764 mRNA expression of a novel adipokine (pigment epithelium-derived factor, PEDF) in various tissues from dairy cows receiving supplements with or without conjugated linoleic acids (CLA). B. Saremi*[1], S. Winand[1], S. Dänicke[2], J. Pappritz[2], D. von Soosten[2], H. Sauerwein[1], and M. Mielenz[1], *[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Bonn, North Rhine-Westphalia, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Lower Saxony, Germany.*

Pigment epithelium-derived factor (PEDF) is a recently discovered adipokine, a secreted glycoprotein belonging to the non-inhibitory serpin group; it is one of the most abundant proteins secreted by human adipocytes and is related to obesity, insulin resistance and inflammatory signaling. To our knowledge, no information is available on bovine PEDF expression and functions. Herein, CLA effects on PEDF mRNA abundance (Ab) were tested in tissues of dairy cows ante and postpartum (a.p., p.p.). In 2 trials, Holstein cows were allocated to a CLA (Lutrell, BASF, Germany) or control (Silafat, BASF) fat supplement at 100 g/day from d 1 to 182 p.p. in trial 1 and d 1 to 105 p.p. in trial 2. Trial 1: 21 multiparous cows at d 21 a.p. and d 1, 21, 70, 105, 182, 196, 224, 252 p.p. were biopsied from tail head adipose tissue (AT) and liver. In trial 2, 25 heifers were slaughtered on d 1 (n = 5; control), 42 and 105 p.p. (n = 5 per each group) and 3 visceral (Vc; mesenterial, omental and retroperitoneal) and 3 subcutaneous (Sc; sternum, withers and tail head) AT, liver, mammary gland, and muscle tissues were sampled. PEDF mRNA was quantified by qPCR. Data were analyzed using mixed model for trial 1 and GLM or nonparametric test for trial 2 (SPSS 17, $P < 0.05$). In trial 2, liver had 34.3, 15.1, 6.8 and 2.9 fold higher PEDF Ab than muscle, mammary gland, VcAT, and ScAT respectively. VcAT had 2.4 fold lower PEDF Ab than ScAT (trial 2). Liver PEDF Ab did not change with time in both trials. In AT, time-dependent changes were solely observed in the control group in which PEDF Ab increased after parturition, decreased until d 200, and increased thereafter (d 231 and 252). CLA effects were limited to trial 1 in which PEDF Ab in ScAT did not show the p.p. and late lactation increases of control cows. In trial 2, time-dependent changes were limited to mesenterial fat and to ScAT from sternum and withers with lowest values each on d 1. Our data confirm expression of PEDF for bovine AT with consistently higher Ab in Sc than in VcAT. Moreover, we identified the liver as a major site of PEDF expression.

Key Words: pigment epithelium-derived factor (PEDF), CLA, liver and fat

765 Effects of long-term hyperketonemia on metabolism and performance in lactating dairy cows. M. Zarrin*[1,2], L. De Matteis[1,3], M. C. M. B. Vernay[1], O. Wellnitz[1], H. A. van Dorland[1], and R. M. Bruckmaier[1], *[1]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Department of Animal Science, Yasouj University, Yasouj, Iran, [3]Istituto di Zootecnica, Università Cattolica S, Cuore, Piacenza, Italy.*

