

Physiology and Endocrinology: Nutritional and Metabolic Effects on Growth, Reproduction and Lactation

T175 Measurement of adiponectin in lactating dairy cows. J. R. Raddatz*, A. N. Elias, and C. S. Whisnant, *North Carolina State University, Raleigh.*

Adipose tissue is now known to be part of the endocrine system as well as an energy storage depot. One of the recently discovered adipose secreted hormones is adiponectin. In rodents and humans adiponectin concentration decreases with increasing adiposity and has been reported to increase tissue sensitivity to insulin. No report of adiponectin secretion in cattle could be found. In the current project we used a commercially available human adiponectin kit (HADP-61 HK, Linco, Millipore) to measure adiponectin concentrations in lactating Holstein ($n = 26$) cows for the first 11 wk of lactation. Insulin and progesterone concentrations were also determined in weekly blood samples. Body condition scores and milk production data were recorded. Adiponectin concentrations increased from 8.3 ± 1.4 ng/mL in the first wk to 16.0 ± 2.7 ng/mL ($P < 0.01$) at wk 4 postpartum and then declined to remain at 12-13 ng/mL for the remainder of the study. Insulin concentrations increased from 5.6 ± 0.7 mIU/mL at wk 1 to 17.6 ± 2.2 mIU/mL at wk 3 and then declined to remain at 9-11 mIU/mL for the remainder of the study. Individual cows had consistently high or low adiponectin levels throughout the sampling period. Adiponectin concentrations did not correlate with body condition score or energy corrected milk yield. Nor did time of resumption of estrous cycles have any effect on adiponectin. Dilution of plasma as recommended by the manufacturer resulted in samples being below the sensitivity of the assay. Using undiluted plasma, adiponectin concentrations ranged 1-80 ng/ml whereas in other species concentrations were reported to be in the ug/ml range. Similarly samples collected from growing bulls were undetectable when diluted but in the 0-40 ng/ml range when assayed undiluted. Equine samples were assayed along with the bovine samples and the equine samples after the recommended dilution (1:500) were in the ng/ml range and after adjustment for dilution were 1-3 ug/ml, similar to values reported for horses in the literature. The human adiponectin kit may not accurately measure bovine adiponectin.

Key Words: Dairy Cattle, Adiponectin, Insulin

T176 L-carnitine stimulates the early postnatal myofiber formation in pig skeletal muscle. D. Loesel*, C. Kalbe, G. Nuernberg, and C. Rehfeldt, *Research Institute for the Biology of Farm Animals, Dummerstorf, Germany.*

Piglets of low birth weight exhibit a lower total number of skeletal myofibers at birth and throughout life compared with piglets of middle and heavy birth weight, which is associated with impaired (lean) growth, carcass and meat quality at market weight. To investigate, whether L-carnitine is effective in stimulating the early postnatal increase in the number of skeletal myofibers, 30 piglets of low (LW) and middle (MW) birth weight (each within one third of frequency distribution) from 6 German Landrace sows were supplemented once daily with 400 mg L-carnitine ($n=16$) or a placebo ($n=14$) from d 8 to 28 (weaning) of age. Offspring were slaughtered at d 29 of age to analyze blood plasma components, *semitendinosus* (ST) muscle structural and functional prop-

erties, and body composition. Live weight gain and final body weight as well as the plasma concentrations of glucose, free fatty acids, urea, and IGF-I remained unchanged by treatment. Carnitine concentration in ST muscle more than doubled in response to treatment ($P < 0.0001$). The total number of ST myofibers was increased by 19% ($P < 0.05$) in treated LW pigs thereby reaching the unchanged level of MW littermates. Myofibers tended to be smaller ($P = 0.13$), and protein concentration and protein/DNA ratio were lower in treated pigs ($P < 0.05$). Specific ICDH activity as a marker of oxidative metabolism was increased ($P = 0.06$), whereas no differences were observed in muscular LDH, CK, and fiber type composition. Body composition was unchanged by treatment. Notwithstanding, the perirenal fat percentage tended to be decreased ($P = 0.10$), and L-carnitine-treated females exhibited lower percentages of dry matter and crude fat ($P < 0.05$). The results suggest that L-carnitine stimulates early postnatal myofiber formation in pig skeletal muscle, which may attenuate the negative consequences of low birth weight on growth, carcass and meat quality of pigs at market weight.

