

## Growth and Development II

**W126 Chromium acetate induces adipogenesis of bovine intramuscular adipocytes through reduced phosphorylation of adenosine monophosphate-activated protein kinase  $\alpha$ .** K. Y. Chung\*, R. T. Tokach, and B. J. Johnson, *Texas Tech University, Lubbock.*

Chromium sources have positive effects on glucose uptake in both cattle and pigs. Chromium aids in insulin signaling in insulin-sensitive cells such as adipocytes. Adenosine monophosphate-activated protein kinase  $\alpha$  (AMPK $\alpha$ ) can affect lipid metabolism in the bovine intramuscular (i.m.) and subcutaneous (s.c.) adipocytes. We hypothesized that chromium acetate (CrAc) may affect AMPK $\alpha$  phosphorylation state in bovine i.m. and s.c. adipocytes. Bovine i.m. and s.c. preadipocytes were incubated with similar differentiation factors such as 10  $\mu$ M insulin, 10  $\mu$ M ciglitizone, 1  $\mu$ M dexamethasone, and 100  $\mu$ M oleic acid. Multilocular lipid droplets accumulated in the cultured i.m. adipocytes, but unilocular lipid droplets accumulated in the s.c. adipocytes after 96 h of CrAc treatment. Data were analyzed as a completely randomized design using the MIXED model, each treatment performed in triplicate. Difference between control and treatments were determined using the LSD procedure. Quantity of mRNA was measured by agarose gel electrophoresis and OD calculation. Western blot analysis revealed that CrAc reduced ( $P < 0.05$ ) phospho-AMPK $\alpha$  to AMPK $\alpha$  in i.m. adipocytes but had no effect in s.c. adipocytes. Relative PPAR $\gamma$  mRNA concentrations were greater ( $P < 0.05$ ) in i.m. adipocytes with CrAc treatments compared with the control cultures. Treatment with 10  $\mu$ M sodium acetate compared with CrAc did not differ ( $P > 0.05$ ) from control for PPAR $\gamma$ , glucose transporter 4 (GLUT4), and GPR43 mRNA concentrations. Total amount of PPAR $\gamma$  mRNA was 5 times greater in s.c. than in i.m. adipocytes ( $P < 0.05$ ). Although GLUT4 level was not different in i.m. adipocytes, there was a dose-dependent effect of CrAc in the s.c. adipocytes. The mRNA concentrations of GPR43, a short-chain fatty acid receptor, tended to be increased ( $P = 0.08$ ) in i.m. adipocyte cultures. Chromium acetate can induce adipogenic development in the i.m. preadipocytes potentially by reducing phosphorylation of AMPK $\alpha$ , and the GPR43 membrane protein may be involved in this process.

**Key words:** chromium acetate, adenosine monophosphate-activated protein kinase  $\alpha$ , adipocyte

**W127 Palmitoleic acid regulation of lipid metabolism in primary bovine adipocytes could involve genes associated with fatty acid oxidation.** A. K. G. Kadegowda\*, T. A. Burns, S. L. Pratt, and S. K. Duckett, *Clemson University, Clemson, SC.*

Palmitoleic acid (C16:1n7) is a proposed lipokine that regulates systemic metabolism. The objective was to determine the effect of C16:1 on fatty acid metabolism gene expression in adipocytes. Bovine primary preadipocyte cultures were isolated from intermuscular fat from rib sections of 18-mo old Angus crossbred heifers ( $n = 3$ ) fed a concentrate diet. Preadipocytes were differentiated (D0) in differentiation media [DMEM containing 10% fetal calf serum, 2.5  $\mu$ g/mL insulin, 0.25  $\mu$ M dexamethasone (DEX), 20  $\mu$ M troglitazone (TRO), 0.5 mM isobutylmethylxanthine (IBMX), and 10 mM acetate] for 2 d. Cells were further differentiated from D2 to D12 in differentiation media [with out DEX and IBMX] containing 1 of 4 levels of C16:1 (0, 50, 150, or 300  $\mu$ M). Cells were harvested on D6 and D12 for fatty acid analysis using GLC and mRNA expression by RT-qPCR. We measured the expression of *Acyl-Coenzyme A oxidase 2 (ACOX2)*, *Acyl-CoA dehydrogenase, long chain (ACADL)*, *Phytanoyl-CoA 2-hydroxylase*

(*PHYH*), *Caveolin1 (CAV1)* and *Adipose differentiation-related protein (ADFP)*. The geometric mean of *Eukaryotic translation initiation factor 3, subunit k (EIF3K)* and *Ubiquitously expressed transcript (UXT)* was used for normalization. Increasing the concentration of C16:1 in the media increased the cellular concentration of C16:1 ( $P < 0.05$ ) and C18:1cis11 ( $P < 0.05$ ), a C16:1 elongation product but decreased ( $P < 0.05$ ) the cellular levels of C16:0, C18:0, C18:1c9. Of the measured genes related to fatty acid oxidation, *ACADL* increased by 2.36 fold ( $P < 0.07$ ) while *PHYH* increased by 95.5 fold ( $P < 0.01$ ) compared with controls on D6 suggesting potential increase in mitochondrial  $\beta$ -oxidation and peroxisomal  $\alpha$ -oxidation, respectively. The increase in the mRNA expression of lipid droplet associated proteins *CAV1* (1.47 fold,  $P < 0.07$ ) and *ADFP* (3.4 fold,  $P < 0.01$ ), could be a consequence of increase in lipid droplet content due to increase in C16:1 level. The results from the study shows that C16:1 regulates lipid metabolism in the adipose tissue and could potentially involve mechanisms related to fatty acid oxidation.