Hyperketonemia in dairy cows occurs frequently in early lactation. Previous studies observed that increasing plasma BHBA concentration decreases glucose levels in pigs and ewes through hormonal mechanisms or possible inhibition of gluconeogenesis. In cows, this has not yet been investigated. The objective was to study effects of a BHB infusion for 48 h in mid lactating dairy cows on metabolism and performance. Thirteen mid lactation dairy cows were randomly assigned to one of 2 treatments, including an intravenous infusion with Na-DL-β-OH-butyrate (1.7 mol/L) at a rate of 8.5 ± 0.6μmol/kg BW/min (HyperB, n = 5), or an infusion with a 0.9% saline solution (20 mL/h; NaCl, n = 8). The infusions started from 0900 h and continued to 0900 h 2 d later by use of a permanent catheter in the jugular vein. Blood was sampled before and on an hourly basis during the infusions for metabolite and hormone levels. In liver, mRNA transcripts of pyruvate carboxylase, mitochondrial phosphoenolpyruvate carboxykinase, and glucose-6- phosphatase were measured by real-time RT-PCR. GAPDH and ubiquitin were housekeeping genes. Cows were fed hay and concentrate and were milked twice daily throughout the study. Changes (difference between before and after 48h infusion) in the measured parameters were evaluated by ANOVA with treatment as fixed effect. The plasma BHB concentration reached and maintained during the study period in HyperB cows was 1.7 ± 0.2 mmol/L (mean ± SE) compared with 0.59 ± 0.02 mmol/L for NaCl cows. The change in feed intake, milk and ECM yield was not different between 2 groups. BHB infusion reduced the plasma glucose concentration (3.47 ± 0.11mmol/L) in HyperB compared with NaCl cows (4.11 ± 0.08mmol/L; $P < 0.05$). The other plasma factors were unaffected. In the liver, changes in mRNA abundance for the selected genes were similar between HyperB and NaCl cows. Results demonstrate that intravenous infusion of BHB decreased plasma glucose concentration in dairy cows but this decrease was not explained by alterations in insulin or key enzymes related to hepatic gluconeogenesis at a molecular level.

Key Words: hyperketonemia, metabolism, gluconeogenesis

766 Tissue-dependent expression of G-protein couple receptor (GPR) 40, 41, 43, 109A mRNA in early lactation dairy cows treated with conjugated linoleic acids (CLA) and long-chain fatty acids (LCFA). B. Saremi*[1], H. Sauerwein[1], D. von Soosten[2], S. Dänicke[2], and M. Mielenz[1], *[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Bonn, North Rhine-Westphalia, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Lower Saxony, Germany.*

The receptors GPR40, 41, 43, and 109A are a group of transmembrane receptors related to energy homeostasis through regulation of insulin and glucagon secretion, leptin release, adipogenesis and lipolysis, respectively. LCFA and CLA as ligands of GPR40 are insulinotropic in humans. We investigated GPRs mRNA abundance (Ab) in dairy cows tissues considering effects of CLA. From 25 heifers, 5 were slaughtered on d 1 postpartum (p.p.). Remaining heifers were randomly allocated to a CLA (Lutrell pure, BASF, Germany, n = 10) or a control fat supplement containing LCFA but without CLA (Silafat, BASF, n = 10) each at 100 g/d. Five animals per group were slaughtered at d 42 or 105. Subcutaneous (ScAT; sternum, withers and tail head) and visceral (VcAT; mesenterial, omental and retroperitoneal) adipose tissues, liver, mammary gland, and muscle were sampled. The mRNA was quantified by qPCR. GLM or nonparametric tests were used for statistical analysis (SPSS 17; $P < 0.05$). GPR40 was the only receptor which had a higher Ab in CLA group in liver ($P < 0.065$), omental and retroperitoneal AT. GPR41 and 43 Ab were higher (3.3 and 3.5 fold respectively) in VcAT in comparison to ScAT. GPR109A Ab was 1.4 and 33 fold higher in ScAT vs. VcAT and in AT vs. the mean of liver, muscle and mammary gland. Liver and mammary gland exhibited similar and muscle showed a lower GPR43 Ab in comparison to ScAT. Liver and muscle had the highest

and lowest GPR40 Ab respectively, with the exception of equality of liver to ScAT from withers. GPR40 Ab increased from d 1 to d 42 and 105 p.p. in AT and was stably expressed in muscle and liver. GPR41 Ab decreased from d 1 and 42 to d 105 p.p. GPR43 Ab increased from d 1 to d 42 and 105 in ScAT and was consistently expressed in VcAT and non fat tissues. Different behavior of GPR40 Ab between CLA and LCFA in bovine AT and liver raises the differential regulation of the receptor in bovine vs. human. Differential expression of the GPRs in different fat or nonfat tissues might be a hint to their tissue specific roles.