Key Words: Birth Weight, Body Composition, Muscle

T177 The assessment of complex I concentration in muscle mitochondria of crossbred steers with high and low residual feed intake (RFI). M. P. Davis*, J. H. Porter, and M. S. Kerley, *University of Missouri, Columbia.*

The objective of this study was to evaluate the relationship between complex I concentration in muscle mitochondria and RFI of steers fed a concentrate based-ration. Individual feed intake was recorded for 81 crossbred steers over approximately 150 d using the GrowSafe feed intake system. Residual feed intake was computed as the residual of actual intake minus expected intake. Expected intake was calculated using coefficients for averaging daily gain and mid-weight. The coefficients were generated from the linear regression of dry matter intake on metabolic mid-weight and average daily gain. Three efficient steers (average RFI of -0.78) and 2 inefficient steers (average RFI of 0.90) were selected for further evaluation. Tissue samples were taken at slaughter from the LM and mitochondria isolated using differential centrifugation. Complex I concentration was quantified from mitochondria. Immunocapture of complex I from the mitochondria was done using MitoProfile complex I Immunocapture kit (Mitosciences, Eugene, OR 97403). Complex I protein concentration was determined using bicinchoninic acid colorimetric procedures. Concentration of complex I tended ($P \leq 0.15$) to be greater in Low RFI (6.33 ± 0.48 $\mu\text{g}/\text{mg}$) steers than high RFI (4.08 ± 0.73 $\mu\text{g}/\text{mg}$) steers. The correlation between RFI and complex I protein concentration was -0.69. While only tending to be significant ($P \approx 0.19$) the magnitude of correlation is similar to that reported but the relationship between RFI and complex I is opposite than reported. The association of low RFI (improved efficiency) and higher complex I concentration does however agree with research reported for broilers. These results show that complex I may be influencing RFI and further research is needed to determine if complex I concentration can be used as a predictor of RFI.

Key Words: Muscle Mitochondria, Complex I, Residual Feed Intake

T178 Madin-Darby Bovine Kidney (MDBK) cells and liver tissue of periparturient cows share remarkable similarity in gene expression profiles. M. Bionaz*, R. E. Everts, H. A. Lewin, J. K. Drackley, and J. J. Loor, *University of Illinois, Urbana*.

The importance of fatty acids (FA) in regulating liver metabolism has been well-established in mammalian species and is becoming of interest in periparturient dairy cattle nutrition. Use of in vitro models to assess the effects of FA on bovine cells is crucial due to high costs of in vivo experiments as well as confounding effects of ruminal FA metabolism (e.g., biohydrogenation). In this regard, work from our laboratory with MDBK has demonstrated they represent a useful tool to study potential metabolic effects of FA. The objective of this study was to compare transcript abundance between MDBK cells and liver tissue of periparturient cows. For this purpose, transcript profiles of MDBK cells cultured in high-glucose DMEM and liver tissue from cows at -14, 1, 14, and 28 d relative to parturition were compared. A 13,257 bovine oligonucleotide (70-mers) array was used for transcript profiling. A total of eight microarrays in a direct dye-swap design were hybridized. Using a t-test, we identified 1,983 (15%) genes with different expression and 1,091 showing ≥ 2 -fold expression between MDBK and liver. We also selected 4,850 genes involved in several liver-specific functions (e.g., apolipoprotein metabolism, urea cycle) or metabolism (e.g., fatty acid metabolism, apoptosis) to assess their level of expression in MDBK vs. liver tissue. Analysis indicated that $60 \pm 14\%$ of the chosen genes had similar expression pattern between MDBK and liver. Genes involved in apolipoprotein metabolism, urea cycle, gluconeogenesis, and β -oxidation had greater abundance in liver vs. MDBK, while genes involved in glycolysis, TCA-cycle, and cell cycle had greater abundance in MDBK. Among transcription factors, liver was characterized by larger expression of *HNF4A* (27-fold), *RXRG* (2.2-fold), and *PPARA* (1.5-fold), while MDBK had greater abundance of *PPARG* (1.3-fold) and *NR1H2* (1.4-fold). This analysis allowed characterization of MDBK-specific transcripts and uncovered high similarity (60%) between this cell line and liver tissue of periparturient cows.