**Key words:** palmitoleic acid, adipocyte, fatty acid oxidation

**W128 Effect of anabolic implant and quality grade on lipogenic gene expression in subcutaneous adipose tissue.** S. K. Duckett\*, S. L. Pratt, and J. W. Long, *Clemson University, Clemson, SC.*

Angus-cross steers ( $n = 24$ ; 488 kg) were randomly allotted to either non-implant (CON) or implant (IMP) treatments to explore the effects of anabolic implants on lipogenic gene expression in subcutaneous adipose depots by quality grade. Steers allocated to IMP received a single Revalor-S (24 mg estradiol, 124 mg trenbolone acetate) on d 0. All steers were individually fed a high-concentrate diet for 72 d and slaughtered. At slaughter, adipose tissue samples were collected from subcutaneous adipose depots, flash-frozen, and stored at  $-80^{\circ}\text{C}$  for subsequent RNA extraction. Carcass weight and skeletal maturity were greater ( $P < 0.05$ ) for IMP than CON. Other carcass variables including marbling score, quality grade and yield grade did not differ among treatments. For qPCR, a sub-sample ( $n = 8$ ) was selected from each treatment based on quality grade, LOW (Select-) vs. HI (Choice-). Data were analyzed with implant treatment, quality grade, and 2-way interaction in the model. Total RNA yield from subcutaneous adipose tissues averaged 36.2  $\mu$ g/g and was not affected by treatment or quality grade. All 2-way interactions were significant ( $P < 0.05$ ) for the lipogenic genes evaluated. Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the conversion of saturated fatty acids to monounsaturated fatty acids. The SCD mRNA expression was downregulated ( $P < 0.05$ ) by 11.3-fold in IMP-LOW subcutaneous adipose tissues compared with CON-LOW. Expression of SCD did not differ ( $P > 0.05$ ) for CON-HI or IMP-HI compared with CON-LOW. Fatty acid synthase (FASN) is 1 of 2 enzymes regulating de novo fatty acid synthesis. The mRNA expression of FASN was downregulated ( $P < 0.05$ ) in IMP-LOW by 11.6-fold and IMP-HI by 1.9-fold compared with CON-LOW. Fatty acid elongase (ELOVL6) is the enzyme responsible for the elongation of fatty acids. ELOVL6 mRNA expression was downregulated ( $P < 0.05$ ) in IMP-LOW by 6-fold compared with CON-LOW. ELOVL6 mRNA expression did not differ ( $P > 0.05$ ) in IMP-HI or CON-HI compared with CON-LOW. Lipogenic gene expression was downregulated in subcutaneous fat from implanted steers with low quality grades.

**Key words:** beef, implant, gene expression

**W129 Signaling pathways mediating the effects of insulin-like growth factor-I on proliferation, protein synthesis, and protein degradation in bovine satellite cells.** X. Ge and H. Jiang\*, *Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg.*

The objective of this work was to identify the signaling pathways mediating the effects of IGF-I on proliferation, fusion, protein synthesis, and protein degradation in bovine muscle cells. Satellite cells were isolated from adult cattle skeletal muscle and were allowed to activate and proliferate or were induced to form myotubes following standard protocols. Cell proliferation was determined by measuring the numbers of viable cells at different times. Protein synthesis and degradation were determined by measuring the accumulation of 3H-phenylalanine in cellular protein and the release of 3H-phenylalanine to the medium, respectively. The signaling pathway involved was identified by including in the medium rapamycin, LY294002, or PD98095, which are specific inhibitors of the IGF-I receptor signaling molecules mTOR, AKT (PKB), and ERK (MAPK), respectively. Western blotting confirmed that IGF-I action caused phosphorylations of p70S6K (a signaling molecule immediately downstream of mTOR), AKT, and ERK, and that these phosphorylations were completely or near completely blocked by their corresponding inhibitors. Proliferation of bovine myoblasts was stimulated by 500 ng/mL IGF-I ( $P < 0.01$ ), and this stimulation was partially blocked by PD98095 ( $P < 0.05$ ), and was completely blocked by rapamycin or LY294002 ( $P < 0.01$ ). Protein degradation in myotubes was inhibited by approximately 20% by 500 ng/mL IGF-I ( $P < 0.05$ ), and this inhibition was completely relieved by LY294002 ( $P < 0.01$ ), but not at all by rapamycin or PD98095. Protein synthesis in myotubes was increased by 30% by 500 ng/mL IGF-I ( $P < 0.01$ ), and this increase was completely blocked by rapamycin, LY294002, or PD98095 ( $P < 0.01$ ). Addition of IGF-I to the culture medium had no effect on fusion of myoblasts into myotubes. These data suggest that IGF-I stimulates proliferation of bovine myoblasts and protein synthesis in bovine myotubes through both the PI3K/AKT and the MAPK signaling pathways, and that IGF-I inhibits protein degradation in bovine myotubes through the PI3K/AKT pathway only from the IGF-I receptor.

