*

In dairy cows, milk yield increases after parturition to a peak at around wk 6 of lactation. Because milk is not sold during the colostral period, milk yield during the first few days after parturition is mostly not recorded. However this period is crucial for the development of metabolic diseases. For analysis of the evolution of milk production from parturition the first 10 milkings after parturition and daily milk yields from d 1 to 28 of lactation were analyzed in 17 primiparous and 39 multiparous cows milked twice daily. Milk yield of first milking after parturition ranged from 1.3 to 20.7 kg (Δ 19.4 kg) in cows and from 1.8 to 10.9 kg in heifers (Δ 9.1 kg). At the tenth milking production ranged from 9.2 to 21.5 kg in cows (Δ 12.3 kg), and from 7.0 to 15.2 kg in heifers (Δ 8.2 kg), resp. The correlation between the amount of first colostrum and daily milk production in cows decreased from r = 0.47 on d 5 to r = 0.32 on d 14 ($P < 0.05$) i.e. the effect of colostrum yield on milk production decreased with DIM. On d 28, all multiparous cows had a comparable production level independent of their first colostrum yield. However, in heifers the variation of the daily milk production was maintained until d 28 of lactation. Immediately after parturition, daily milk production increased exponentially, but after approximately 1 wk in lactation, the slope of the daily milk production curve flattened and continued more linear. A non-linear regression equation was used to determine the time point when the lactation curve passed into a linear slope. This turn occurred earlier in heifers (d 6.9 ± 0.3) than in multiparous cows (d 8.2 ± 0.2, $P < 0.01$). In conclusion, a lower milk production during the first days of lactation was not necessarily related to the lactational performance in multiparous cows. Considering a simultaneously lower metabolic load in cows giving less milk in the very beginning of lactation, this attribute might gain importance as a breeding objective.

Key Words: lactation curve, milk yield, dairy cow

494 Palmitate induces endoplasmic reticulum stress and oleate and sodium salicylate suppress oxidative stress in immortalized bovine mammary epithelial cells. L. K. Mamedova[1], S. R. Montgomery*[1], K. J. Harvatine[2], and B. J. Bradford[1], *[1]Kansas State University, Manhattan, [2]Pennsylvania State University, University Park.*

Palmitate has been shown to induce endoplasmic reticulum (ER) and oxidative stress in some cell types, whereas sodium salicylate (SS) and unsaturated fatty acids have been shown to mitigate these stresses. Therefore, we hypothesized that SS would counteract the effects of palmitate in immortalized bovine mammary epithelial (MAC-T) cells. In the first experiment, MAC-T cells were treated with SS (50 µ*M*), palmitate (250 µ*M*), or oleate (500 µ*M*) alone or in combination in DMEM with 10% fetal bovine serum, 1% penicillin streptomycin, 1% insulin, and 2% bovine serum albumin and incubated for 24 h. To determine if palmitate effects were due to ceramide (CER) synthesis, a second experiment was conducted where MAC-T cells were treated with palmitate (250 µM), myriocin (1 µ*M*; CER synthesis inhibitor), or both in the same basal media. Results from replicate wells (6 to 12/treatment) were analyzed by ANOVA and treatment effects were declared at $P < 0.05$. Palmitate increased mRNA abundance of the ER stress response targets XBP-1 (4-fold, $P < 0.01$), ATF3 (46-fold, $P < 0.001$) and CHOP (34-fold, $P < 0.001$) but neither SS nor oleate affected these transcripts ($P > 0.10$). The XBP-1 transcript is also spliced during the ER stress response. Palmitate increased the proportion of XBP-1 that was spliced (3.8 vs. 13.6 ± 2.2%, $P < 0.001$); this effect was not counteracted by SS or oleate. Myriocin treatment ablated the XBP-1 mRNA and XBP-1 splicing responses to palmitate (both $P < 0.05$), but surprisingly, further increased CHOP and ATF3 mRNA abundance (both $P < 0.001$). Palmitate had no effect on TBARS (a measure of oxidative products), but both SS and oleic acid decreased TBARS and acted synergistically when combined ($P < 0.001$ for direct effects and interaction). These results demonstrate that palmitate induces ER stress in MAC-T cells, in part through ceramide effects. Although palmitate did not induce oxidative stress, oleate and SS may function to alleviate oxidative stress in MAC-T cells.

Key Words: palmitate, mammary epithelial cell, ceramide