Key Words: Genomics, Microarray, Nutrition

T179 Effect of 17 β -estradiol on distal colon contractions and L-arginine-NOS-NO-cGMP-cGMP-PK1 pathway. A. Bulbul¹, K. Altunbas¹, H. A. Celik¹, G. Avci¹, O. Yildiz-Gulay*¹, and M. S. Gulay², ¹*Afyon Kocatepe University, Afyonkarahisar, Turkey*, ²*Mehmet Akif Ersoy University, Burdur, Turkey*.

Objectives were to determine the effect of estrogen on distal colon contractions and L-arginine-NOS-NO-cGMP-cGMP-PK1 α/β pathway. Six month-old female Sprague Dawley rats (n = 72) were divided equally into four groups (n = 18/group) and ovaries were removed. The control group (Ov) received sesame oil, whereas rats in E1, E2 and E3 groups received 25, 50 and 100 μg im injections of 17 β -estradiol in sesame oil, respectively. To determine time effect of estradiol injections, each treatment group was further divided into three sub-groups; the first sub-groups received single dose, the second sub-groups received 3 doses, and the third sub-groups received 6 doses of sesame oil or estradiol (24 h intervals). Rats in all sub-groups were sacrificed 18 h after the last estradiol injections. Distal colons were removed immediately after sacrifice. Smooth muscle contractions were recorded by force transducer and acquisition system to evaluate L-arginine-NOS-NO-cGMP pathway in colon. Expression of nNOS and cGMP-PK1 α/β in distal colon was also tested. The statistical significance was set at $P < 0.05$. The results

indicated that 17 β -estradiol injections decreased the distal colon contractions in a dose and time dependent manner. Moreover, L-arginine-NOS-NO-cGMP pathway was effective on the smooth muscle contractions in distal colon. Results from immunohistochemistry revealed that nNOS expression was not affected by estradiol injections. However, expression of cGMP-PK1 α/β declined significantly. In conclusion, although estrogen injections decreased the smooth muscle contractions in distal colon, this effect did not use L-arginine-NOS-NO-cGMP pathway.

Key Words: 17 β -estradiol, nNOS, cGMP

T180 Effect of ovarian steroids on distal colon contractions and L-arginine-NOS-NO-cGMP-cGMP-PK1 pathway. A. Bulbul¹, A. Yagci¹, K. Altunbas¹, H. A. Celik¹, G. Avci¹, O. Yildiz-Gulay*¹, and M. S. Gulay², ¹*Afyon Kocatepe University, Afyonkarahisar, Turkey*, ²*Mehmet Akif Ersoy University, Burdur, Turkey*.

The current study addressed the effect of ovarian steroids on spontaneous distal colon contractions and L-arginine-nitric oxide synthase (NOS)-nitric oxide (NO) pathway. Ovariectomized three to six-month old Sprague-Dawley rats were assigned to control (Ov; sesame oil; n = 10), estrogen (E; 10 $\mu\text{g}/\text{d}$; n=10), progesterone (P; 2 mg/d; n = 10), and progesterone and estrogen (EP; 10 $\mu\text{g}/\text{d}$ estrogen and 2 mg/d progesterone; n = 10) groups. Daily intramuscular injections were continued for 10 d. Rats in all groups were sacrificed 18 h after the last injection. Distal colons were removed immediately after sacrifice, tissue samples were replaced in isolated organ baths, and contractions against different solutions were examined. The level of statistical significance was set at $P < 0.05$. Results indicated that among the treatment groups, intensity of colon contractions decreased only in E group. Moreover, L-arginine-NOS-NO pathway reduced the intensity of contraction induced by electrical field stimulation (EFS). In E group, L-arginine decreased the efficacy of sodium nitroprusside (SNP), whereas cGMP was not effective on smooth muscle contractions. Among the groups, level of nNOS expression was similar in non-adrenergic non-cholinergic (NANC) neurons. However, cGMP-PK1 α/β expression in smooth muscle cells was decreased in E group. In conclusion, results from the current study indicated that NO induced the relaxation in distal colon via cGMP-cGMP-PK pathway. Estrogen also reduced the distal colon contractions. However, the data suggested that estrogen uses cGMP-PK to reduce the effectiveness of L-arginine-NOS-NO-cGMP-cGMP-PK pathway.

