PHYSIOLOGY AND ENDOCRINOLOGY I

1371 (M188) Comparison of endocrine changes, timing of ovulations, ovarian follicular growth, and efficacy associated with Estradoublesynch and Heatsynch protocols in Murrah buffaloes (*Bubalus bubalis*). R. Mirmahmoudi[*1] and B. S. Prakash[2], *[1]Dep. of Animal Science, Faculty of Agriculture, University of Jiroft, Iran, [2]National Dairy Research Institute, Karnal, India.*

Experiments were conducted on 135 cycling and 31 anestrus buffalos to compare a) the endocrine changes, timing of ovulations, ovarian follicular growth and efficacy of Estradoublesynch (*P*$GF_{2\alpha}$ 0, GnRH 2, $PGF_{2\alpha}$ 9, Estradiol Benzoate; EB 10) and Heatsynch (GnRH 0, $PGF_{2\alpha}$ 7, EB 8) protocols in cycling buffaloes, and b) the efficacy of Estradoublesynch and Heatsynch protocols for fertility improvement in cycling and anestrus buffaloes. Ovulation was confirmed following all GnRH and EB treatments by ultrasonographic examination at 2-h intervals. Plasma progesterone and total estrogen concentrations were determined in blood samples collected at daily intervals, beginning 2 d before onset of protocols until the day of second ovulation detection. Ovulatory follicle size was measured at the time of a) first $PGF_{2\alpha}$ administration/2 d before onset of protocol, b) GnRH administration, c) 2 h before ovulation detection post GnRH administration, d) second $PGF_{2\alpha}$/$PGF_{2\alpha}$ injection, e) EB injection and, f) 2 h before ovulation detection post EB injection. Plasma LH, total estrogen and progesterone concentrations were determined in blood samples collected at 30-min intervals for 8 h, beginning GnRH and EB injections, and thereafter at 2-h intervals until 2 h after detection of ovulation. The first ovulatory rate was significantly ($P < 0.05$) higher in Estradoublesynch (84.6%) protocol than that in Heatsynch protocol (36.4%). The first LH Peak concentration in Estradoublesynch (74.6 ± 10.4 ng/ml) protocol was significantly ($P < 0.05$) higher than that of Heatsynch (55.3 ± 7.4 ng/ml) protocol. In Estradoublesynch protocol, the total estrogen concentration gradually increased from the day of GnRH administration coinciding with LH peak, and then gradually declined to the basal level until the time of ovulation detection. However, in Heatsynch protocol, the gradual increase in total estrogen concentration after GnRH was observed only in those buffaloes which responded to treatment with ovulation. In both Estradoublesynch and Heatsynch protocols, ovulatory follicles size increased from GnRH/EB injections until ovulation. The pregnancy rate after Estradoublesynch (60.0%) protocol was significantly ($P < 0.05$) higher than that achieved after Heatsynch protocol (32.5%). Satisfactory success rate using Estradoublesynch protocols was attributed to the higher release of LH following GnRH injection, leading to most of the animals ovulating post GnRH injection and hence creating the optimum follicular size at EB injection for ovulation and pregnancy to occur.

Key Words: estradoublesynch, heatsynch, endocrine changes

1372 (M189) Development of a novel strategy for synchronization of ovulation and fertility augmentation in cycling buffalo cows. R. Mirmahmoudi[*1] and B. S. Prakash[2], *[1]Dep. of Animal Science, Faculty of Agriculture, University of Jiroft, Iran, [2]National Dairy Research Institute, Karnal, India.*

The aim of present study was to investigate the endocrine changes (progesterone, total estrogens, and LH), ovarian follicular growth, timing of ovulation and efficacy in terms of pregnancy rate in cycling Murrah buffaloes subjected to a novel protocol named Estradoublesynch ($PGF_{2\alpha}$ 0, GnRH 2, $PGF_{2\alpha}$ 9, Estradiol Benzoate; EB 10, timed artificial insemination (TAI) 48 and 60 h later). Twelve cycling buffaloes were subjected to the Estradoublesynch protocol. Ovarian follicle size and the rate of induction of ovulation were examined using transrectal ultrasonography at two hourly intervals post EB injection. Plasma progesterone and total estrogens concentrations were measured in blood samples collected at daily intervals. In addition, plasma LH and total estrogens concentrations were determined in intensive blood samples collected post EB administration. Ovulation occurred in all buffaloes 48.5 ± 1.6 h post EB treatment. Follicle size was gradually increased from second $PGF_{2\alpha}$ injection (9.7 ± 0.3 mm) until ovulation (12.9 ± 0.4 mm). Peak LH concentration of 34.2 ± 7.7 ng/ml occurred 18.3 ± 0.8 h after EB treatment. Peak total estrogen of 50.8 ± 6.9 pg/ml occurred 5.7 ± 1.0 h after EB treatment. Fourteen cycling buffaloes were subjected to the Estradoublesynch protocol, with TAI 48 and 60 h following EB injection, and 58 cycling buffaloes were inseminated after spontaneous estrus (control group). Pregnancy rates were 62% for TAI of cycling buffaloes and 34.5% for control group. These results demonstrated that the Estradoublesynch protocols can be potentially used to obtained satisfactory fertility after TAI in cycling buffaloes. This is a practical application of endocrine study toward fertility augmentation at farm level.

Key Words: Estradoublesynch, ovulation, ovarian follicle

1373 (M190) Maternal dietary effects on embryonic ovarian development in cattle. S. E. Echternkamp[*], D. R. Eborn, and R. A. Cushman, *USDA, Agricultural Research Service, Clay Center, NE.*

Ovarian gametogenesis and folliculogenesis begins early in fetal development with peak numbers of follicles present in bovine fetal ovaries in the second trimester of gestation and may be altered by maternal nutrition. The objective was to

determine whether maternal dietary energy intake by replacement beef heifers before breeding affects ovarian development in female progeny. Over three breeding seasons, puberal heifers were fed either a high (HE) or low (LE) energy diet for 6 mo before breeding plus the first 22 d of a 47-d breeding period to achieve 55 vs. 65% of mature BW; heifers were subsequently managed together on pasture. Female progeny (n = 68 LE and 67 HE) were developed on a standard management protocol for replacement beef heifers. Numbers of antral follicles (AFC), corpora lutea (CL), and ovarian length and height were measured in progeny ovaries by transrectal ultrasonography at 14 mo of age before a 29-d breeding period with fertile bulls; pregnancy was diagnosed at about 75 d of gestation. Data were analyzed by ANOVA with diet, year and diet x year as fixed effects. Progeny of LE vs. HE dams did not differ in birth (33.8 vs. 34.4 ± 0.6 kg, respectively) or prebreeding BW (381.2 vs. 385.0 ± 3.6 kg, respectively). Ovaries of LE progeny contained fewer small (2 to 5 mm) (18.0 vs. 21.9 ± 1.0, LE vs. HE; P = 0.02) and total antral follicles (AFC, 19.9 vs. 24.0 ± 1.0, LE vs. HE; P = 0.01). Overall, 96.5% of progeny had a CL at examination. Although AFC was correlated positively (r = 0.36; P < 0.01) with ovarian size (length x height), size did not differ between diets (357.4 vs. 348.4 mm^2 ± 12.6, LE vs. HE, P > 0.10). The AFC was similar between left and right ovary (r = 0.77; P < 0.01), but not between progeny and dam AFC (r = 0.05). Proportion of daughters pregnant to the 29-d breeding period did not differ between maternal diets (72.4 vs. 70.7 ± 0.5%, LE vs. HE) and was not influenced by prebreeding AFC. Results indicate that nutrient intake by first-parity heifers during early embryonic development may affect fetal ovarian development. USDA is an equal opportunity provider and employer.

Key Words: beef heifers, diets, developmental programming

1374 (M191) Effects of excessive energy intake and supplementation with chromium propionate on insulin resistance parameters in lactating dairy cows: I. Performance and weekly physiological measurements. T. Leiva[1], R. F. Cooke[2], F. G. Dantas[1], F. P. Santos[1], A. P. Brandao[1], J. Ranches[1], A. C. Aboin[1], and J. L. M. Vasconcelos[*1], *[1]UNESP–FMVZ, Botucatu, Brazil, [2]Oregon State University–EOARC Burns, Burns.*

The objective of this experiment was to compare performance and insulin resistance parameters in lactating dairy cows with adequate or excessive energy intake, as well as in lactating dairy cows with excessive energy intake receiving Cr-propionate supplementation. Seventeen primiparous and multiparous, lactating Holstein cows were ranked by parity, BW, and BCS, and assigned to 1 of 3 dietary treatments on d 0: 1) diet to meet their NE_l requirements without Cr supplementation (MAN; n = 5), 2) diet to exceed their NE_l requirements without Cr supplementation (HIGH; n = 6), and 3) HIGH with 2.5 g/d of Cr-propionate (HIGHCR; n = 8, with 10 mg of Cr/cow daily). Cows were maintained in a single group and offered corn silage for ad libitum consumption, but received a corn-based concentrate twice daily via individual self-locking head gates from d 0 to 210. Concentrate intake was formulated to provide 100% of daily NE_l requirements of MAN and 160% of daily NE_l requirements of HIGH and HIGHCR cows. Cow BW, BCS, and milk production were recorded weekly. Blood samples were also collected weekly, before and at 2 and 4 h after the morning concentrate feeding, and analyzed for serum glucose, insulin, and NEFA. Pre-prandial revised quantitative insulin sensitivity check index (RQUICKI) was determined using serum glucose, insulin, and NEFA concentrations obtained before concentrate feeding. No treatment effect was detected for BW change (P = 0.74), although BCS change from d 0 to 210 was greater (P = 0.02) in HIGH and HIGHCR compared with MAN. Milk production was similar between treatments (P = 0.92). Serum glucose concentrations and RQUICKI were also similar ($P \geq 0.68$) across treatments, whereas mean serum NEFA concentrations (pre-prandial samples only) were greater (P = 0.04) for MAN compared with HIGH and HIGHCR. Treatment × parity × day interactions were detected (P < 0.01) for serum insulin and insulin:glucose ratio. These parameters were generally greater ($P \leq 0.05$) for HIGH, intermediate for HIGHCR cows, and lesser for MAN beginning on d 70 of the experiment for multiparous cow, and beginning on d 168 for primiparous cows. In conclusion, lactating dairy cows consuming excessive energy experienced reduced insulin sensitivity compared to cows consuming adequate amounts of energy, characterizing a state of insulin resistance, whereas Cr-propionate supplementation alleviated this outcome. However, milk production was not impacted by excessive energy intake or Cr-propionate supplementation.