**Key words:** IGF-I, muscle, signaling

**W130 Effects of energy intake and age on the expression of adipogenic genes in subcutaneous and intramuscular fat in bovine Spanish Pirenaica breed.** B. Soret\*, P. Tiberio, A. Arana, JA Mendizabal, and L. Alfonso, *Universidad Publica de Navarra, Pamplona, Navarra, Spain.*

An improved understanding of the molecular mechanisms that drive adipose tissue development in livestock may allow for new strategies to modify adipose tissue distribution to improve meat quality by enhancing intramuscular fat (IMF). The objective of this study was to investigate the effects of dietary energy and age on the expression of key adipogenic genes in Pirenaica, a very low fattening breed widely used in cattle production systems in Navarra, Spain. Sixteen half-sibling young Pirenaica bulls were distributed into 4 groups ( $n = 4$ ) and slaughtered at 6, 12, and 18 mo of age; the later assigned to 2 groups differing in energy density in the ration (ME 3.29 and 2.87 Mcal/kg DM). Subcutaneous fat (SCF) and IMF were harvested at slaughter for mRNA isolation. Gene expression was measured by reverse transcription and quantitative PCR. Relative gene expression (Ct method) was calculated by normalizing against  $\beta$ -actin using the 6-mo-old group as calibrator. Backfat thickness (BFT), IMF chemical fat content, and adipocyte diameter were measured. Statistical

analysis was performed by ANOVA. No differences in gene expression for *PPAR $\gamma$* , sterol regulatory element binding protein (*SREBP*), fatty acid binding protein (*FABP*), lipoprotein lipase, and acetyl-CoA carboxylase (*ACC*) were found in IMF, maybe related to the low state of development of that depot in these animals which only had 2.4% chemical fat. Energy content of the diet did not affect SCF expression of any of the genes evaluated. In contrast *FABP* ( $P < 0.01$ ) and *ACC* ( $P < 0.001$ ) were affected by age, showing the higher values at 12 mo. Also BFT and the diameter of the SCF adipocytes showed the higher increase between 6 and 12 mo ( $P < 0.05$ ); thus, some genes involved in lipogenesis changed with age accordingly to changes in cell size in a depot-dependent manner. Differences between depots in expression of *FABP*, *PPAR $\gamma$* , and *SREBP* ( $P < 0.001$ ) were found. This, together with changes with age for SCF only, may suggest depot-specific patterns of gene expression during fattening.

**Key words:** adipogenic gene, intramuscular fat, bovine

**W131 Age post weaning but not birth weight and sex affects the small intestinal glutathione redox status of piglets.** J. Michiels\*<sup>1,2</sup>, E. Claeys<sup>2</sup>, A. Obyn<sup>2</sup>, and S. De Smet<sup>2</sup>, <sup>1</sup>*Faculty of Biosciences and Landscape Architecture, University College Ghent, Ghent, Belgium*, <sup>2</sup>*Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Melle, Belgium.*

Glutathione (GSH) serves as the major endogenous antioxidant in gut tissue and cells keep it predominantly in the reduced state, i.e., a low oxidized to reduced glutathione (GSSG/GSH) ratio. The aim of the study was to assess the effect of birth weight, sex, and age post weaning on small intestinal mucosal glutathione redox status of piglets. Newborns from 17 Danbred hybrid sows were weighed and tagged. At weaning (18.8  $\pm$  0.44d) pairs of intra-uterine growth-retarded (IUGR) and normal birth weight sex-matched littermates were selected and fed a starter ad libitum until 1h before sampling at 0, 2, 5, 12, and 28d post weaning. An IUGR pig was defined as having a birth weight  $< 1$  kg and  $<$  mean litter birth weight  $- 1.5$  SD. Mucosa was collected from 2 small intestinal sites; at 5% ( $\approx$ end of duodenum) and at 75% of total length. GSH and GSSG were determined by HPLC using  $\gamma$ -Glu-Glu as internal standard following the reaction of thiols with iodoacetic acid to form S-carboxymethyl compounds and derivatization with 2,4-dinitrofluorobenzene. Data were analyzed by linear models with birth weight, sex, and age post weaning as fixed factors and presented as adjusted means. Birth weight and sex showed no significant effects. A temporal decline in GSH content at d2 and increase in the GSSG/GSH ratio at d5 in the proximal small intestinal mucosa indicates that oxidative stress occurred in that time window (Table 1). At 75% of length of the small intestine there was a gradual decrease of the GSSG/GSH ratio with time. The higher GSH content and GSSG/GSH ratio in the proximal small intestine might illustrate the higher need for antioxidant action against dietary pro-oxidants at that site.

**Table 1.** Effect of age post weaning (d) on glutathione redox status of small intestinal mucosa in piglets (n = 16)

	Age post-weaning (d)					SEM	P-value
	0	2	5	12	28		
5% of length							
GSH (μmol/g)	1.94 <sup>c</sup>	1.79 <sup>c</sup>	2.11 <sup>c</sup>	2.65 <sup>b</sup>	3.27 <sup>a</sup>	0.075	<0.001
GSSG/GSH	0.042 <sup>b</sup>	0.043 <sup>b</sup>	0.089 <sup>a</sup>	0.044 <sup>b</sup>	0.046 <sup>b</sup>	0.0040	<0.001
75% of length							
GSH (μmol/g)	1.23 <sup>b</sup>	1.44 <sup>b</sup>	1.79 <sup>a</sup>	1.89 <sup>a</sup>	1.90 <sup>a</sup>	0.062	0.001
GSSG/GSH	0.036 <sup>a</sup>	0.038 <sup>a</sup>	0.031 <sup>ab</sup>	0.030 <sup>ab</sup>	0.027 <sup>b</sup>	0.0036	0.055

<sup>a-c</sup>Values with different superscripts within a row are significantly different at  $P < 0.05$ ; LSD-test.