Key Words: Ovarian Steroids, cGMP, nNOS

T181 Effect of diets containing soybean meal or canola meal on blood metabolites in early lactation Iranian Holstein cows. F. Hosseini, A. Heravi Moussavi*, M. Danesh Mesgaran, and J. Arshami, *Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran*.

The aim of this study was to evaluate substitution of soybean meal with canola meal and measure its effects on serum glucose, cholesterol, urea nitrogen (BUN), and aspartate aminotransferase (AST) in early lactation Iranian Holstein cows. From d 5 to 56 postpartum, cows were fed diets that were isoenergetic containing soybean meal (SBM; n = 5) or canola meal (CM; n=5). Cows were housed in tie stalls and fed the TMR two times a day to allow 5 to 10%orts (as-fed basis). Blood samples were collected weekly throughout the study via venipuncture

from coccygeal vessels prior to the morning feeding and serum was separated using centrifugation. Serum metabolites were analyzed by enzymatic colorimetric assays using procedures modified from available kits. The data were analyzed using the MIXED procedure of SAS (2001) for a completely randomized design with repeated measures. The model contained the effects of treatment, week of treatment, cow within treatment and the interaction of treatment by week. The overall effect of treatment was tested using cow within treatment as the error term. Least squares means are reported throughout, and significance was declared at $P < 0.05$. Plasma glucose concentrations were similar among diets (60.38 and 60.76 ± 1.65 mg/dL, respectively). The effect of time was significant ($P < 0.05$). Diet and time had no effect on BUN. Plasma cholesterol concentrations were similar among the diets (143.38 and 141.21 ± 7.5 mg/dL, respectively). The effect of time was significant ($P < 0.01$) and plasma cholesterol increased over the study. Diet had no effect on AST (72.00 and 72.43 ± 4.63 U/L, respectively). The effect of time also was not significant. The results of this study demonstrated that substituting soybean meal with canola meal in the early lactation cows had no apparent effect on the blood metabolites.

Key Words: Dairy Cow, Soybean Meal, Canola Meal

T182 Effects of carbohydrate source and processing on serum progesterone and insulin concentrations of dairy cattle. P. Moriel^{*1}, T. S. Scatena¹, O. G. Sa Filho¹, R. F. Cooke², and J. L. M. Vasconcelos¹, ¹FMVZ-UNESP, Botucatu, Brazil, ²University of Florida, Gainesville.

Two trials were conducted to investigate the effects of carbohydrate source and processing on serum progesterone (P4) and insulin (INS) concentrations. In Exp. 1, 12 ovariectomized grazing Gir \times Holstein cows were stratified by BW and randomly assigned, in a crossover design, to receive a supplement based on finely ground corn (FC) or citrus pulp. Treatments were offered individually 2 \times /day at a rate 11 kg of DM/cow. Within each of the 2 experimental periods, cows were adapted to treatments from d 0 to d 14 and received an intravaginal P4-releasing device (CIDR) prior to the beginning of the study. On d 7 after CIDR implant, blood samples were collected immediately prior to, and 1, 2, 3, 4, 5, and 6 h after the first feeding of the day. In Exp. 2, the cows utilized in Exp. 1 were re-stratified by BW and randomly assigned to receive, in a crossover design, a supplement based on coarsely ground corn (GC), finely ground corn (FC), or high-moisture corn (HM). Treatments were offered individually 2 \times /d at a rate 11 kg of DM/cow. Within each of the 3 experimental periods, cows were adapted to treatments from d 0 to d 7. Blood samples were collected on d 7 after CIDR implant as in Exp. 1. Time effects ($P < 0.01$) were detected in Exp. 1 and 2 because P4 concentrations decreased 3 h after feeding, whereas INS concentrations increased 1 h after feeding. In Exp. 2, HM cows tended ($P = 0.09$) to have decreased P4 concentrations compared to GC cows (1.7 vs. 1.9 ng/mL), and had greater ($P < 0.01$) INS concentrations compared to GC and FC cows (8.8 , 5.7 , and 6.1 μ UI/mL, respectively). Data combined from both experiments indicate that cows with INS ≥ 4.5 μ UI/mL prior to treatment feeding had greater P4 concentrations at 1 h, but decreased P4 concentrations at 5 h compared to cows with INS < 4.5 μ UI/mL. In the present study, carbohydrate processing, but not carbohydrate source, affected serum P4 and INS concentrations of non-lactating dairy cows.