Key Words: chromium, dairy cows, energy intake, insulin resistance

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1375 (M192) Association of monocyte chemoattractant protein-1 and vascular endothelial growth factor in subcutaneous and visceral adipose tissue of early lactating dairy cows. S. Häussler[*1], C. Sacré[1], P. Friedrichs[2], S. Dänicke[3], and H. Sauerwein[1], *[1]University of Bonn, Institute of Animal Science, Germany, [2]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany.*

Adipose tissue (AT) is an endocrine organ, producing and secreting a wide range of adipokines, which are known to regulate metabolism and immune function. Metabolic adaptations during early lactation in dairy cows may be accompanied by changes in vascularization within AT to ensure nutrient supply for the adipocytes and/or to support the release of NEFA and

glycerol through increased blood flow. Small concentrations of monocyte chemoattractant protein-1 (MCP-1) can stimulate AT vascularization through the vascular endothelial growth factor (VEGF). Based on the virtual absence of macrophages in bovine AT, we hypothesized that MCP-1 in bovine AT is rather related to vascularization than to recruiting monocytes. Therefore, we aimed to investigate MCP-1 together with VEGF in different bovine AT. Primiparous German Holstein cows (n = 25) from a feeding trial were allocated to 3 groups that were slaughtered on Day 1 (n = 5), 42 and 105 (each n = 10) postpartum (P.p.). Subcutaneous (sc) AT (tailhead, withers, sternum) and visceral (vc) AT (omental, mesenteric, retroperitoneal) were sampled. Quantification of MCP-1 and VEGF was done by qPCR; mRNA abundance was summarized for sc and vc AT (means ± SEM). Differences throughout lactation were analyzed using the Kruskal–Wallis test, comparison between the sc and vc AT was done by Mann–Whitney-U test, and Spearman correlation was used to investigate the relation between the two variables (SPSS 21, $P \leq 0.05$). From d 1 to d 105 p.p. the mRNA abundance of MCP-1 and VEGF decreased 1.6-fold (P = 0.008) and twofold (P = 0.002), respectively, irrespective of AT depot. Comparing different AT across all days, MCP-1 mRNA abundance was 2.5-times ($P < 0.001$) and VEGFA mRNA abundance was 1.4-times ($P < 0.001$) higher in vc than in sc AT. Together with the previous finding that hardly no macrophages were observed in bovine AT, the positive correlation between MCP-1 and VEGF (r = 0.297; $P < 0.001$) indicates that MCP-1 may play a role in vascularization of bovine AT. High levels of MCP-1 and VEGF at the onset of lactation might increase vascularization to improve the essential blood flow in bovine AT right after calving. The higher expression of both MCP-1 and VEGF in vc than in sc AT might be due to the higher metabolic activity of vc AT.

Key Words: VEGF, MCP-1, adipose tissue

1376 (M193) Reactive oxygen metabolites (ROM) and advanced oxidation protein products (AOPP) as influenced by energy intake and niacin supplementation in the periparturient dairy cow.

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Increasing metabolic requirements related to late pregnancy, calving, and initiation of lactation may result in augmented production of ROM and, if not compensated by endogenous antioxidants, in oxidative stress. Niacin, as a precursor for NAD+ synthesis, upregulates the expression of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme in the pentose phosphate pathway and the principal source of cellular NADPH. Increased levels of NADPH decrease cellular ROM through regulating ROM-generating oxidases or by maintaining anti-oxidant enzymes in active form. We hypothesized that niacin by increasing NAD(p)H levels ameliorates oxidative stress in dairy cows and that this effect will differ depending on the portion of concentrate in the diet fed during late pregnancy and the first 100 d of lactation. Fifty-six Holstein cows were studied from d 42 ante partum (a.p.) through d 100 post partum (p.p.), and were assigned to 1 of 4 treatment groups (n = 14 each) in a 2 × 2 factorial arrangement of the level of concentrate feeding (high or low concentrate portion in the diet), with or without 24 g/d of niacin from d −42 to d 24. Blood was collected in weekly intervals (3-d intervals around calving). Derivatives of ROM (dROM; indirect photometric assessment of free radicals) were measured in all serum samples, and AOPP (marker of oxidative protein damage) in sera from d −42, -21, 14, 21, 28, and 35. Data were analyzed by the PROC MIXED of SAS using repeated measure analysis ($P < 0.05$). The model included the effects of diet, niacin, time, and two- and three-way interactions of main effects with time. Serum concentrations of dROM were not affected by concentrate level or niacin supplementation, and no interactions between concentrate and niacin, as well as no three-way interactions between treatments and time on dROM concentrations were observed. Serum concentrations of dROM were affected by time reaching a nadir around calving, and increasing immediately thereafter to relatively constant concentrations slightly higher than a.p. Serum AOPP was not different between treatment groups, but changed over time. Decreasing dROM values around calving might result from increased antioxidant protection. The two serum markers of oxidative stress selected herein yielded no effects of diet or niacin, however, markers of antioxidant status should additionally be considered.

Key Words: niacin, energy intake, oxidative status, dairy cow

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1377 (M194) The effect of aspirin on prostaglandin $F_{2\alpha}$ secretion in lactating dairy cows during the luteal phase of the estrous cycle.

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Approximately, 70 to 80% of total embryonic loss in dairy cattle occurs between Days 8 and 16 after artificial insemination (AI). Early embryonic loss may be due to the premature secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during Days 14–16 after fertilization. The objective of this study was to examine the effect of Aspirin, a non-steroid anti-inflammatory drug (NSAID), on $PGF_{2\alpha}$ secretion in lactating dairy cows by characterizing blood plasma prostaglandin metabolites (PGFM) and progesterone (P_4) during the luteal phase of the estrous cycle. Twenty-four lactating Holstein cows were synchronized

to ovulation. The ovulation was confirmed by ultrasonography (d 1). On d 14 and after detection of corpora lutea, cows were assigned randomly to receive Aspirin (total of 140 g) or no Aspirin (control) and the blood sample was obtained from each cow. Aapirin was given orally on d 14 (2×) and d 15 (1×), 12 h apart. On d 15, 6 h after the last dose of aspirin, hourly blood samples were taken for 6 h for PGFM concentrations. Daily blood samples were also collected (d 15 to 23) to examine P_4concentrations. One cow was eliminated from the study for having < 1 ng/mL P_4 on d 15. Analyses of repeated measures, using the mixed model procedure of SAS were utilized. The model included treatment, the repeated factor time, and treatment × time interaction. Cow was the random effect. On d 14, mean P_4 was > 1 ng/mL for all cows and it was similar between groups. Before treatment, there was no difference in mean PGFM concentrations between the groups, (203 vs. 224 ng/mL; SE ± 39 for aspirin and control, respectively). There was an effect of treatment and treatment × time on mean PGFM ($P < 0.05$). Mean PGFM concentrations were decreased ($P < 0.05$) 30 h after initiation of treatment and remained low for 5 h after last treatment, whereas they remained unchanged in the control. Overall, mean PGFM concentrations were 106 and 190 ng/mL (SE ± 33) for aspirin and control, respectively. Blood P_4 concentrations post-treatment were similar between the two groups (3.6 vs. 3.2 ng/mL; SE ± 0.6), but declined from d 15 to d 23 ($P < 0.01$). The study indicated that oral administration of aspirin treatment may suppress $PGF_{2\alpha}$ during d 14 to 15 after estrus and prevent early luteal tissue regression; however, no effect on P_4 was observed.

Key Words: dairy cows, NSAID, prostaglandin $F_{2\alpha}$

1378 (M195) Association between oxidative stress through excessive fat accumulation and the number of mitochondrial DNA copies in adipose tissue of dairy cows.