**Key words:** pig, glutathione, gut health

**W132 Feed restriction alters reactivity of body fat after catabolic stimulation in growing pigs.** B. U. Metzler-Zebeli, S. Görs, K. Giggel, R. Krüger, H. M. Hammon, and C. C. Metges\*, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Birth weight (BIRTH) and feed restriction (FR) may affect later body composition due to persistent alterations in catabolic and anabolic processes. Therefore, we investigated the effect of BIRTH and FR on the reactivity of body fat after stimulation of catabolic processes by pST-Clenbuterol (ST+C). Two female littermate pigs from 20 sows each with low ( $\leq 1.1$  kg) or normal birth (1.4 to 1.6 kg) weight were used. Half of the pigs were fed ad libitum whereas the other half was restrictively fed (50% ad libitum) between ages d 78 and 98. Subsequently, all pigs were (re)fed ad libitum. At d 84 of age, 20 pigs were fitted with catheters in A. carotis and V. jugularis. After intravenous ST+C administration, plasma lipids, glycerol, and glucose concentrations were determined over 12 h at ages d 96, 104, and 118. Body fat content was determined using DXA measurement at ages d 75 and 96. The statistical model included BIRTH, feeding type, time, litter size group, interactions, and random factor sow. Mobilization of body fat and glycogen due to ST+C was confirmed by increased ( $P < 0.01$ ) plasma triglycerides (TG; 1 to 8 h after ST+C), glycerol (1 to 6 h after ST+C) and glucose (0.5 to 6.5h after ST+C) concentrations at d 96. Plasma TG, glycerol, and glucose were not affected by BIRTH and FR. In contrast, plasma NEFA were greater ( $P < 0.01$ ) in restrictively compared with ad libitum-fed pigs after ST+C at d 96 indicating increased lipolysis. The enhanced catabolic status in restrictively-fed pigs was confirmed by their lower body fat as compared with ad libitum-fed pigs at age d 96 ( $P < 0.05$ ). Although TG increased after ST+C stimulation at ages d 104 and 118, NEFA release did not differ among pigs at age d 104. Interestingly, after ST+C stimulation at d 118 plasma NEFA were again greater in pigs that were restrictively fed between d 78 and 98 of age ( $P < 0.05$ ), but were not affected by BIRTH. In conclusion, FR causes alterations in the catabolic reactivity of body fat not only during the immediate FR period but also at 3 wk of refeeding. However, BIRTH did not affect body fat mobilization after catabolic stimulation. Supported by BMBF (VISION EPIFOOD).

**Key words:** lipolysis, body fat, NEFA

**W133 The effect of different methods of using zilpaterol hydrochloride on growth performance in Japanese quail.** M. Mohammadi\*, A. Towhidi, H. Moravej, and A. Zareh Shahne, *Department of Animal Science, University of Tehran, Karaj, Alborz, Iran.*

Zilpaterol hydrochloride is a  $\beta_2$ -adrenergic agonist which has been shown to increase lean muscle and decrease fat deposition. It seems that the chronic supplementation of  $\beta$ -agonists diminishes the response because of desensitizing of the receptors. This study was designed to compare 2 methods of using zilpaterol hydrochloride including once a day and skip 2 d on growth performance in Japanese quail. Ninety-six quail of 33 d of age were assigned to 3 groups with 4 replications. Treatments were defined as: T1 as control, T2 received zilpaterol skip 2 d, and T3 received zilpaterol once a day. Diets were based on corn and soybean meal in the finisher period (24% CP and 2.9 Mcal/kg of ME) and the birds orally received 0.225 mg/kg of live weight/d zilpaterol for 14 d and slaughtered at 50 d of age. The complete randomized design in GLM procedure was used to analyze the data. Results showed zilpaterol supplementation improved weight gain ( $P < 0.0001$ ) and feed conversion ratio ( $P < 0.001$ ) in both treatments compared with control, but did not affect feed intake at d 33 to 40 ( $P = 0.10$ ), whereas at d 40 to 47, zilpaterol did not have a significant effect on growth performance ( $P > 0.05$ ). Furthermore, there were no significant differences in weight gain ( $P = 0.15$ ), feed conversion ratio ( $P = 0.21$ ), or feed intake ( $P = 0.31$ ) between the 2 treatments that received zilpaterol at 40 to 47 d. The feed conversion ratio ( $P = 0.23$ ) and weight gain ( $P = 0.13$ ) were negatively affected in the period of 40 to 47 d in all groups. It seems that maturity and hormonal modification had considerable effects on growth performance. It was concluded that zilpaterol hydrochloride could improve growth performance when used by either method for the 33 to 40 d of quail rearing. However, considering the economics, the skip 2 d was better than once-a-day consumption and will have less cost.