Key Words: GPR40-43 and GPR109A, CLA, dairy cow

767 Is calcitonin involved in hypocalcemia of periparturient cows? E. M. Rodríguez*[1], A. Bach[1,2], M. Devant[1], and A. Arís[1], *[1]Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, [2]ICREA, Barcelona, Spain.*

Evidence for an association of calcitonin (CALC) in hypocalcemia in the cow is inconsistent: whereas some studies point out that this hormone secretion is increased, others state that it is diminished. The objective of this study was to elucidate the involvement of CALC on periparturient hypocalcemia establishing a comparative profile of the changes in calcium-regulating factors under high and normal dietary Ca supplies. Sixteen Holstein multiparous cows (674 ± 22.8 kg of BW and 3.9 ± 0.29 y of age) were distributed into 2 groups: a diet containing 1.64% Ca (HIGH) or a diet containing 0.87% Ca (CTR) during 3 wk before calving. Feed intake was individually recorded and blood samples were obtained daily from −7 to 7 d from parturition. Milk yield was recorded daily during 3 weeks after calving. Calcium was analyzed in plasma using a colorimetric method (SPINLAB 100) and in milk using atomic absorption spectrophotometry. Plasma parathyroid hormone (PTH) and 1,25-dihydroxyvitamin-D (1,25-vitD) were analyzed using ELISA, and plasma CALC was determined by RIA. Data were analyzed with a mixed-effect model with repeated measures. No differences in DMI were observed between groups. During the first 7 d after calving, milk yield tended ($P = 0.06$) to be greater in CTR than in HIGH cows (23.9 vs. 19.4 ± 1.56 L, respectively) and milk Ca concentration tended ($P = 0.08$) to be lower in CTR than in HIGH cows (1482 vs. 1627 ± 57.4 mg/kg, respectively), although total milk Ca production (31.7 ± 2.91g/d) did not differ between treatments. Seventy 5 percent of the cows incurred subclinical hypocalcemia (blood Ca concentrations <8 mg/dL). During hypocalcemia the CALC secretion was not increased and the hormone was maintained under basal concentrations. Blood PTH concentrations increased at d 1 postpartum as a reaction to Ca loss and 1,25-D concentrations increased at d 2. In conclusion, this study shows that CALC secretion is not increased on the hypocalcemic period of cows and as expected, the main Ca reduction was followed by an increase in PTH and 1,25-D secretion.

Key Words: hypocalcemia, calcitonin, parathyroid hormone

768 Reproductive performance of Ossimi rams fed biologically treated rations. E. B. Abdalla*[1], F. R. Abed El-Aziz[2], H. M. Gado[1], A. E. Hassan[2], and M. S. Ziada[3], *[1]Ain Shams University, Cairo, Egypt, [2]Anim. Prod. Res. Inst., Agric. Res. Center, Ministry of Agric., Giza, Egypt, [3]Anim. Reprod. Res. Inst., Agric. Res. Center, Ministry of Agric, Giza, Egypt.*

This experiment was conducted on 20 adult Ossimi rams (1–2 years old and averaged 44–48 kg live body weight) to evaluate the dietary supplementation of ZAD compound (patent product contains cellulases, xylanases, protease and α amylase) to improve overall nutritional status and consequently will improve semen quality characteristics in sheep. Rams were randomly divided into 2 equal groups (10 for each). The first group (G1) served as control and was fed rice straw without supplementation, while the second group (G2) was fed rice straw treated with ZAD compound. All rams received concentrate feed mixture (CFM) to cover 100% of their maintenance energy. Forage and CFM were adjusted biweekly according to live body weight changes and roughage to CFM ratio was 60:40 during the experimental period. The experimental feeding period lasted for 8 mo, the first 2 mo were a preliminary period and the other 6 mo were the main period of semen collection (once a week). General Linear Model (GLM) procedure of SAS, ANOVA and LSD test were used for statistical analysis. Results revealed that overall mean of semen characteristics were 0.54 vs. 1.21 mL, 77.81 vs. 85.18%, 2.15 vs. 2.85 × 10^9/mL, 75.90 vs. 78.05%, 12.10 vs. 9.68% and 5.20 vs. 5.91 for ejaculate volume, advanced motility, sperm cell concentration, live sperm, abnormal sperm and pH value of semen for control and treated group, respectively. Body weight changes were promoted ($P < 0.05$) by treatment as compared with the control group. Blood plasma concentrations of total protein, albumin and globulin were higher ($P < 0.05$) in G2 (8.22, 2.82 and 5.40 g/dL) as compared with those corresponding values in G1 (5.59, 2.18 and 3.41 g/dL, respectively). Blood plasma levels of thyroxine (T4) hormone showed a tendency of increases in G2 than in G1 (5.21 vs. 4.65 ng/dL). Similarly, triiodothyronine (T3) and testosterone concentrations were significantly higher in G2 than in G1 (164.8 vs. 138.3 ng/mL). Results of the present study indicated that feeding rams on ration containing ZAD compound had a beneficial effect on their semen quality characteristics, including sperm motility %, sperm cell concentration (× 10^9/mL), live sperm% and normal sperm % comparing with the control group .