L. Laubenthal[*1], L. Locher[2], J. Winkler[3], U. Meyer[3], J. Rehage[2], S. Dänicke[3], H. Sauerwein[1], and S. Häussler[1], *[1]University of Bonn, Institute of Animal Science, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany.*

In the transition period, overconditioned cows usually have more problems to adapt to the needs of lactation than leaner cows and are thus more prone to health problems. The mitochondrial DNA (mtDNA) copy number reflects the abundance of mitochondria in a cell and may change under different energy requirements and physiological conditions. Increased metabolic demands as well as increased mtDNA copies per cell are associated with elevated oxidative stress. However, the association between oxidative stress through excessive fat accumulation and mtDNA copy numbers in bovine adipose tissue (AT), being considered as a major contributor to systemic oxidative stress, has not been investigated so far. We hypothesized that the mtDNA copy number in AT will increase concomitant with oxidative stress [assessed by quantifying the derivates of reactive oxygen species (dROM)] during fat accretion in cows. Eight non-lactating, non-pregnant pluriparous German Holstein cows (age: 4 to 6 yr) were fed diets with increasing portions of concentrate feed during the first 6 wk of the experiment until 60% were reached, which was maintained for 9 wk. Within this period cows had an average body weight (BW) gain of 243 ± 33.3 kg. Blood samples were collected monthly and dROM were photometrically quantified in serum using N,N,diethyl-1,4-phenylendiamine as chromogen. Biopsies from the subcutaneous tailhead AT were taken every 8 wk and immediately snap frozen for genomic DNA isolation. The number of mtDNA copies/cell was measured by a multiplex quantitative PCR using β-globin as reference gene. Data (mean ± SEM) for mtDNA copies and dROM as well as for BW were analyzed using non-parametric tests or repeated measurement ANOVA, respectively. Correlations were calculated using the Spearman (r) correlation coefficient. Throughout the fat accumulation period mtDNA copies/cell and dROM increased fourfold (329 ± 57.5 to 1385 ± 160; $P = 0.002$) and 2.5-fold (49.9 ± 9.24 to 125 ± 16.0 µg H_2O_2equivalents/mL; $P = 0.003$), respectively. We observed a positive correlation between mtDNA copy numbers and BW ($r = 0.596$, $P = 0.003$) and dROM ($r = 0.550$, $P = 0.005$). Increased mtDNA copies in AT might be an adaptation in response to oxidative stress that evolves from excessive fat accumulation in overconditioned cows. It is known, that mtDNA copy numbers increase as a compensatory response mechanism to mtDNA damage. Therefore, increased numbers of mitochondria and thus increased numbers of mtDNA copies per cell might then amplify the production of ROM leading to further mtDNA damage.

Key Words: mtDNA, oxidative stress, fat accumulation

1379 (M196) Telomere length shortening in response to an excessive fat accumulation in subcutaneous adipose tissue of dairy cows.

L. Laubenthal[*1], L. Locher[2], J. Winkler[3], U. Meyer[3], J. Rehage[2], S. Dänicke[3], H. Sauerwein[1], and S. Häussler[1], *[1]University of Bonn, Institute of Animal Science, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany.*

With the onset of lactation, overconditioned cows mobilize more body tissue than thin cows and are prone to develop metabolic disorders. The metabolic stress in cows with higher body condition score (BCS) before calving and greater loss of BCS after calving comprises oxidative stress. Increased production of reactive oxygen metabolites may damage cells and accelerate telomere shortening which serves as biological marker for age and stress-related conditions. Herein we aimed to investigate the telomere length (TL) in subcutaneous

adipose tissue (AT) during exemplarily induced excessive fat accumulation in cows. We hypothesized that TL is associated with the production of derivates of reactive oxygen metabolites (dROM), as indicator for oxidative stress, in overconditioned cows. Eight non-lactating, non-pregnant German Holstein cows (4 to 6 yr) received diets with increasing concentrate feed proportions (0 to 60% of the total daily dry matter intake) during the first 6 wk of the experiment which was maintained for 9 wk. The BCS (5-point scale) increased from 2.3 ± 0.12 to 4.57 ± 0.14 and the average body weight (BW) gain was 243 ± 33.3 kg. Blood samples were taken monthly for photometric quantification of dROM in serum using N,N,diethyl-1,4-phenylendiamine as chromogen. Subcutaneous AT from tailhead was collected at the beginning and of the experiment and after 15 wk. Samples were snap frozen for isolation of genomic DNA. A multiplex quantitative PCR was used to analyze the relative quantity of telomere (qT) products compared with β-globin products which served as reference gene to estimate TL in AT. Data (mean ± SEM) for TL, BCS and BW as well as for dROM were evaluated using repeated measurement ANOVA or non-parametric tests, respectively. Correlations were calculated using the Spearman (*r*) and Pearson (*r*) correlation coefficient. Relative qT decreased throughout the experiment (51.8 ± 3.26 to 43.6 ± 1.76; $P = 0.01$) whereas dROM increased more than two-fold ($P = 0.003$). Shorter TL were correlated with BCS ($r = -0.586$, $P = 0.017$), BW ($r = -0.653$, $P = 0.008$) and dROM ($r = -0.596$, $P = 0.015$). Increasing dROM indicating oxidative stress were observed in overconditioned cows. Fat accumulation was accompanied by reduced TL in bovine AT. Shortening of telomeres might indicate fibrosis and would thus result in AT dysfunction which might compromise the adaptive capability of AT to the needs of lactation in overconditioned cows.

Key Words: telomere length, adipose tissue, oxidative stress

1380 (M197) Pregnancy per AI of high producing Holstein cows treated with norgestomet ear implant or progesterone intravaginal device.

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The aim of present study was to compare pregnancy per AI (p/AI) of high producing Holstein cows subjected to timed artificial insemination (TAI) using a new or previously used progesterone intravaginal device or a Norgestomet ear implant. In Experiment 1, 359 cows (173 primiparous and 186 multiparous) received 2 mg estradiol benzoate i.m. (Gonadiol, MSD Animal Health, Brazil) at random days of the estrous cycle (d 0) and were homogenously distributed in two groups. Cows on group NEW-PROGESTERONE received a new progesterone releasing intravaginal device (CIDR, Zoetis, Brazil), while cows on group NEW-NORGESTOMET received a new Norgestomet ear implant (Crestar, MSD Animal Health, Brazil). On d 8, 500 mg Cloprotenol (Ciosin, MSD Animal Health, Brazil) and 1 mg estradiol cypionate (E.C.P., Zoetis, Brazil) were administered and the ear implants or intravaginal devices were removed. On d 10, 100 μg gonadorelin (Fertagyl, MSD Animal Health, Brazil) was administered and TAI was performed 10 h later. In Experiment 2, 293cows (146 primiparous and 147 multiparous) were subjected to the same experimental design described above except for using implants and devices previously used for 8 d (USED-PROGESTERONE = 146 cows vs. USED-NORGESTOMET = 147 cows). The experimental period began in May and ended in September 2012. Statistical analyses were performed using logistic regression (*P*ROC GLIMMIX of SAS). There were no interactions for treatment and parity, farm, and times in bred. The P/AI 30 d after TAI was similar ($P > 0.10$) between groups NEW-PROGESTERONE [31.6% (50/158)] and NEW-NORGESTOMET [35.3% (71/201)], and between groups USED-PROGESTERONE [35.6% (52/146)] and USED-NORGESTOMET [36.7% (54/147)]. These results indicate that both CIDR and Crestar, either new or previously used for 8 d, can be used for TAI treatments producing similar P/AI.

Key Words: norgestomet, pregnancy per AI, Holstein

1381 (M198) Telomere length in different visceral and subcutaneous adipose tissue depots of overconditioned cows.

L. Laubenthal[*1], L. Locher[2], J. Winkler[3], U. Meyer[3], J. Rehage[2], S. Dänicke[3], H. Sauerwein[1], and S. Häussler[1], *[1]University of Bonn, Institute of Animal Science, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany.*

Telomeres are short and repetitive sequences of the chromosomes which shorten with every cell-division. Therefore telomere length (TL) is considered as a biological marker for aging and cell proliferation depending on the tissue type. Increasing oxidative stress in response to nutrient surplus, e.g., in overconditioned dairy cows at the onset of lactation, accelerates telomere attrition. Adipose tissue (AT) is mobilized in early lactating cows to cope with the nutrient demands for milk synthesis. The different AT depots are divided into visceral (vc) and subcutaneous (sc) depots which exhibit different metabolic functions and cellular composition. We hypothesized that TL shortening within AT is depot specific in overconditioned cows due to different metabolic activities of sc and vcAT. Herein, we aimed to characterize the TL in seven different fat depots after rapid, diet-induced fat accumulation in cows. Eight German Holstein

cows (non-lactating, non-pregnant, age: 4 to 6 yr) were gradually adapted to a high-energy ration by increasing the portion of concentrate in the ration from 0 to 60% of daily dry matter intake within 6 wk. Animals were fed the 60% diets for further 10 wk and were then slaughtered; tissue samples from scAT (sternum, withers and tailhead) and vcAT (*P*ericardial, mesenteric, omental and retroperitoneal) were collected and snap frozen for further analyses. After isolation of genomic DNA, a multiplex quantitative PCR was used to analyze the relative quantity of telomere (qT) products compared with β-globin products which served as reference gene to estimate TL in AT. Differences between qT in the single AT depots were analyzed using the Student's *t* test (SPSS; mean ± SEM). Mesenteric AT exhibited 1.3-fold lower qT compared to omental (59.1 ± 7.4; $P = 0.01$) and pericardial (57.7 ± 7.9; $P = 0.004$) depots and 1.2-fold lower qT than retroperitoneal (53.5 ± 4.9; $P = 0.01$) AT. In scAT depots, fat from withers displayed 1.3-fold higher qT values than sternum (47.3 ± 3.5; $P = 0.005$) AT and 1.1-fold higher qT compared to tailhead (54.1 ± 8.2; $P = 0.05$) fat. Although vcAT is known to have a higher metabolic activity than scAT in dairy cows, we did not observe any differences in TL when comparing all sc versus all vcAT. Depot specific differences of TL might nevertheless be a hint to depot specific roles

Key Words: telomere length, adipose tissue, dairy cow

1382 (M199) Liveability of buck spermatozoa after cold storage using egg yolk citrate extender.