**Key words:** zilpaterol hydrochloride, Japanese quail, growth performance

**W134 Effects of dietary supplementation of sodium stearoyl-2-lactylate in a low-energy density diet on growth performance, blood profiles, and relative organ weight in broilers.** S. M. Hong\*, J. P. Wang, and I. H. Kim, *Dankook University, Cheonan, Choongnam, South Korea.*

The aim of this study was to investigate the effects of supplementation of a dietary emulsifier sodium stearoyl-2-lactylate (SSL) in a low-energy density diet on growth performance, blood profiles, and relative organ weight in broilers. A total of 260 male and female ROSS 308 broiler chicks (2-d old, average BW =  $45 \pm 1.0$  g) were randomly allotted to 1 of 5 treatments with 4 replications per treatment and 13 chicks per pen. The diets were fed during the experiment in 2 phases consisting of a starter phase from d 0 to 21 and a finisher phase from d 22 to 35. A corn-soybean meal-based diet was formulated as a control diet and dietary treatments were as follows: 1) NC (negative control; -200kcal ME/kg energy down spec diet), 2) PC (positive control; 3,150kcal ME/kg), 3) P1 (-50 kcal ME/kg energy down spec diet + 0.05% SSL), 4) P2 (-150kcal ME/kg energy down spec diet + 0.05% SSL), and 5) P3 (NC + 0.1% enzyme and 0.05% SSL). Body weight gain (BWG) was greater in PC, P1, and P3 treatments than in NC treatment ( $P < 0.05$ ) and feed intake (FI) was lesser ( $P < 0.05$ ) in P2 treatment than in P3 treatment throughout the whole experiment. The PC and P1 treatments had greater ( $P < 0.05$ ) FCR than NC and P3 treatments overall during the experiment. Lymphocyte percentages in NC and PC treatments were greater ( $P < 0.05$ ) than that in P1 treatment. Birds fed PC, P1, and P3 diets resulted in an increased triglyceride level compared with birds fed NC diet ( $P < 0.05$ ). The relative spleen weight was decreased ( $P < 0.05$ ) in P3 treatment compared with CON treatment. The bursa of Fabricius was heavier in P3 treatment than

that in PC, P1, and P2 treatments. In conclusion, SSL administration partially improved BG, FCR, and triglyceride level.

**Key words:** broiler, emulsifier, growth performance

**W135 Insulin-like growth factor-I (IGFI), IGF binding proteins (IGFBP), and growth hormone receptor (GHR) mRNA concentration in fetal liver and duodenum in response to variable maternal nutrition during gestation.** M. Field\*, R. Anthony, T. Engle, S. Archibeque, and H. Han, *Colorado State University, Fort Collins.*

Undernutrition during gestation is known to influence fetal development and predispose offspring to the metabolic syndrome. We investigated the mRNA concentration of IGFI, IGFBP-2 and -3, and GHR in liver and duodenum from twin fetal lambs. Multiparous whiteface ewes were randomly assigned to 1 of 3 treatments at 21 d of gestational age (dGA). Ewes were either fed 100% (Control; n = 7) or 50% of nutrient requirements from 28 to 78 dGA and readjusted to 100% beginning at 79 dGA (50-100; n = 5) or 50% of requirements from 28 to 110 dGA, followed by a 5% increase at 5-d intervals until 135 dGA (50-50; n = 7). Fetal liver (L) and duodenum (D) were collected at 135 dGA. Concentration of each mRNA was corrected by ribosomal protein S15 mRNA concentration. Concentration of IGFI mRNA did not differ in fetal liver ( $P = 0.54$ ) and duodenum ( $P = 0.32$ ), but was numerically greater in 50-100 (L =  $0.90 \pm 0.02$ , D =  $0.58 \pm 0.05$ ) than Control (L =  $0.87 \pm 0.02$ , D =  $0.54 \pm 0.04$ ) and 50-50 (L =  $0.87 \pm 0.02$ , D =  $0.64 \pm 0.04$ ) fetuses. There were no differences in expression of IGFBP3 mRNA between treatments in either liver or duodenum. Liver expression of IGFBP2 mRNA did not differ and concentrations were low or undetectable in duodenum. Liver GHR mRNA was numerically greater ( $P = 0.15$ ) in 50-100 fetuses ( $0.92 \pm 0.02$ ), than Control ( $0.90 \pm 0.02$ ) and 50-50 ( $0.87 \pm 0.02$ ) fetuses. Duodenal GHR mRNA did not differ (Control  $0.78 \pm 0.03$ ; 50-100  $0.72 \pm 0.04$ ; 50-50  $0.81 \pm 0.03$ ;  $P = 0.266$ ). Natural intrauterine growth restriction in twin pregnancy may contribute to lower IGFI and GHR concentrations while realimentation from mid-gestation may induce elevated GHR and IGFI expression, which contributes to compensatory fetal growth during late gestation in twin pregnancy. This project was supported by National Research Initiative Competitive Grant no. 2009-35206-05273 from the USDA National Institute of Food and Agriculture.