Key Words: ZAD, hormone, semen

769 The effect of yeast cell wall supplementation on the metabolic responses of crossbred heifers to endotoxin challenge. N. C. Burdick*[1], T. R. Young[2], J. A. Carroll[1], J. R. Corley[3], R. J. Rathmann[2], and B. J. Johnson[2], *[1]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [2]Texas Tech University, Department of Animal and Food Sciences, Lubbock, [3]Lesaffre Feed Additives, Milwaukee, WI.*

This study examined the effect of feeding yeast cell wall (YCW) products on the metabolic responses of newly-received heifers to endotoxin (lipopolysaccharide; LPS) challenge. Heifers (n = 24; 218.9 ± 2.4 kg) were obtained from commercial sale barns and transported to the Texas Tech Univ. Beef Center. Heifers were separated into treatment groups receiving a Control Diet (C; n = 8), YCW A (2.5 g/hd per day; n = 8) or YCW C (2.5 g/hd per day; n = 8) and were fed for 52 d. Heifers were weighed on d 0, 14, 36, 38, and 52. On d36 heifers were fitted with indwelling jugular catheters and moved into a barn with individual stalls. On d37 heifers were challenged i.v. with LPS (0.5 μg/kg BW) and blood samples were collected every 0.5 h from −2 to 8 and again at 24 h relative to LPS challenge (0 h). Serum was isolated and stored at −80°C until analyzed for glucose, insulin, nonesterified fatty acid (NEFA), and blood urea nitrogen (BUN) concentrations. Heifer weight increased from d0–36 and from d38–52 ($P < 0.01$), but was not affected by treatment ($P > 0.32$). Post-LPS YCW A (−6.0 ± 0.9 kg) lost more weight (from d36–38) than C (−2.4 ± 0.9 kg) and YCW C (−4.2 ± 0.9kg; $P = 0.04$). Post-LPS glucose increased ($P < 0.001$) and was less in YCW A (98.5 ± 2.5 mg/dL) than C (105.6 ± 2.4 mg/dL) and YCW C (109.5 ± 2.4 mg/dL; $P < 0.01$). Pre-LPS insulin was greater in YCW A (0.80 ± 0.06 ng/mL) and YCW C (0.087 ± 0.06 ng/mL) than C (0.44 ± 0.06 ng/mL; $P < 0.01$). Post-LPS insulin increased ($P < 0.01$) with YCW C (0.95 ± 0.04 ng/mL) and YCW A (0.71 ± 0.05 ng/mL) having greater insulin

than C (0.59 ± 0.04 ng/mL; $P < 0.001$). Pre-LPS NEFA tended ($P = 0.07$) to be less in YCW C (0.14 ± 0.01 mmol/L) than C (0.18 ± 0.01 mmol/L) and YCW A (0.17 ± 0.01 mmol/L). The difference in NEFA was significant post-LPS (0.18 ± 0.01, 0.21 ± 0.01, and 0.21 ± 0.01 mmol/L respectively for YCW C, C, and YCW A). Pre-LPS BUN was greater in YCW A (8.2 ± 0.3 mg/dL) than C (6.9 ± 0.3 mg/dL; $P = 0.03$). Post-LPS BUN was greater in YCW A (8.9 ± 0.2 mg/dL) than C (8.2 ± 0.2 g/dL) and YCW C (8.1 ± 0.2 mg/dL; $P < 0.01$). These data indicate that certain YCW products can enhance the energy metabolism during an immune challenge without causing lipolysis or muscle catabolism.