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Preservation and extension of West African Dwarf (WAD) buck semen has been a challenge, because AI centres are far from farms where they are needed. This study was performed to investigate the liveability of buck spermatozoa extended with egg yolk citrate of West African dwarf goat buck spermatozoa. Twenty matured bucks were used for this experiment. Semen from these bucks was collected by means of artificial vagina after which evaluation was done. Buffer and extender were prepared using egg Yolk Citrate to preserve the semen and it was stored at 5°C in a refrigerator. Data were collected for 5 d at 0, 24, 48, 72, and 96 h on sperm concentration, percentage liveability, percentage dead, intact, damage, missing acrosome and sperm morphology. Data were analyzed using Statistical Package for Social Sciences (SPSS). Results show that the percentage sperm liveability at 0, 24, 48, 72, and 96 h were 57.50, 56.30, 54.03, 51.27, 47.00 respectively indicating a decrease with time. However, the percentage sperm dead increased with time: 42.50, 43.68, 45.97, 48.73, and 50.92% for 0, 24, 48, 72, and 96 h, respectively. The sperm motility (percentage) also reduced as the length of storage increased. The morphological characteristics of the spermatozoa also decreased with increase in the length of storage. It was concluded that liveability of sperm reduced as the storage time increased in West African Dwarf buck even with egg yolk citrate extender.

Key Words: egg yolk citrate, liveability, goat buck

1383 (M200) Bedding surface does not alter circulating patterns of cortisol, corticosteroid-binding globulin, or free cortisol index in preweaned Jersey calves.

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Previous research found no significant treatment differences in behavior or performance of Jersey calves residing at one farm when housed individually in hutches bedded with gravel ($n = 11$), sand ($n = 12$), or rubber mat ($n = 11$) from birth to 10 wk of age. The aim of the present study was to further examine the effects of these bedding surfaces on plasma total cortisol (CORT) and corticosteroid binding globulin (CBG) concentrations, and free cortisol index (FCI) analyzed from blood samples (10 mL) collected weekly from each of the calves during this earlier study beginning within 24 h of birth. Calves were provided 4 L of waste milk 1x/d from birth to 6 wk of age, and 2 L of waste milk 1x/d with calf starter and water ad libitum during the remaining 4 wk. Serum CORT was analyzed using a commercial RIA kit procedure and bovine CBG by an indirect ELISA developed and validated in our laboratory. The FCI was calculated using the ratio of serum CORT (nmol/L) to CBG (mg/L) concentrations. Data were analyzed by PROC MIXEDs in SAS 9.3 for repeated measures. No differences were found ($P > 0.10$) in CORT, CBG or FCI among treatment groups at any time point measured. Age-related changes ($P < 0.01$) among the three variables were found such that CORT was greatest (132.7 ± 32.0 nmol/L) at birth, reached a nadir (2.1 ± 0.5 nmol/L) at 6 wk of age, and increased ($P < 0.05$) to 8.0 ± 2.0 nmol/L by 9 wk of age. Serum CBG increased ($P < 0.01$) between birth and 1 wk of age (1.1 vs. 1.4 mg/L; SE = 0.1) and remained unchanged until the calves reached 6 wk of age at which time CBG was at its lowest (0.8 ± 0.1 mg/L; $P < 0.05$). Subsequently, CBG increased (1.4 ± 0.1 mg/L; $P < 0.05$) at 7 wk of age where it persisted thereafter. The FCI reflected the changes observed in CORT. The data are consistent with our previous results indicating that any of these bedding types may be used without compromising the welfare of preweaned Jersey calves. Whether the changes in CORT, CBG and calculated FCI noted here are age and/or diet related await further study.

Key Words: dairy calf, free cortisol index, bedding

1384 (M201) Niacin increases chemerin mRNA abundance in differentiated bovine preadipocytes in vitro. C. Kopp[1], H. Khalilvandi-Behroozyar[1,2], H. Sauerwein[*3], and M. Mielenz[1,4], *[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Germany, [2]Dep. of Animal Science, Urmia University, Iran, [3]University of Bonn, Institute of Animal Science, Germany, [4]Leibniz Institute for Farm Animal Biology (FBN), Institute of Nutritional Physiology, Dummerstorf, Germany.*

Chemerin is thought to be involved in controlling immune responses as a chemoattractant for antigen-presenting cells and has anti-inflammatory as well as pro-inflammatory functions; the latter are linked with obesity and insulin resistance. It is highly expressed as prochemerin in liver and adipose tissue and was recently identified as adipokine that regulates adipogenesis and adipocyte metabolism. The role of chemerin on glucose metabolism is controversially discussed. However, chemerin enhances insulin-stimulated glucose uptake, insulin signaling and increases insulin sensitivity in murine adipocytes. Niacin (NA) is an antilipolytic and lipid-lowering compound which is used since decades to treat dyslipidemia in humans. Furthermore, NA affects the secretion of several adipokines (e.g., adiponectin) and improves insulin sensitivity in humans. These effects are mediated through binding the G protein coupled receptor 109A (GPR109A). Our objective was thus to examine both, the effect of NA on chemerin mRNA expression in bovine adipocytes and the involvement of GPR109A in NA sensing. A primary cell culture system using differentiated bovine preadipocytes was established. Subcutaneous adipose tissue was collected from five Holstein-Friesian cows. Stromal-vascular cells were isolated, pooled and seeded at 2500 cells/cm^2. Preadipocytes after 12 d of differentiation were used. After serum starvation (4 h), cells were incubated either with 100 ng/mL pertussis toxin (*P*TX), a non-selective G protein uncoupling agent, or PBS for 16 h to characterize the NA mediating pathway. Cells were then treated with NA (10 or 15 μM) for 12 or 24 h or with PBS as controls, respectively. Chemerin mRNA abundance was quantified by qPCR. Data normalization was based on five stable reference genes. Statistical analyses were performed using ANOVA with Bonferroni post hoc tests ($P < 0.05$). The mRNA abundance of chemerin was increased 3.3-fold compared to controls after treatment with 10 μM NA for 24 h ($P = 0.006$). Treatment for 12 h or with 15 μM NA showed no difference in the mRNA abundance of chemerin. Pre-incubation with PTX abolished the observed NA-induced increase of chemerin mRNA abundance. Our results showed a NA-stimulated increase of chemerin mRNA expression in differentiated bovine adipocytes, which may point to improved insulin sensitivity by NA. Due to the annihilated increase after PTX treatment, we suggest that GPR109A mediates the effect of NA.

Key Words: chemerin, niacin, bovine adipocytes

1385 (M202) Macrophage infiltration into subcutaneous adipose tissue in overconditioned cows after excessive fat accumulation. S. Häussler[*1], L. Laubenthal[1], L. Locher[2], J. Winkler[3], U. Meyer[3], J. Rehage[2], S. Dänicke[3], and H. Sauerwein[1], *[1]University of Bonn, Institute of Animal Science, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany.*

Adipose tissue (AT) secretes adipokines regulating both metabolism and immune function. In monogastrics, diet-induced obesity is associated with changing adipokine profiles and increased macrophage (Ms) infiltration. However, in early lactating dairy cows we found virtually no Ms infiltration in different AT depots; non-lactating overconditioned heifers had increased accumulation of Ms in AT related with larger adipocytes, albeit in low total numbers. We hypothesized that the portion of Ms in bovine AT will remain small, even if fat is excessively accumulated in short time. Therefore we aimed to investigate the Ms infiltration in subcutaneous (sc)AT after rapid, diet-induced fat accumulation in cows. Eight non-pregnant, non-lactating pluriparous German Holstein cows, were adapted to diets with increasing concentrate feed proportions (from 0 to 60% of total dry matter intake) during the first 6 wk of the experiment which was maintained for 9 wk at 60% concentrate feeding. The body condition score (5-point scale) increased from 2.31 ± 0.12 to 4.53 ± 0.14, and the body weight increased from 540 ± 20 kg to 792 ± 29 kg. Three biopsies were taken every 8 wk of the entire experimental period from scAT of the tailhead region. Immunohistochemistry was performed on cryosections (12 μm) using the Ms-specific marker CD68. Bovine lymph nodes were used for positive and negative controls. The number of Ms and adipocytes per mm^2 were counted (100-fold magnification; 10 fields per sample). The portion of Ms was calculated from the mean number of positive stained cells/mean number of total adipocytes × 100. In total, 12 out of 23 samples yielded CD68-positive stainings. Considering the different time points, 5 out of 7, 5 out of 8 and 2 out of 8 samples were Ms-positive at the beginning, the middle and the end of the experiment, respectively. However, the average portion of Ms was only marginal: 3.7 ± 3.0% (n = 7) at the beginning, 0.9 ± 0.3% (n = 8) at the middle and 0.5 ± 0.3% (n = 8) at the end of the experiment. Thus even a rapid and pronounced increase of fat mass was not accompanied by Ms infiltration into subcutaneous AT. In consideration of the virtual absence of Ms in AT in earlier studies about cows during the first weeks of lactation, and the low portion of Ms in overconditioned heifers and in the present study, we assume that Ms infiltration is of no importance for bovine AT.