Key Words: Carbohydrate, Insulin, Progesterone

T183 Effects of prepartum 2,4-thiazolidinedione on plasma leptin and insulin sensitivity in transition dairy cows. K. M. Schoenberg^{*}, K. L. Smith, R. M. Ehrhardt, Y. R. Boisclair, and T. R. Overton, Cornell University, Ithaca, NY.

Administration of thiazolidinediones (TZD) has been shown to alter lipid metabolism in transition dairy cows. The objective was to determine the effect of prepartum TZD treatment on plasma leptin and insulin response. Holstein cows ($n = 40$) entering second or greater lactation were administered 0, 2.0, or 4.0 mg TZD/kg BW by intrajugular infusion once daily from 21 d before expected parturition until parturition. Plasma samples collected daily from 22 d before expected parturition through 21 d postpartum were analyzed for glucose, NEFA, and insulin. Plasma samples collected on d -14, -3, -1, 1, 3, 7, 14, and 49 relative to parturition also were analyzed for leptin. Data from 40 cows were used for prepartum analyses and 31 cows were used for periparturient (d -7 to +7) and postpartum analyses. Prepartum BCS was not affected by treatment but postpartum BCS was higher for TZD-treated cows (2.8, 2.9, 3.1; $P = 0.001$). There was a trend ($P = 0.15$) for a treatment by time effect on plasma leptin prepartum such that values were similar on d -14 but cows receiving 2.0 mg/kg BW TZD tended to have lower leptin as calving approached. Postpartum leptin tended ($P = 0.14$) to increase linearly in TZD-treated cows (2.3, 2.4, 2.5 ng/mL). Insulin response was assessed using the revised quantitative insulin sensitivity check index (RQUICKI = $1/[\log(\text{glucose}) + \log(\text{insulin}) + \log(\text{NEFA})]$) as applied to dairy cattle, and results suggested that TZD-treated cows had greater insulin sensitivity as calving approached (treatment by time; $P = 0.05$). During the periparturient period, there was a tendency for RQUICKI to increase linearly with increasing TZD (0.43, 0.43, 0.46; $P = 0.10$). Postpartum RQUICKI was increased linearly in cows treated with TZD prepartum (0.43, 0.44, 0.47; $P = 0.02$). These results suggest that TZD treatment may alter plasma leptin, but postpartum effects may be confounded with BCS differences. Changes in RQUICKI suggested that TZD treatment altered insulin sensitivity in periparturient dairy cows.

Key Words: Transition Cow, Thiazolidinedione, Leptin

T184 The effects of prepartum 2,4-thiazolidinedione administration to dairy cows on energy balance, growth hormone, and insulin-like growth factor-I during the transition period. L. A. Winkelman^{*}, K. L. Smith, R. M. Ehrhardt, and T. R. Overton, Cornell University, Ithaca, NY.