**Key words:** sheep, insulin-like growth factor I, growth hormone

**W136 Effects of variable maternal undernutrition on uterine and umbilical IGF-I, insulin, and ghrelin concentrations in near-term sheep twin pregnancies.** M. Field\*, R. Anthony, T. Engle, S. Archibeque, and H. Han, *Colorado State University, Fort Collins.*

Maternal undernutrition during gestation alters the developmental environment of a fetus and predisposes the offspring to the metabolic syndrome in later life. We investigated the impact of maternal undernutrition on IGF-I, insulin, and ghrelin concentrations in uterine and umbilical blood in near-term twin pregnant sheep. Multiparous whiteface ewes were randomly assigned to 1 of 3 treatments and acclimation to individual pens (7 d) begun at 21 d of gestational age (dGA). Ewes were either fed 100% (Control; n = 7), or 50% of nutrient requirements from 28 to 78 dGA and readjusted to 100% beginning at 79 dGA (50-100; n = 5) or 50% of requirements from 28 to 110 dGA, followed by a 5% increase at 5-d intervals until 135 dGA (50-50; n = 7). During cesarean section uterine and umbilical blood was collected while the fetus was viable. Blood hormone concentrations were determined by RIA and analyzed using the PROC MIXED model of SAS.

Uterine artery IGF-I and insulin concentrations were not different between treatments. Umbilical vein (UV) IGF-I was greater ( $P = 0.02$ ) in 50-100 ( $74.4 \pm 6.7$  ng/mL) than Control ( $50.9 \pm 5.9$  ng/mL) and 50-50 ( $46.7 \pm 5.6$  ng/mL). Umbilical artery (UA) IGF-I exhibited a similar trend ( $P = 0.10$ ) where 50-100 ( $91.4 \pm 9.0$  ng/mL) was greater than Control ( $67.9 \pm 8.1$  ng/mL) and 50-50 ( $66.6 \pm 7.5$  ng/mL). Insulin in UA was greater ( $P = 0.08$ ) in 50-100 ( $0.70 \pm 0.15$  ng/mL) than Control ( $0.30 \pm 0.14$  ng/mL) and 50-50 ( $0.24 \pm 0.13$  ng/mL). The UV and UA ghrelin concentrations were not different between treatments, although UV and UA ghrelin were numerically greater in 50-50 (UA =  $44.5 \pm 10.3$ , UV =  $31.8 \pm 7.5$  pg/mL) than both Control (UA =  $29.6 \pm 11.1$ , UV =  $25.3 \pm 8.0$  pg/mL) and 50-100 (UA =  $24.5 \pm 12.5$ , UV =  $23.0 \pm 8.8$  pg/mL). The IGF-I and insulin concentrations in 50-100 umbilical V and A indicate a shift in IGF-I and accelerated fetal growth as a result of nutrient deprivation followed by realimentation.

**Key words:** undernutrition, IGF-I, blood

**W137 Transfer of omega-3 fatty acids from dams to calves in dairy cows.** M. Zachut<sup>\*1,2</sup>, A. Romanenco<sup>1,2</sup>, H. Lehrer<sup>1</sup>, A. Arieli<sup>2</sup>, and U. Moallem<sup>1</sup>, <sup>1</sup>*Agriculture Research Organization, Bet Dagan, Israel*, <sup>2</sup>*Faculty of Agriculture, Hebrew University, Rehovot, Israel.*

In many species fatty acid (FA) composition of the maternal diet during pregnancy can affect the FA composition of the fetus. Omega-3 FA have a crucial role in neonatal brain development, yet the transfer of long chain FA through the placenta in ruminants is very limited. The objectives were to examine 1) the plasma FA composition in newborn calves, and 2) the transfer of various omega-3 FA from dams into calves' plasma. Twenty 7 multiparous Israeli-Holstein dry cows (256 d pregnant) were assigned to 3 groups and supplemented with 300 g/d per cow of encapsulated fat that contained: (i) control - saturated FA; (ii) FLX - 51.3 g/d per cow 18:3n-3 (ALA) from flaxseed oil, and (iii) FO - 3.6 C22:5n-3 (DPA) and 3.0 g/d per cow C22:6n-3 (DHA) from fish oil. Blood samples were collected from cows twice a week and from calves immediately after calving, before colostrum offering. FA composition was determined in dams in the last sample before parturition. Data were analyzed using the GLM model of SAS. Across treatments analysis revealed that the proportions of saturated and mono-unsaturated FA in plasma were greater in calves than in cows (49.8 vs. 42.5% and 30.0 vs. 12.7%, respectively), while the proportion of polyunsaturated FA (PUFA) was 2-fold greater in dams than in calves (44.8 vs. 20.1%, respectively). The proportion of ALA in plasma of FLX cows was elevated to 5.2% as compared with 2% in the control; however, this FA was not transferred into calves' blood. Greater plasma proportions of DPA (0.32 vs. 0.16%) and DHA (0.30 vs. 0.02%) were found in the FO cows than in the controls, respectively, and the proportion of DHA was nearly doubled in the FO calves' blood as compared with controls (0.47 vs. 0.26%, respectively). Furthermore, the FO calves had greater proportions of total PUFA in plasma as compared with both other groups (23.9 vs. 18.5%, respectively). In summary, the low permeability of the placenta to FA resulted in a very different plasma FA composition in newborn calves as compared with dams. Furthermore, dietary DHA, but not ALA, passages from dam to fetus in cows, perhaps in an active transfer due to the essentiality of this FA to fetal development.

**Key words:** omega-3, calf, fatty acid composition

**W138 Temporal changes in the proteome of the uterine histotroph in cattle.** M. P. Mullen<sup>\*1</sup>, A. C. O. Evans<sup>2</sup>, G. Elia<sup>3</sup>, M.