Key Words: macrophages, adipose tissue, cow

1386 (M203) Rumen-protected methionine, histidine, and slow-release urea: Effects on plasma 3-methylhistidine and ubiquitin proteasome-related gene expression in skeletal muscle of dairy cows receiving a diet deficient in metabolizable protein. H. Sadri*[1], F. Giallongo[2], A. N. Hristov[2], C. Lang[3], J. Werner[2], C. Parys[4], B. Saremi[5], and H. Sauerwein[1], *[1]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Germany, [2]Dep. of Animal Science, Pennsylvania State University, University Park, State College, [3]Dep. of Cellular and Molecular Physiology, Hershey Medical Center, Penn State College of Medicine, [4]Evonik Industries AG, Hanau, Germany, [5]Evonik Industries AG, Hanau, Germany.*

Skeletal muscle, the largest organ in vertebrates, plays a major role in homeostasis. The ubiquitin-proteasome system (UPS) is regarded as the main proteolytic pathway in muscle. It requires the coordinated reactions of three enzymes including ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin_ligases (E3). We hypothesized that supplementation of diets deficient in metabolizable protein (MP) with slow-release urea or rumen-protected (RP) Met and His will affect the gene expression of UPS-related factors in skeletal muscle of dairy cows in support of decreased proteolysis. Sixty Holstein cows were blocked based on DIM and milk yield and randomly assigned to one of five diets: MP-adequate diet (AMP); MP-deficient diet [DMP; 5% below the requirements (NRC, 2001)]; DMP supplemented with slow-release urea as Optigen (Alltech Inc., Nicholasville, KY; DMPO); DMPO supplemented with RPMet (Mepron; Evonik Industries AG, Hanau, Germany; DMPOM); and DMPOM supplemented with RPHis (Balchem Corp., New Hampton, NY; DMPOMH). The experimental period was 10 wk, with first 2 wk as covariate period. Muscle biopsies were collected from *M. longissimus dorsi* during the last week of the experiment. The mRNA abundance of the following UPS-related target genes was quantified by qPCR: F-box protein 32 (FBXO32), muscle ring-finger protein 1 (MuRF-1), both being muscle-specific E3 ubiquitin ligases, ubiquitin-like modifier activating enzyme 1 (UBA1), and ubiquitin-conjugating enzymes (UBE2G1 and UBE2G2). Data were normalized based on the geometric mean of the 4 most stable reference genes: lipoprotein receptor-related protein 10, marvel domain containing 1, RNA polymerase II, and emerin. The concentration of 3-methylhistidine (3-MH) as marker of muscle catabolism was measured in plasma samples collected at the end of the experiment. Data were analyzed by the PROC MIXED of SAS. With the exception of MuRF-1, the mRNA abundance of the target genes was not affected by treatment. In DMP cows, about twofold more (P = 0.05) MuRF-1 mRNA than in DMPO was observed. Plasma 3-MH did not differ among treatments. In conclusion, the UPS seemed to be upregulated at the level of the mRNA during protein deficiency but this effect was apparently not sustained to increased 3-MH plasma concentrations.

Key Words: rumen-protected amino acid, ubiquitin-proteasome system, dairy cow

1387 (M204) Antioxidant supplementation during in vitro maturation increased oocyte mitochondrial membrane potential and bovine embryo development. B. C. D. S. Leão*, N. A. D. S. Rocha Frigoni, P. C. Dall'Acqua, and G. Z. Mingoti, *University of Sao Paulo State (UNESP), Araçatuba, Brazil.*

This study evaluated the effects of bovine oocytes IVM medium supplementation with intracellular (cysteine and cysteamine) and extracellular (catalase) antioxidant on the oocyte competence, based on evaluation of nuclear maturation rates, occurrence of apoptosis, mitochondrial membrane potential and the subsequent embryonic development. Oocytes were matured during 22h in TCM-199 medium with bicarbonate, hormones and 10% FCS, without supplementation (Control group) or supplemented with: 0.6 mM cysteine associated with 100 µM cysteamine (C+C group); 100 UI catalase (CAT group); or 0.6 mM cysteine associated with 100 µM cysteamine and 100 UI catalase (C+C+CAT group), at 38.5°C and 5% CO_2 in air. A sample of matured and immatured oocytes were stained with 500 nM of the fluorescent probe MitoTracker Red (CMXROs, Molecular Probes, Invitrogen, Oregon, USA) or TUNEL (In Situ Cell Death Detection Kit, Fluorescein, Roche Applied Science, IN, USA), to evaluate mitochondrial membrane potential (n = 344) and apoptotic index (n = 565), respectively. Stained oocytes were evaluated under an epifluorescence inverted microscope (excitation 579/510–550nm and emission 599/590nm, respectively for MitoTracker and TUNEL) and the mitochondrial membrane potential (quantified in arbitrary fluorescence units- AFU) were measured by Q-Capture Pro image software. The intensity values of the fluorescence signal obtained from oocytes were subtracted from mean values of "background" in the images. The rest of oocytes were submited to IVF and presumptive zygotes were IVC in SOFaa, at 38.5°C and 5% CO_2 in air, for 7 d. Cleavage and blastocyst rates were evaluated at 72 and 168 hpi, respectively. Were made 10 replicates with 50 oocytes per dish, and it was considered the experimental unit. The mitochondrial membrane potential was analyzed by ANOVA followed by Tukey's test and percentage of apoptosis, cleavage and blastocyst rates by Chi-square test ($P < 0.05$). Data are presented as mean ± SEM. The AFU for membrane mitochondrial potential were 1.00 ± 0.05^{a} (immature oocytes), 1.60 ± 0.05^{b} (Control), 0.94 ± 0.03^{a} (C+C), 1.41 ± 0.05^{c} (CAT) and 1.81 ± 0.07^{d} (C+C+CAT). The oocyte maturation rates were 0.0% (immature) 76.7% ± 1.7 (Control), 80.3% ± 4.1 (C+C), 80.5% ± 5.2 (CAT) and 78.2% ± 1.1 (C+C+CAT), and the percentage of apoptotic oocytes were $1.55\%^{a}$ (immature), $5.83\%^{ab}$ (Control), $5.45\%^{ab}$ (C+C),

1.92%[a] (CAT) and 10.78%[b] (C+C+CAT). Cleavage and embryo development were 72.5%[a] and 28.2%[a] (Control), 75.7%[a] and 31.1%[a] (C+C), 75.4%[a] and 33.3%[a] (CAT) and 73.1%[a] and 46.2%[b](C+C+CAT). In conclusion, supplementation with association of cysteine, cysteamine and catalase improved blastocyst development that can be associated with the increase mitochondrial membrane potential and oocyte competence.

Key Words: mitochondrial membrane potential, antioxidant, in vitro maturation, blastocyst development

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1388 (M205) Hepatic and adipose mRNA expression of genes related to FGF21 in response to conjugated linoleic acid (CLA) supplementation in dairy cows during early lactation. H. Sadri[*1], S. Dänicke[2], J. Rehage[3], and H. Sauerwein[1], *[1]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Germany, [2]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany, [3]University for Veterinary Medicine Foundation, Hannover, Germany.*

The hepatokine fibroblast growth factor 21 (FGF21), a member of the FGF super-family, is emerging as an important regulator of metabolism. It is induced by fasting, ketogenic diets, and by peroxisome proliferator-activated receptor (PPAR) agonists. The glycemic and insulin sensitizing effects of FGF21 are mediated through the adipokine adiponectin that is induced by FGF21. We recently reported that the postpartal increase of adiponectin is attenuated in dairy cows receiving the PPAR-agonistic CLA and thus hypothesized that supplementation of cows with CLA in early lactation will affect the expression of FGF21, of the FGF receptor (FGFR) isotopes FGFR1c and FGFR2c, and of the essential co-receptor b-Klotho in liver and adipose tissue. German Holstein cows receiving 100 g/d CLA (n = 11; Lutrell pure, BASF, Germany; each 12% of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA) or a control fat supplement (Silafat, BASF; CTR; n = 10) from DIM 1 to 182 were biopsied (liver and subcutaneous fat) on d -21, 1, 21, 70, and 105 relative to calving. The target mRNAs were quantified by real-time RT-PCR. Data were analyzed by the PROC MIXED of SAS 9.2. Hepatic FGF21 and FGFFR2c mRNA abundance were affected by time ($P < 0.05$) and by treatment (FGF21: $P = 0.08$; FGFR2c: $P < 0.01$). In CTR cows, a 4.5-fold increase in FGF21 mRNA was observed from d -21 to d 21, followed by a decline to nearly prepartum values by d 105. The CLA cows had less FGF21 mRNA than the CTR cows. The mRNA abundance of FGFR2c increased during lactation in CTR but not in CLA; the greatest difference (1.5-fold) between the CLA and the CTR group was observed on d 70. The mRNA abundance of b-Klotho in the liver and adipose tissue changed over time ($P < 0.05$), while CLA had no effect. Expression of FGFR1c mRNAin adipose tissue was neither affected by time nor by treatment. The observed upregulation of hepatic FGF21 expression during the first 3 wk in liver supports a role of FGF21 in metabolic regulation and nutrient partitioning during early lactation. The inhibiting effects of CLA supplementation on hepatic mRNA expression of FGF21 and its receptor might promote glucose availability for the mammary gland by reducing peripheral insulin sensitivity.