Administration of thiazolidinediones (TZD) to prepartum dairy cows has been shown to reduce plasma NEFA concentrations in the periparturient period and postpartum body condition loss. Data from Holstein cows ($n = 31$) entering second or greater lactation were used to determine whether late prepartum administration of TZD would affect periparturient energy balance (EB), plasma growth hormone (GH) and insulin-like growth factor-I (IGF-I) concentrations, and the ratio of GH:IGF-I. During the pre- and postpartum periods, cows were fed a common TMR for ad libitum intake. Cows were administered 0, 2.0, or 4.0 mg TZD/kg BW by intrajugular infusion once daily from 21 d before expected parturition until parturition. Plasma samples collected on d -14, -7, -3, -1, 1, 3, 7, 14, 28, and 49 relative to parturition were analyzed for GH and IGF-I by RIA. Prepartum plasma GH and IGF-I concentrations, and the GH:IGF-I ratio were unaffected by TZD treatment. Calculated postpartum EB increased linearly ($P = 0.05$) with increasing TZD dose (-4.9 , -5.4 , and -1.7 ± 1.2 Mcal/d for 0, 2.0, and 4.0 mg TZD/kg BW treatments, respectively). After parturition, there was a tendency for a

linear effect of prepartum TZD dose on plasma GH ($P = 0.07$) such that an increasing TZD dose prepartum was associated with lower postpartum GH concentrations (9.55, 8.26, and 6.82 ± 1.12 ng/ml for 0, 2.0, 4.0 mg TZD/kg BW treatments, respectively). Prepartum TZD treatment did not affect postpartum IGF-I, but there was a linear trend ($P = 0.12$) for a decreased postpartum ratio of GH:IGF-I (0.258, 0.240, and 0.174 ± 0.040 for 0, 2.0, 4.0 mg TZD/kg BW treatments, respectively). A more positive calculated EB after parturition was associated with lower GH concentrations in cows receiving the 4.0 mg TZD/kg BW treatment, but there was no apparent relationship between postpartum EB and IGF-I concentrations.

Key Words: Transition Cow, Thiazolidinedione, Growth Hormone

T185 The metabolic status during the dry period influences the ovulation of the first follicular wave postpartum in dairy cows. N. Castro*^{1,2}, C. Kawashima³, H. A. van Dorland¹, S. Richter¹, I. Morel⁴, A. Miyamoto³, and R. M. Bruckmaier¹, ¹University of Bern, Bern, Switzerland, ²Las Palmas de Gran Canaria University, Arucas, Spain, ³Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, ⁴Agroscope Liebefeld-Posieux, Posieux, Switzerland.

The aim of this study was to investigate the effect of the nutritional status, liver function and key metabolic factors in the liver during the

dry period (dp) and early lactation on the resumption of the ovarian activity in dairy cows. 23 high yielding dairy cows were allocated in two groups based on the first ovulation postpartum (pp) as detected by milk progesterone (P4) profiles. Milk samples were collected thrice per wk from d 7 pp until a new pregnancy. Ovulations were identified by an increase of P4 to more than 1 ng/mL. 47.8% of cows showed the first ovulation within 3 wk pp (OC), while in the others, ovulation occurred later (AC). Blood samples were obtained biweekly from 9 wk antepartum (ap) to wk 9 pp and plasma concentration of β HB, NEFA, Glucose (Glu), T-cholesterol, IGF-I, Insulin (Ins), T3, T4, AST and GGT were measured. Liver biopsies were taken ap and pp to analyze mRNA expression levels of hormone receptors (GH-R, IR, IGF-R1) and key metabolic enzymes (PC, PEPCKc, PEPCKm). In addition BCS and energy balance (EB) during dp and pp were assessed. Data were analysed by repeated measures ANOVA. Areas under the curve were calculated for the entire periods dp and pp, respectively. OC during dp showed higher Glu, Ins, IGF-I and T3 than AC (3.64 ± 0.03 vs 3.42 ± 0.06 mmol/l, 5.99 ± 0.99 vs 3.89 ± 0.44 μ g/l, 173.09 ± 11.50 vs 133.04 ± 11.56 μ g/l and 1.26 ± 0.06 vs 1.07 ± 0.06 nmol/l, respectively). During the pp period only higher T4 and BCS in OC were found (48.38 ± 2.23 vs 44.46 ± 0.98 nmol/l and 2.93 ± 0.07 vs 2.72 ± 0.09 , respectively). Liver mRNA expressions and EB did not differ in dp and pp. In conclusion the metabolic status during the dp is crucial for the ovulation of the first follicular wave pp.

Key Words: Ovulation, Metabolism, Dry Period