Hilliard<sup>3</sup>, N. Forde<sup>2</sup>, M. H. Parr<sup>1</sup>, M. G. Diskin<sup>1</sup>, and M. A. Crowe<sup>2</sup>, <sup>1</sup>Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Athenry, Co. Galway, Ireland, <sup>2</sup>School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, <sup>3</sup>Conway Mass Spectrometry Resource, University College Dublin, Belfield, Dublin 4, Ireland.

The composition of the uterine fluid (or histotroph) that bathes the early embryo is critical for its growth and development as it is the sole supply of nutrients. The objective of this study was to characterize the proteome of the bovine histotroph during key stages of the estrous cycle. The uterine horn ipsilateral to the corpus luteum of Holstein-Friesian heifers on Day 7 (n = 6) and Day 13 (n = 6) of separate estrous cycles was non-surgically flushed with 50 mL of 100 mM Tris pH 7.2. Global protein abundance was analyzed using a label-free shot gun proteomics approach encompassing SCX fractionation coupled with reversed phase LC-MS/MS analysis. Thresholds for defining proteins more abundant on either day included (i) an average spectral count value  $\geq 2$ , (ii) signal in at least 3 animals and (iii) spectral count ratio of  $\geq 5$  between days. This led to the classification of 20 proteins more abundant on Day 7 vs. Day 13 including serpins, immune related complement proteins, structural cytokeratins, and hypothetical proteins. In addition, 35 proteins were more abundant on Day 13 vs. Day 7 and included novel bovine histotroph proteins such as members of the Cathespin family B, D, Z, and L2; previously reported bovine histotroph protease modulators Legumain (LGN), Metalloproteinase inhibitor 2 (TIMP2), Tripartite motif-containing protein 25 (TRIM25); metabolic proteins Actin and Lysosomal  $\alpha$ -mannosidase (MAN2B1) and growth factor binding proteins IGFBP-1 and -5. Furthermore, uncharacterized and structural proteins were only identified on Day 7 while proteins involved in stressful microenvironment management were more abundant on Day 13. Lowering the spectral count ratio threshold to include proteins with a ratio of 2 to 4 resulted in an additional 28 proteins more abundant on Day 7 vs. Day 13 and 46 proteins more abundant on Day 13 vs. Day 7. Because temporal changes in uterine gene expression between Day 7 and Day 13 are associated with embryo development, we propose that the abundance of these proteins is similarly supportive of embryo development and required for the establishment of pregnancy in cattle. Funded by Science Foundation Ireland 07/SRC/B1156.

**Key words:** uterus, histotroph, global proteomics

**W139 Effect of maternal diet on the ontogenetic development of the hepatic proteome in intrauterine growth-restricted porcine offspring.** M. Peters, B. Kuhla, I. S. Lang, E. P. Rudolph, and C. C. Metges\*, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

We determined the effect of intrauterine growth restriction (IUGR) caused by excess and low-protein gestation diets (Rehfeldt et al. *J Anim Sci* 89:329, 2011) on development of hepatic proteome of pre- and postnatal porcine offspring. Gilts (n = 58; 241 d, 150.6 kg) were randomly assigned to 3 diet groups. The isocaloric diets contained adequate (AP, 12.1%), high (HP, 30%) or low (LP, 6.5%) protein levels at the expense of carbohydrates. Pigs were killed and liver samples of light (L) and heavy (H) offspring at d 94 post conception (dpc), d 1, 28, and 188 post natum (dpm) were analyzed by 2D-SDS-PAGE and MALDI-TOF MS. The model (SAS PROC MIXED) included maternal diet, offspring BW class (94 dpc, 1 dpm), sex, all interactions, and Tukey-Kramer test ( $P \leq 0.05$ ). In HP fetuses and LP neonates the same

number of proteins related to glycolysis (GL) and glycogen synthesis (GS) were diet dependently affected, whereas proteins of gluconeogenesis (GNG) were increased in HP and L neonates and L offspring at 28 dpm (Table 1). The LP fetuses had an increased expression of GL-related enzymes and a reduced expression of proteins related to GS. Proteins related to TCA cycle were upregulated in HP, LP, and L fetuses, as well as in LP and L offspring at 28 dpm, but downregulated in HP, LP, and L neonates and HP offspring at 28 dpm, whereas they could not be detected at 188 dpm. Validation of 6 selected proteins at 1 dpm via Western blot confirmed the expression pattern obtained from 2D analysis. In conclusion, maternal LP and HP diets persistently changed the offspring proteome profile of major metabolic pathways. Different diet-dependent profiles indicate different intrauterine regulatory mechanisms leading to offspring IUGR. Supported by DFG (ME 1420/8-1) and BMBF (Fugatoplus-FEPROeXPRESS)

**Table 1.** Effect of maternal gestation diet and BW class on offspring hepatic protein expression ratios related to glucose metabolism

Effect	94 dpc	1 dpm	28 dpm	188 dpm
HP vs. AP	GS = GL = 0.94	GNG = 1.04	GL = 0.87	GL = 1.13
LP vs. AP	GL > GS = 1.18	GS = GL = 1.13	GL = 0.95	GL = 1.32
L vs. H	GL = 1.11	GL, GNG = 1.11	GNG = 1.45	GL = 1.18