Key Words: FGF21, CLA, liver and fat tissues, dairy cow

1389 (M206) Effect of melatonin (MEL) or maternal nutrient restriction on vascularity of the ovine placenta. K. A. Vonnahme[*1], M. E. Wilson[2], S. Romero[1], S. T. Dorsam[1], J. Haring[1], P. P. Borowicz[1], D. A. Redmer[1], and C. O. Lemley[3], *[1]North Dakota State University, Fargo, ND, [2]West Virginia University, Morgantown, WV, [3]Mississippi State University, Starkville.*

Objectives were to determine placental vascularity following dietary MEL treatment in a maternal nutrient restriction model. A second experiment was performed to assess MEL receptor dependent modulation in placental vascularity. For Exp. 1, 31 ewes were supplemented with 0 (CON) or 5 mg of MEL per d and allocated to receive 100% (adequate fed; ADQ) of daily nutrient requirements or a 40% restriction in total feed intake (RES) from d 50 to 130 of gestation. Placentomes were collected on d 130 of gestation. For Exp. 2, 14 ewes were fitted with Alzet mini osmotic pumps and infused with vehicle, MEL, or MEL receptor 1 and 2 antagonist (luzindole, LUZ) from d 62 to 90 of gestation. Placentomes were collected on d 90. Placentomes from both Exp. 1 and 2 were fixed, paraffin embedded, and examined for capillary area density (CAD, total capillary area as a proportion of tissue area), capillary number density (CND, total number of capillaries per unit of tissue area), capillary surface density (CSD, total capillary circumference per unit of tissue area), and average cross-sectional area per capillary (APC) for both maternal (caruncular) and fetal (cotyledonary) compartments. For Exp. 1, a MEL x nutritional plane interaction ($P = 0.02$) was observed for caruncle CAD, which was decreased in MEL-RES vs. CON-RES (0.145 vs. 0.269 ± 0.031). A MEL x nutritional plane interaction ($P = 0.01$) was observed for caruncle APC, which was decreased in MEL-RES vs. all other groups (95.8 vs. 171.7, 195.2, 165.6 ± 18.2 μm^2). Cotyledon CND tended to be higher in MEL versus CON, while a tendency ($P = 0.06$) for a MEL x nutritional plane interaction was observed for cotyledon APC, which was decreased in MEL-RES vs. all other groups (85.9 vs. 122.3, 122.2, 124.9 ± 10.2 μm^2). For Exp. 2, LUZ infusion tended ($P = 0.08$) to increase caruncle CAD (0.171 vs. 0.083 ± 0.026) and APC (129.2 vs. 48.1 ± 23.4 μm^2) compared to vehicle infusion. Measurements of cotyledon vascularity were not different ($P > 0.10$) across infusion treatments. *Supported in part by USDA-NIFA-AFRI grant 2011–67012–30683.*

Key Words: placenta, sheep, vascularity

1390 (M207) Follicle-stimulating hormone stimulates β-catenin via protein kinase B in granulosa cells.
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Follicle-stimulating hormone regulation of ovarian estradiol production requires involvement of β-catenin (CTNNB1), a transcriptional co-factor. In cultured bovine granulosa cells, FSH treatment increases protein abundance of CTNNB1 as well as protein kinase B (AKT), a molecule known to regulate components of the CTNNB1 degradation complex. However, whether FSH induction of CTNNB1 is through direct modulation of AKT remains to be determined. To elucidate the effects of AKT signaling on CTNNB1 induction and subsequent estradiol production, bovine granulosa cells were cultured in the presence or absence of known AKT activators and inhibitors. Total protein was collected for analysis by Western blot and culture medium for estradiol quantification by RIA. Values were analyzed using one-way ANOVA procedure of SAS. To investigate specific contributions of AKT to CTNNB1 accumulation, granulosa cells were treated with IGF-1, a well-established AKT activator, in the presence or absence of FSH. Granulosa cells treated with FSH, IGF-1, and FSH+IGF-1 increased (0.68-fold) CTNNB1 accumulation compared to controls ($P = 0.09$; $n = 6$). Estradiol medium concentrations increased ($P = 0.001$; $n = 4$) in cells treated with FSH, IGF-1, and FSH+IGF-1 (166, 379, and 397%, respectively) compared to non-treated controls. A subsequent study utilizing lithium chloride (LiCl), another activator of the AKT pathway, demonstrated similar results. Granulosa cells were cultured in the presence or absence of LiCl with and without FSH. Consistent with data from IGF-1 treated cells, LiCl, FSH, and FSH+LiCl increased CTNNB1 accumulation (0.79-fold) compared to non-treated controls ($P = 0.03$; $n = 3$). In contrast, inhibition of AKT signaling with LY294002 suppressed ($P = 0.02$; $n = 3$) CTNNB1 by 1.93-fold compared to controls. Co-treatment of FSH and LY294002 reduced the ability of FSH to increase CTNNB1 ($P = 0.03$). LY294002 treatment reduced estradiol medium concentrations 1.14-fold when compared to control levels, while co-incubation of FSH+LY294002 and FSH treatment induced estradiol to similar levels above controls ($P = 0.0001$; $n = 4$). Results demonstrate activation of AKT is required for CTNNB1 accumulation and estradiol production in bovine granulosa cells. These data suggest that induced CTNNB1 accumulation by FSH and IGF1 activation of AKT may be the lynch pin molecule responsible for FSH and IGF1 synergistic steroidogenic activity.

Key Words: protein kinase B, β-catenin, granulosa cells

1391 (M208) Ileal tight junction gene expression in glucagon-like peptide 2-treated dairy bull calves with and without coccidiosis.
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Intestinal gut permeability is partially regulated by the intestinotrophic hormone glucagon-like peptide 2 (GLP-2). Specifically, disease models in mice and human cell lines have implicated GLP-2 in the regulation of the tight junction milieu within the intestinal tract. Therapeutic administration of GLP-2 ameliorates gastrointestinal lesions and mechanical damage in rodent models of ileitis, porcine models of bowel resection, and humans with small bowel disease. These damages can reduce nutritional absorption and increase bacteria in the blood stream, both of which are in part attributed to tight junction protein disregulation. The purpose of the present study was to determine whether GLP2 therapy alters tight junction gene expression in ileum of neonatal dairy calves with scours induced by *Eimeria bovis* infection. Neonatal Holstein bull calves ($n = 18$) were separated into 4 treatment groups; uninfected-buffer ($n = 5$), uninfected-GLP-2 ($n = 4$), *E. bovis*-buffer ($n = 5$), and *E. bovis*-GLP-2 ($n = 4$). On d 0, calves in the *E. bovis*-buffer and *E. bovis*-GLP-2 groups were orally dosed with 200,000 sporulated oocysts of *E. bovis*. For 10 d (d 18 to d 27 of the study), uninfected-GLP-2 and *E. bovis*-GLP-2 calves were injected every 12 h with 50 µg of GLP-2/kg BW and at d 28 calves were sacrificed for collection of intestinal tissues for RNA extraction. Tight junction genes including CAR, CLDN1, CLDN2, CLDN4, F11R/JAMA, JAM2/JAMB, and TJP1/ZO-1 were evaluated in ileal epithelium by realtime quantitative PCR. Relative mRNA expression normalized to ATP5B and HMBS revealed greater expression of TJP1/ZO-1 in *E. bovis*-infected calves compared to uninfected calves ($P \leq 0.03$) and an *E. bovis* infection vs. GLP2 treatment interaction ($P \leq 0.004$). Expression of all other genes did not differ ($P > 0.05$) with GLP-2 treatment or *E. bovis*infection status. The lack of significant findings among the majority of genes investigated may be explained by large variation among individuals or the timing of sample collection relative to infection status and GLP-2 treatment. Alternatively, tight junctions may not be regulated at the RNA level, whereby analysis at the protein level may be more appropriate. Finally, the cellular localization of tight junction proteins may become altered during infection and with GLP2 treatment due to post-translational modifications or other regulating molecules. A more in-depth histological study could reveal significant findings that analysis of RNA levels alone cannot detect.

Key Words: dairy cattle, ileum, tight junction

1392 (M209) Effects of heat stress on the metabolic transcriptional profile of peripheral tissues in growing pigs. M. Sanz Fernandez[*1], J. S. Johnson[1], J. T. Seibert[1], R. L. Boddicker[1], S. C. Isom[2], L. Cox[2], J. W. Ross[1], R. P. Rhoads[3], and L. H. Baumgard[1], *[1]Iowa State University, Ames, [2]Utah State University, Logan, [3]Virginia Tech, Blacksburg.*

Heat stress (HS) alters postabsorptive metabolism and nutrient partitioning, independently of reduced nutrient intake. Surprisingly, despite marked hypophagia, heat-stressed animals have reduced plasma non-esterified fatty acids (NEFA), and decreased sensitivity to lipolytic signals. In addition, HS increases plasma insulin parameters in a variety of animal models. Further, HS seems to alter systemic fuel utilization, favoring aerobic glycolysis rather than oxidative phosphorylation. Study objective was to determine if these metabolic changes have their origin at the transcriptional level. Seventeen crossbred gilts (57 ± 5 kg BW) were subjected to one of two environmental treatments: 1) constant HS conditions (32°C, 23% RH) and ad libitum feeding (n = 7), or 2) pair-feeding in thermoneutral conditions (20°C, 36% RH; PFTN; n = 10) to eliminate the confounding effects of dissimilar intake. Feed intake decreased 38% on average and was not different between treatments (P = 0.75). After 8d of environmental exposure, pigs were sacrificed and liver, subcutaneous adipose tissue (AT) and muscle (longissimus dorsi, LD) immediately collected. Gene expression was determined using qPCR (BioMark System, Fluidigm Corporation) on an average of 42 genes per tissue. Genes were selected based on the RNA-Seq output of a similar experiment. Gene expression was normalized to housekeeper genes and statistical analysis was performed in delta delta Ct values (*P*ROC GLM, SAS 9.2). Data is reported as fold change. As expected, heat shock protein-related genes (e.g., HSF2, HSPA4, HSPB8, HSPCB, HSPE1, HSP90AA1) were up-regulated (41–156%, $P \leq 0.10$) across all tissues in HS compared to PFTN conditions. Supporting the phenotypic observation, adipose triglyceride lipase was downregulated (36%, P = 0.07), hepatic fatty acid synthase was up-regulated (1.5-fold, $P < 0.01$), and TCA cycle and electron transport chain proteins (i.e., IDH2, NDUFB7, NDUFS7) were downregulated (43%, P = 0.06; 22%, P = 0.05; 23%, P = 0.10; respectively) in liver and LD of HS pigs. Unexpectedly, AT insulin receptor and LD pyruvate dehydrogenase kinase 4 were downregulated (32%, P = 0.07; 61%, P = 0.05; respectively) in HS compared to PFTN pigs. Abundance of most of the genes involved in bioenergetic pathways did not differ between treatments. These data suggest that changes in metabolism and fuel selection after chronic HS (8d) may partially arise from differences in post-transcriptional regulation. Whether gene expression control at the transcriptional level has a role on metabolic adaptation to acute HS remains unknown.