Key Words: cattle, metabolism, yeast

770 Effect of sward condition on metabolic endocrinology during the early postpartum period in primiparous grazing dairy cows and its association with productive and reproductive performance. A. Meikle*[1], L. Adrien[1], D. Mattiauda[2], and P. Chilibroste[2], *[1]Faculty of Veterinary, Montevideo, Uruguay, [2]Faculty of Agronomy, Montevideo, Uruguay.*

The effect of differential sward herbage allowances and a total mixed ration (TMR) management on milk production, body condition, first postpartum ovulation and endocrine/metabolic parameters were investigated. Primiparous dairy cows (n = 44) were randomly assigned to one of the following grazing treatments (n = 11 each): high (HA, 30 kg DM/cow/d), medium (MA, 15 kg DM/cow/d) and low herbage allowance (LA, 5 kg DM/cow/d), and a TMR group fed ad-lib. Non esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), cholesterol, protein, albumin, urea, insulin, insulin-like growth factor-I (IGF-I) and leptin were determined in plasma every 15 d. Progesterone was determined 2 times per week after parturition to determine first ovulation. TMR group had higher milk production than the HA and MA groups which did not differ, which were in turn greater than LA cows. Overall, the TMR and HA groups had a greater BCS, protein, albumin and urea concentrations than MA and LA groups, suggesting a better energy balance. While metabolic differences were observed among HA cows and MA cows early in the pospartum period (15–30 dpp), differences among HA and LA cows were observed later on (45–60 dpp). While IGF-I concentrations increased after calving in all groups, no increases were found in LA group, probably due to the energy restriction due to the nutrients offered. Greater insulin and IGF-I concentrations were found in the TMR group, which is consistent with the higher nutrient density offered to this group. The probability of cyclicity one month after calving was lower in MA cows than TMR and HA cows, which is consistent with the higher NEFA and lower urea concentrations found in this period. The lowest probability of first ovulation throughout the study was observed in LA cows. This study shows that different nutritional treatments affected several metabolites and hormones that reflect the nutritional partitioning toward milk and reproductive processes.

Key Words: dairy, metabolism, endocrinology

771 Association of biomarkers of stress, inflammation, and negative energy balance with milk yield and reproductive performance in Holstein dairy cows. J. M. Huzzey*[1], D. V. Nydam[2], R. J. Grant[3], and T. R. Overton[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, [3]W. H. Miner Agricultural Research Institute, Chazy, NY.*

The objectives were to evaluate the association between peripartum concentrations of fecal cortisol metabolites (11,17-dioxoandrostane; 11,17-DOA), plasma haptoglobin (Hp), and nonesterified fatty acids (NEFA), and milk yield and reproductive performance. Blood and fecal samples were collected weekly from 412 Holstein dairy cows from wk −3 through wk +1 relative to calving. Pregnancies by 150 DIM and projected 305ME milk yield based on the 3rd DHI test day (102 DIM) were measured. A range of concentration cutpoints were evaluated for each biomarker; associations of these cutpoints with 305ME or risk of conception were assessed using mixed effects or semiparametric proportional hazards models, respectively. Associations between the biomarkers and reproductive performance were strongest for primiparous (PP) cows during wk −1 or +1 relative to calving. Among multiparous (MP) cows, no biomarker measured during wk −1 or +1 was associated with reproductive performance ($P \geq 0.2$). Primiparous cows with Hp >0.4 g/L, 11–17-DOA >2300 ng/g fecal DM, or NEFA >0.40 mEq/L during wk −1 had a 41%, 42%, or 42% decreased risk of conception, respectively ($P \leq 0.05$), compared with PP cows below these cutpoints. Primiparous cows with Hp >1.3 g/L or NEFA >0.45 mEq/L during wk +1 had a 41% or 39% decreased risk of conception, respectively ($P = 0.02$). Associations between Hp and 11,17-DOA with 305ME milk yield were strongest when these analytes were measured during wk +1; however, wk −1 NEFA concentrations were a better predictor of milk yield than wk +1 NEFA. Postpartum Hp >1.1 g/L was associated with 947 kg lower 305ME milk yield for both PP and MP cows ($P = 0.001$). For MP cows only, 11,17-DOA >400 ng/g fecal DM during wk +1 was associated with a 663 kg lower 305ME milk yield ($P = 0.03$). Primiparous and MP cows with NEFA >0.55 mEq/L during wk −1 had a 1360 kg lower projected 305ME milk yield ($P = 0.002$). Biomarkers of stress, inflammation, and negative energy balance around calving can be used to identifying opportunities to improve milk yield and reproductive performance.