**Key words:** proteomics, fetal growth retardation, pig

**W140 Changes in plasma amino acid concentrations in preterm and term born calves.** J. Steinhoff-Wagner\*, S. Görs, J. Flor, C. C. Metges, and H. M. Hammon, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Neonatal development is characterized by ontogenic maturation and high nitrogen (N) turnover. Adequate amino acid (AA) availability is important for protein and nucleic acid synthesis, but might be impaired in immature calves. The objective of the present study was to investigate dependency of plasma AA changes on ontogenic development and colostrum feeding in neonatal calves. Calves were delivered by Cesarean section 9 d before term (preterm; PT) or were born at term (T). Calves of PT and T were not fed during first 24 h of life. Calves of TC were born at term and were fed colostrum at 8% of BW during first 24 h of life (n = 7/group). Blood samples were taken at 2 to 3 h and 24 h after birth and before feed intake (TC) for determination of total protein (TP), urea, and free AA plasma concentrations. Data were analyzed by Mixed Model of SAS with ontogenic stage and postnatal feeding as fixed effects. Plasma concentrations of TP increased ( $P < 0.05$ ) during 24 h only in TC due to immunoglobulin absorption. Plasma urea concentrations were greatest ( $P < 0.05$ ) at birth and 24 h after birth in PT. At birth, plasma concentrations of Phe, Val, Leu, Ile, Glu, and Tyr were greater ( $P < 0.05$ ) in PT than T and TC, whereas after 24 h plasma concentrations of Lys, Thr, Glu, Asp, Asn, Ala, Cys, Ser, Orn, and Pro were greater ( $P < 0.05$ ) in PT than T. Colostrum feeding resulted in greater ( $P < 0.05$ ) plasma concentrations of Leu, Val, Ile, Trp, His, and Tyr in TC than in T and PT, and greater ( $P < 0.05$ ) plasma concentrations of Asp and Pro than in T. Plasma concentrations of Thr, Gly, Ala, Cys, Ser, and Orn were greater ( $P < 0.05$ ) in PT than in TC. Plasma concentrations of Glu increased ( $P < 0.05$ ) in TC and were greatest ( $P < 0.05$ ) 24 h after birth in TC, whereas Gln increased in PT, but decreased in TC and were lowest ( $P < 0.05$ ) 24 h after birth in TC. Greater essential AA (EAA) in PT than in T and greater non-EAA and urea plasma concentrations in PT than in T and TC suggested enhanced protein breakdown and AA degradation in PT. Colostrum

feeding leads to an improved EAA status and less AA degradation, but indicates enhanced N use for anabolic metabolism.

**Key words:** calf, preterm, amino acid

**W141 Placental and fetal plasma amino acid uptake and release in mid and late pregnancy of gilts fed limited- and excess-protein diets associated with intrauterine growth retardation (IUGR).** C. C. Metges\*, S. Görs, I. S. Lang, K.-P. Brüssow, G. Nürnberg, C. Rehfeldt, W. Otten, and B. U. Metzler-Zebeli, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Low- and high-protein diets fed to pregnant gilts lead to IUGR (Rehfeldt et al. 2011, *J Anim Sci*). To explore underlying mechanisms, maternal, umbilical, and fetal amino acid (AA) concentrations were analyzed. Eighteen gilts each were fed isoenergetic diets with low (6.5%, LP), adequate (12.1%, AP), or high (30%, HP) protein levels, starting at insemination. Gilts and fetuses were examined at d 64 and 94 of pregnancy. Blood was collected during Caesarian section from maternal V. jugularis, umbilical vein (V) and artery (A), and fetal V. cava cranialis (4 fetuses/gilt). Fetal weight was recorded. Plasma AA concentration was analyzed by HPLC. Placental and fetal AA uptake

(UPT) and release (REL) were calculated by V-A differences. Effects were evaluated by ANOVA with diet, fetal litter size, sex and weight class, interactions and random factor gilt. In HP, fetal weight was lower than in AP at d 94 but not at d 64 ( $P < 0.05$ ). In LP maternal plasma, Leu, Phe, Tyr, Thr, and Trp were lesser and Ala was greater at d 64, whereas at d 94 Leu, Trp, Val, Cys, and Tyr were lesser and Ala and Gly were greater than in AP ( $P < 0.05$ ). In HP gilts at d 64, Ile, Thr, and Val concentrations were greater and Ala and Gly were lesser than in AP gilts whereas at d 94, Ile, Lys, and Val were greater and Ala, Gly, and Glu were lesser ( $P < 0.05$ ). At d 64 and d 94, placental UPT of Ile, Leu, Trp, and Val was greater in HP than in LP. Placental Lys REL did not differ at d 64 but was lower in HP and LP than in AP at d 94 ( $P < 0.05$ ). At d 64, Thr was taken up by the placenta in HP while it was released in LP; at d 94, placental Thr REL was greater in LP and AP than in HP ( $P < 0.05$ ). At d 64, fetal AA UPT did not differ among diets, whereas at d 94 Leu and Lys UPT was lower in LP and HP than in AP ( $P < 0.05$ ). Thus, placental AA metabolism largely compensated the imbalanced maternal AA patterns. In LP, but also in HP, fetal utilization of Leu and Lys was limited, which relates to lower birth weights in both groups.

**Key words:** amino acid, high protein, intrauterine growth retardation