Key Words: heat stress, pig, postabsorptive metabolism

1393 (M210) Effect of feeding high or low portions of concentrate during the transition period on serum adiponectin concentrations and mRNA expression of adiponectin and its receptors in subcutaneous and retroperitoneal fat of dairy cows. P. Friedrichs[1], M. Weber[1], L. Locher[2], S. Dänicke[3], U. Meyer[3], R. Tienken[3], H. Sauerwein[*1], and M. Mielenz[4], *[1]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany, [4]Leibniz Institute for Farm Animal Biology (FBN), Institute of Nutritional Physiology, Dummerstorf, Germany.*

The mRNA expression of adiponectin (ADIPOQ) and its receptors ADIPOR1/2 in adipose tissue (AT) decreases with the onset of lactation in dairy cows; the serum concentrations of ADIPOQ have also been demonstrated to drop during the lactation-induced negative energy balance (NEB) but not during feed-restriction induced NEB at later stages of lactation (Singh et al. (2014), doi:10.3168/jds.2013–7598). As to whether the extent of NEB during the lactation-induced NEB may affect ADIPOQ system was not known and we thus hypothesized that the ADIPOQ system will be affected by feeding different portions of concentrate in the diet throughout the transition period, and that visceral (VC) and subcutaneous (SCAT) respond concordantly. Twenty pluriparous German Holstein cows were fed with rations containing either 60% (HC) or 30% (LC) concentrate (DM basis, n = 10 per group) from d 1 to 21 postpartum. The SCAT (tail head) and RPAT were biopsied at d -21, 1, and 21 relative to parturition. Blood samples were collected weekly. ADIPOQ and AdipoR1/2 mRNA abundances were quantified by qPCR, and serum ADIPOQ by ELISA. The statistical analyses were performed using SPSS 21.0 ($P < 0.05$). The NEB was more negative in LC than in HC animals (Δ = 18.5 MJ/d, third week of lactation). Effects of diet were limited to ADIPOQ in SCAT, with 4.1-fold lower mRNA abundance in LC than in HC at d 21 ($P < 0.02$). With the exception of AdipoR2 mRNA in RPAT, we detected time-related changes in SC and RPAT with higher abundances ante partum ($P < 0.05$) for all target mRNAs, and for ADIPOQ in serum. AdipoR2 mRNA abundance in RPAT was highest at d 1. Irrespective of time, ADIPOQ and AdipoR2 expression was higher in RPAT than in SCAT ($P < 0.05$), whereas AdipoR1 mRNA abundance was not different between both tissues. ADIPOQ and AdipoR1 mRNA abundance were positively correlated in SCAT (r = 0.461, $P < 0.01$) and RPAT (r = 0.745, $P < 0.01$). AdipoR1 and AdipoR2 were correlated in SCAT (r = 0.422, $P < 0.01$). The lower ADIPOQ mRNA abundance in SCAT of the LC group points to greater responsiveness towards dietary effects in this depot; however, this was apparently not reflected in serum ADIPOQ indicating that other fat depots might compensate the decrease. The observed time-related changes in SCAT and of

serum ADIPOQ confirm earlier reports, whereas the likewise regulation in RPAT for ADIPOQ and AdipoR1 has not been previously investigated in cows. The correlation between ADIPOQ and AdipoR1 mRNA abundances points to a co-regulation of both genes in AT.

Key Words: adiponectin

1394 (M211) Heat stress affects insulin sensitivity in primary bovine adipocytes.

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Animals experiencing heat stress (HS) have diminished lipolytic response. Current research on lipid metabolism in lactating cows shows a clear disconnect between in vivo and in vitro data, wherein bovine cells cultured under HS conditions were found to be more sensitive to lipolytic stimuli. The objective of this study was to determine if HS affects insulin sensitivity in subcutaneous adipose tissue (AT) of dairy cattle. Bovine primary adipocytes, isolated from 7 multiparous Holstein cows in late lactation, were cultured at either 42°C (HS) or 37°C (thermal neutral, TN) and incubated with varying concentrations of insulin (0 to 2.5 mU) in combination with isoproterenol (ISOP, 10^{-6} M). Glycerol release was measured as an indicator of lipolytic response. The effects of temperature and insulin concentration, as well as their interaction on AT lipolysis were evaluated. Likewise, the abundance of several lipolytic proteins in relation to HS was analyzed. A significant insulin concentration by temperature interaction was observed in HS ($P < 0.001$) but not TN ($P = 0.34$) cells. Insulin significantly reduced the amount of glycerol released ($P < 0.001$), indicating a decline in response to lipolytic stimuli. Meanwhile, in the absence of insulin, adipocytes cultured under HS exhibited an elevated response to ISOP ($P < 0.001$) relative to their TN counterparts. Basal lipolytic (-ISOP/-insulin) response was not different between HS and TN cells ($P > 0.05$). Furthermore, a significant decrease in the phosphorylation of hormone sensitive lipase (HSL) at Serine 563 ($P = 0.03$) and perilipin ($P = 0.04$) with respect to increasing insulin concentrations was observed for cells cultured under HS but not TN conditions. These data support the view that HS affects insulin sensitivity of bovine adipocytes, suggesting that HS may indirectly prevent in vivoadipose tissue mobilization as a result of heat-induced increase in circulating insulin concentrations, combined with higher AT sensitivity to insulin.

Key Words: heat stress, adipose tissue, insulin, lipolysis

1395 (M212) mRNA expression of chemerin and its receptor in a subcutaneous and a visceral fat depot of dairy cows fed with high or low portions of concentrate during the transition period.

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Chemerin (RARRES2) is involved in adipogenesis and in mediating fat mobilization in mature adipocytes, exerting its effects via its receptor ChemR23. In humans, circulating chemerin levels are regulated by the energy intake. We hypothesized that the expression of RARRES2 or ChemR23 in subcutaneous (SC) and retroperitoneal (RP) adipose tissue (AT) of dairy cattle will change throughout the transition period and will be affected by different portions of concentrate in the diet. Twenty pluriparous German Holstein cows were divided into a high-concentrate group (HC, $n = 10$) receiving a diet with 60% and a low-concentrate group (LC, $n = 10$) receiving a diet with 30% concentrate on DM basis from d 1 until d 21 post partum. The SCAT from tail head and RPAT were biopsied at d -21, 1, and 21 relative to calving. RARRES2 and ChemR23 mRNA abundance were quantified by qPCR. The statistical analyses were performed with SPSS 21.0 ($P < 0.05$). The mRNA abundances of both genes were not different between the HC versus the LC group neither in SCAT nor in RPAT, thus, groups were pooled for further analyses. In both tissues RARRES2 and ChemR23 mRNA expression were time-dependent, i.e., less RARRES2 and ChemR23 mRNA was observed ante partum than post partum in both AT ($P < 0.05$). When comparing both AT depots within the individual sampling times, RPAT had 1.33-fold higher RARRES2 mRNA abundance than SCAT on d 1 ($P = 0.004$), whereas no differences between both depots were seen on d -21 and 21. For ChemR23, RPAT and SCAT did not differ at any time point. In conclusion, the different concentrate portion and the difference in energy balance ($\Delta = 18.5$ MJ/d in the third week of lactation) between HC vs. LC group were apparently not relevant for the expression of RARRES2 or ChemR23. Considering time-related differences we observed that RARRES2 and its receptor are regulated in a likewise manner in bovine AT. Due to their functions, the higher mRNA abundances of RARRES2 and its receptor in both AT after parturition may reflect the paracrine/autocrine involvement of RARRES2 in stimulation of lipolysis to provide non-esterified fatty acids as energy source to other peripheral tissues.

Key Words: chemerin, adipose tissue, transition period

1396 (M213) Individual *trans* monounsaturated fatty acids have distinct effects on lipogenesis in 3T3-L1 adipocytes. P. Vahmani*[1], T. D. Turner[1], P. D. Duff[1], D. C. Rolland[1], C. Mapiye[2], W. J. Meadus[1], and M. E. R. Dugan[1], *[1]Agriculture and Agri-Food Canada, Lacombe, AB, Canada, [2]Stellenbosch University, Western Cape, South Africa.*