Key Words: biomarkers, milk yield, reproduction

772 Serum amyloid A3 (SAA3) mRNA in liver and adipose tissue of dairy cows supplemented with or without conjugated linoleic acids (CLA): A whole lactation cycle study. B. Saremi*[1], S. Winand[1], J. Pappritz[2], S. Dänicke[2], M. Mielenz[1], M. M. Rahman[1], and H. Sauerwein[1], *[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Bonn, North Rhine-Westphalia, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Lower Saxony, Germany.*

Serum amyloid A3 (SAA3) is an acute phase protein (APP) mainly expressed extrahepatically. Bovine adipose tissues (AT) are immune responsive and express SAA3. In monogastrics, CLA have anti-inflammatory and anti-lipolytic effects. We hypothesized that CLA might exert analogous effects in bovine tissues and be related to AT metabolism. Holstein Frisian cows received a CLA (Lutrell pure, BASF, Germany, n = 11) containing 12% each of the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 CLA isomers or a non-CLA fat supplement (Silafat, BASF, n = 10) from d 1 to 182 in milk. Biopsies were collected from subcutaneous (Sc) AT from tail head and liver tissue at d 21 ante partum (a.p.) and d 1, 21, 70, 105, 182, 196, 224, 252 postpartum (p.p.). SAA3 mRNA abundance (Ab) was measured by real-time PCR in all biopsies, but in the CLA group only on d 21 a.p. and d 21, 105, 196, 252 p.p. Detection of the SAA protein in AT was done by Western blot. Statistical analyses were done using the mixed model or Pearson correlation (SPSS 17; $P < 0.05$). Typical SAA protein bands were obtained from AT. CLA had no effect on SAA3 Ab in liver and ScAT. Liver SAA3 Ab exhibited a peak at the day of parturition (8 fold higher in comparison to the mean of the other d a.p. and p.p.) but remained stable throughout all other

times. In ScAT, the day of parturition was significantly different in comparison to 196 and 252 d p.p. (7.7 fold higher SAA3 Ab). Liver and ScAT SAA3 Ab were positively correlated to haptoglobin plasma concentrations and ScAT and liver haptoglobin mRNA (r ranging from 0.30 to 0.62); ScAT SAA3 Ab was positively correlated to plasma NEFA (r = 0.31) (haptoglobin and NEFA from published data). The correlation of ScAT SAA3 Ab with plasma NEFA might be related to the homeorhetic adaptation regarding to the energy requirement during lactation. The peripartal peak of SAA3 in liver and ScAT is comparable to haptoglobin as another APP. The expression of SAA protein in bovine AT is a novel finding though the relevance of individual SAA isomers remains to be clarified.

Key Words: serum amyloid A3 (SAA3), CLA, cow liver and adipose tissue

773 Responses of mammary gland metabolism to long-term manipulated plasma concentrations of insulin and glucose in lactating dairy cows. J. J. Gross,* M. C. M. B. Vernay, L. Kreipe, O. Wellnitz, H. A. van Dorland, and R. M. Bruckmaier, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