The objective of this research was to determine the isomer specific effects of *trans* 18:1 isomers on lipogenic gene expression and fatty acid composition in adipocytes. Differentiated 3T3-L1 adipocytes were treated with 200 μM of either *trans*-9 18:1, *trans*-11 18:1, *trans*-13 18:1, or *cis*-9 18:1 (control) for 120 h. Cells were then analyzed for changes in gene expression and fatty acid composition using real-time PCR and gas liquid chromatography, respectively. The experiment was repeated three times and data were analyzed using the PROC MIXED of SAS. The *trans*-9 18:1 treatment increased the expression of acetyl-CoA carboxylase (1.6-fold, $P = 0.08$), fatty acid synthase (1.5-fold, $P = 0.03$) and stearoyl-CoA desaturase-1 (1.7-fold, $P = 0.03$) compared to the control, whereas *trans*-11 18:1 and *trans*-13 18:1 did not affect ($P > 0.10$) the expression of these genes. Consistent with the gene expression results, the content of *cis*-9 16:1 ($P = 0.01$; SEM = 8.63), total monounsaturated fatty acids ($P = 0.002$; SEM = 31.5) and total fatty acids ($P = 0.04$; SEM = 41.7) were higher in *trans*-9 18:1 (191, 986, and 1502 μg/well respectively) compared with *cis*-9 18:1 (144, 783, and 1272 μg/well respectively), *trans*-11 18:1 (141, 716, and 1370 μg/well respectively) or *trans*-13 18:1 (128, 659, and 1349 μg/well respectively). The *trans*-9 18:1 treatment also increased the *cis*-9 16:1/16:0 ratio ($P < 0.01$; SEM = 0.016) compared to the *cis*-9 18:1, *trans*-11 18:1 or *trans*-13 18:1 treatments (0.61, 0.52, 0.41 and 0.42 respectively). The amount of treatment fatty acid deposited in cells was highest ($P = 0.01$; SEM = 31.5) for *trans*-9 18:1 (585 μg/well) followed by *cis*-9 18:1 (493 μg/well), *trans*-11 18:1 (382 μg/well) and *trans*-13 18:1 (339 μg/well). About 32% of *trans*-13 18:1 was desaturated to *cis*-9, *trans*-13 18:2, whereas 19% of *trans*-11 18:1 was desaturated to *cis*-9, *trans*-11 18:2. Our results suggest that *trans*-9 18:1, the most abundant industrial *trans* fatty acid, is more lipogenic than *trans*-11 18:1 or *trans*-13 18:1. We also found that *trans*-13 18:1 is used as a preferred substrate for delta-9 desaturation. Our results demonstrate that *trans*-13 18:1, the second most abundant *trans*-18:1 isomer in beef when feeding forage based diets, is metabolized differently and may have differing bioactivity than major industrially produced *trans* fatty acids. Consequently, potential bioactivities of *trans*-13 18:1 and its delta-9 desaturation product deserve further investigation.

Key Words: adipocytes, mouse, *trans* monounsaturated fatty acids

1397 (M214) Modeling diurnal variation in ruminal temperature of beef cows. B. H. Boehmer*, and R. P. Wettemann, *Oklahoma Agricultural Experiment Station, Stillwater.*

Ruminal temperature (RuT) of beef cows is an effective measure of core body temperature. Monitoring RuT may be useful for the prediction of physiological events in cattle including estrus, parturition, and health status. Daily variation in core body temperature of cattle is well documented and may influence prediction models using temperature. The objective of this experiment was to develop a method to reduce the impact of diurnal variation in RuT of beef cows. Hourly reporting temperature boluses (SmartStock, LLC) were administered to postpartum, lactating, Angus cows. The data set used for modeling contained 12,358 RuT values (58 cows; 14 d), with a daily mean of 19.3 ± 0.3 RuT readings per cow. Models were developed to generate hourly correction factors for RuT, which reduce the impact of diurnal variation. Briefly, the RuT for a cow at an hour was subtracted from the mean RuT of all cows at all hours in the experimental group (Ac), or mean RuT of an individual cow for all hours (Cc), or all RuT during a 72 h running average for an individual cow (Ra). Correction factors for each daily hour (0000 to 2300 h) were calculated as the hourly least squares mean for the hourly deviations from the means (Ac, Cc, Ra). Hourly least square means of the deviations for an hour were calculated for all cows (AM) or for individual cows (CM). Unadjusted RuT and RuT excluding drinking events (W; less than 2 x SD of mean RuT) were used for model evaluation. Ruminal temperature for each model was analyzed using PROC UNIVARIARTE, PROC REG, and PROC MIXED (SAS Inst. Inc.). All six correction models reduced the variation (> 54%) and skewdness (> 30%) of RuT. Hourly variation in RuT occurred for unadjusted RuT, W, and RaAM ($P < 0.05$), but was eliminated in AcAM, AcCM, CcAM, CcCM, and RaCM models. Bayesian information criterion values (goodness of fit), were least when AcCM was used to model RuT. When the AcCM model was used, variation in RuT was greatly diminished. Daily hour did not influence RuT when AcCM, CcCM, and RaCM models ($P = 0.87$, 0.83, 0.91, respectively) were used to adjust RuT. These results indicate models can be developed to greatly reduce diurnal variation in RuT. The usefulness of RuT can be enhanced through the use of models to reduce diurnal variation in body temperature of cows.

Key Words: beef cow, diurnal variation, ruminal temperature

1398 (M215) β-Hydroxybutyrate profile of high-yielding dairy cows of a Brazilian intensive system.

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This study aimed to investigate the β-hydroxybutyrate (BHB) profile of high-yielding primiparous and multiparous cows according to milk yield level. A total of 174 Holstein cows was evaluated and divided as: 1) primiparous with mean milk yield equal to or higher than 35 kg/d (42.92 ± 0.78 kg/d) (HP group, n = 37); 2) primiparous with mean milk yield lower than 35 kg/d (26.44 ± 0.91 kg/d) (LP group, n = 50); 3) multiparous with mean milk yield equal to or higher than 35 kg/d (44.28 ± 0.87 kg/d) (HM group, n = 37), and 4) multiparous with mean milk yield lower than 35 kg/d (24.87 ± 0.91 kg/d) (LM group, n = 50). The animals belonged to a commercial herd located in São Paulo State, Brazil, and were kept in free stall barns, according to their production level, and were given high-concentrate diet based on NRC recommendations (NE_L: H groups: 1.74 MCal/kg DM and L groups: 1.56 MCal/kg DM). Blood samples were collected from the coccygeal vein to evaluate BHB by an enzymatic method using a commercially available kit (Randox-Ranbut Laboratories, Oceanside, CA). The mean lactation period of all evaluated cows was 216 ± 12. Statistical analysis was performed using SAS software by One-way ANOVA and Pearson Correlation Test. The mean dry matter intake of each free stall barn during the period in which blood samples were collected was similar between groups (HP: 24 kg/d; LP: 22.7 kg/d; HM: 26.9 kg/d and LM: 22.7 kg/d). There were no statistical differences between groups ($P > 0.05$). The BHB concentrations for primiparous groups were: HP 0.45 ± 0.05 mmol/L, and LP 0.48 ± 0.08 mmol/L; for the multiparous ones, HM 0.42 ± 0.04 mmol/L, and LM 0.42 ± 0.09 mmol/L. When the same production level was compared, HP and HM tended to have a negative correlation, and so did LP and LM (Pearson´s correlation coefficients: -0.26 and -0.22, respectively); when the same category was compared, both HP– LP and HM– LM combinations tented to have a positive correlation (0.21 and 0.40, respectively). The results suggest that the cows with the mean lactation period in this study did not show a negative energy balance (NEB), probably due to the fact that milk production requirements were provided by their diet, which did not alter BHB concentration levels, an NEB marker.

Key Words: β-hydroxybutyrate, dairy cows, negative energy balance

1399 (M216) Analysis of transcription regulator gene networks in peripartal bovine liver during summer and spring seasons.

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Gene network analysis was used on hepatic transcriptome data from cows calving during spring (SP) or summer (SU) to uncover transcription regulators (TR) and their target genes. Liver tissue was harvested from multiparous Holstein cows at -30, 3, and 35 d relative to parturition during SP. (March–April, n = 6) and SU (June–July, n = 6). Mean temperature-humidity indeces for SP. (day/night, below 72) and SU (day, 79.5 ± 2.9; night, 70.1 ± 4.7) were recorded. Transcriptomics was conducted using the 45K-Agilent bovine microarray. Statistical analysis with FDR $\leq$ 0.10 resulted in 618, 1030 and 894 differentially expressed genes during SU vs. SP. at -30, 3, and 35 d, respectively. Ingenuity pathway analysis (IPA) was used for gene network reconstructions. Among molecular and cellular functions, the IPA analysis identified cell death and survival and cellular growth and development as the most-enriched functions. Carbohydrate metabolism was the most enriched at -30 and 3 d, while nucleic acid metabolism and cellular development were the most enriched at 3 and 35 d. A total of 50, 78, and 74 TR were identified at -30, 3, and 35 d. The IPA analysis uncovered HNF4A, MYC, and NCOA1 (-30, 3, and 35 d), STAT3, and RELA (-30 and 35 d), BCL6 (3 and 35 d), KAT2B (-30 d), and GATA2 (3 d) as key TR. Comparing SU vs. SP. at -30d uncovered HNF4A and MYC (both triggered by RELA) as key TR. Both are linked with several downstream up-regulated target genes involved in oxidation of xenobiotic compounds (CYP3A4), tryptophan catabolism (ACMSD1), arginine catabolism (ARG1), apoptosis regulation, and ER Calcium homeostasis (CFLAR, TMBIM6). In contrast, among the downregulated targets there were several involved in cellular proliferation, anti-apoptotic activities, immune related disorders (CDKN1, LGALS1, TSPO), and liver disease (SERPINA1, FTH1). At 3 d, both HNF4A and MYC were downregulated. Up-regulation of BCL6 was directly linked with the IL-6-dependent immune-response and cell growth. In contrast, BCL6 was associated with downregulation of IL7R, IL13R1 and CXCL10-dependent immune responses. At 35 d, the up-regulation of RELA was associated with target genes involved in activation of anti-inflammatory responses (CCL3, B2M), extracellular matrix breakdown (MMP1), regulation of cell cycle (MYC, PTEN, CASP8) and gluconeogenesis (*P*CK1). These results suggests that the molecular phenotype of the liver differs when calving during the summer compared with spring.

Key Words: temperature-humidity index, transcription regulator network analysis, transition cows