The regulation of genes involved in mammary gland metabolism was investigated during manipulated concentrations of insulin and glucose for 48 h. Six mid-lactating dairy cows each were assigned to a hyperinsulinemic hypoglycemic clamp (HypoG), a hyperinsulinemic euglycemic clamp (EuG) and a control treatment (NaCl). Blood samples were collected before and hourly during the infusions for measurements of glucose and insulin. Mammary gland biopsies were taken immediately before and at 48 h of infusion and mRNA abundance of genes involved in the mammary gland metabolism were measured by RT-qPCR with glycerinaldehyde-3-phosphate-dehydrogenase, ubiquitin and cyclophilin as static expressed housekeeping genes. Changes in the measured parameters, and the area under the curve of plasma parameters from 24 to 48 h of infusion were evaluated by ANOVA with the treatment as fixed effect. For HypoG, insulin infusion rate (IIR) was 0.46 ± 0.02 mU/kg/min from 24 to 48 h whereas IIR for EuG was maintained constant at 0.62 mU/kg/min, and the simultaneous glucose infusion rate in EuG was 2.47 ± 0.05 mmol/kg/min. From 24 to 48 h of infusion, HypoG had a lower glucose concentration (2.25 ± 0.05 mmol/L) in comparison to EuG (3.80 ± 0.16 mmol/L) and NaCl (4.17 ± 0.10 mmol/L; $P < 0.05$); HypoG and EuG had higher insulin concentrations than NaCl (41.9 ± 8.1, 57.8 ± 7.8, and 12.2 ± 2.8 mU/L, resp.; $P < 0.05$). In mammary tissue, the mRNA abundance of glucose transporter (GLUT) 4 was decreased in EuG compared with HypoG, and that of αS1-Casein and insulin receptor was decreased compared with HypoG and NaCl, while mRNA abundance of insulin induced gene 1 and UDP-glucose pyrophosphorylase was upregulated compared with NaCl and HypoG ($P < 0.05$), while mRNA abundance of κ-Casein and acetyl-CoA-carboxylase were increased in EuG by trend ($P = 0.06$). No differences between groups were found for mRNA abundance of GLUT1, E74-like factor 5, α-lactalbumin, sterol response element binding factor 1 and fatty acid synthase. In conclusion, most differences in gene expressions of mammary gland metabolism were found between HypoG and EuG indicating the regulatory role of glucose at simultaneously elevated insulin concentrations during an increased glucose turnover.

Key Words: mammary gland, metabolism, dairy cow

774 Tumor necrosis factor-α (TNF-α) mRNA expression in early lactation in different tissues of dairy cows with a focus on different fat depots. B. Saremi*[1], H. Sauerwein[1], D. von Soosten[2], S. Dänicke[2], and M. Mielenz[1], *[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Bonn, North Rhine-Westphalia, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Lower Saxony, Germany.*

The pro-inflammatory cytokine TNF-α is expressed in different cell types, mainly in stimulated monocytes and macrophages. In humans, TNF-α is involved in inflammation, apoptosis, insulin sensitivity, and induction of lipolysis. The *trans*-10, *cis*-12 isomer of conjugated linoleic acids (CLA) attenuates the release of TNF-α from bovine immune cells. Therefore, we investigated possible changes of TNF-α mRNA abundance (Ab) in tissues of dairy cows in postpartum (p.p.), considering effects of CLA. From 25 heifers, 5 were slaughtered on d 1 p.p. and remaining heifers were randomly allocated to conjugated linoleic acids (CLA) (Lutrell pure, BASF, Germany, n = 10) or control fat supplementation without CLA (Silafat, BASF, n = 10) each at 100 g/d. Five animals per group were slaughtered at d 42 or 105. Subcutaneous (Sc) (sternum, withers and tail head) and visceral adipose tissue (VcAT: mesenterial, omental and retroperitoneal), liver, mammary gland and muscle tissues were sampled. Quantification of TNF-α was done by qPCR. Fat mass and NEFA were adopted from published data on the same cows. Pearson correlation, GLM or non parametric tests were used for statistical analysis (SPSS 17; $P < 0.05$). From 1 to 105 d p.p. TNF-α Ab increased in ScAT and liver and decreased in mammary gland and retroperitoneal AT (from d 1 and 42 to 105 p.p.). In omental and mesenterial AT as well as in muscle, TNF-α Ab was stably expressed. Abundance of TNF-α mRNA was not affected by CLA except at d 42 in mesenterial AT (lower Ab in the CLA group). Liver, mammary gland and muscle TNF-α Ab was 5.3, 2.5 higher and 4.4 fold lower than that in AT. Within AT, Sc sternum and omental had the highest and mesenterial and retroperitoneal had the lowest TNF-α Ab. In contrast to AT, liver TNF-α Ab was correlated negatively to fat mass and positively to blood NEFA. As in humans, TNF-α in cattle might be linked to fat mobilization. Differential expression of TNF-α throughout lactation could be associated to paracrine mechanisms related to insulin sensitivity in the analyzed tissues.

Key Words: TNF-α, liver and fat tissues, dairy cattle