corn-soybean meal diet with 5% DDGS and 4) corn- soybean meal diet with 5% DDGS + 0.05% enzyme complex. The pigs were allotted randomly into four pigs per pen with six replicate pens per treatment in a completely randomized design. Pigs were slaughtered at the end of experiment and the loin muscle was obtained for meat quality. Meat pH ($p<0.01$), firmness ($p<0.01$) and redness ($p<0.05$) were higher in DDGS treatment than corn-soybean meal treatment. However, color, marbling, lightness, yellowness, TBARS, water holding capacity, drip loss, cooking loss and loin muscle area were not significantly different among treatments ($p>0.05$). The pigs fed the diet containing DDGS had higher total UFA concentration and total UFA/SFA ratio of loin and backfat. In conclusion, DDGS can change pH, firmness, redness and total UFA concentration and total UFA/SFA ratio of meat and backfat, however, enzyme addition has no affect on meat quality.

Key Words: DDGS, fatty acid composition, finishing pigs

M213 Supplementation with phytase and xylanase can increase energy availability in swine diets containing corn distillers dried grains with solubles (DDGS). M. D. Lindemann*[1], G. A. Apgar[2], G. L. Cromwell[1], P. H. Simmins[3], and A. Owusu-Asiedu[3], [1]*University of Kentucky*, *Lexington*, [2]*Southern Illinois University*, *Carbondale*, [3]*Danisco Animal Nutrition*, *Marlborough, UK*.

One way of enhancing dietary energy at times of high feed prices is to use exogenous enzymes to improve diet digestibility and utilize more of the nutrients already present in a diet containing byproducts such as DDGS. To examine the potential for enzymes to enhance nutrient release, a study was conducted with 96 crossbred pigs (mean initial and final BW of 64 and 123 kg) allotted to pens of 4 pigs (2 barrows and 2 gilts). Treatments were: 1) a positive control [PC] corn-soybean meal diet with 20% DDGS and 3% choice white grease [CWG], and 2) a negative control [NC] similar to the PC but with 1% CWG and no inorganic P source. The NC was lower in ME [90 kcal/kg] and available P [about 0.02%]. The enzymes added were phytase (Phyzyme® 6-phytase, EC 3.1.3.26; PHY; 250 or 500 U/kg diet) and xylanase (Porzyme® 9300, endo 1,4-beta-xylanase; XYL; 2000 or 4000 U/kg diet). Diets 3-8 were the NC plus: 3) 250 PHY and 0 XYL, 4) 250 PHY and 2000 XYL, 5) 250 PHY and 4000 XYL, 6) 500 PHY and 0 XYL, 7) 500 PHY and 2000 XYL, and 8) 500 PHY and 4000 XYL. The ADG for the PC and NC (1.04 vs 1.05 kg), ADF (2.78 vs 2.93 kg), and F/G (2.68 vs 2.78) were as anticipated with higher F/G in the NC diet. Fecal digestibility for DM (77.1 vs 73.7%, P=0.02), energy (75.5 vs 71.4%, P=.006), and N (72.7 vs 68.8%, P=0.007) was consistently higher for PC compared to NC. For Trt 3-8 the ADG (1.04, 1.07, 1.03, 1.00, 0.95, and 1.01 kg) and F/G (2.77, 2.73, 2.66, 2.75, 2.75, and 2.68) illustrated an apparent release of energy with incremental PHY and XYL additions. For Trt 3-8 the DM (76.1, 76.7, 74.6, 76.2, 74.4, and 74.6%), energy (73.8, 74.4, 71.2, 73.7, 72.0, and 71.6%), and N (70.9, 71.7, 70.3, 71.1, 69.5, and 71.3%) digestibility confirmed an improved digestibility. The inclusion of PHY improved digestibility ($P<0.05$) of all 3 components. Further improvements in fecal digestibility were not observed with XYL but the recovery of F/G was observed only when the high level of XYL was included with the PHY. These data demonstrate that appropriate exogenous enzymes are a means of nutrient release in diets containing byproducts.

Key Words: phytase, pigs, xylanase

Physiology and Endocrinology: Endocrinology and Metabolism

M214 Methionine requirements for the preimplantation bovine embryo. L. Bonilla*[1], D. Luchini[2], E. Devillard[3], and P. J. Hansen[1], [1]*University of Florida*, *Gainesville*, [2]*Adisseo USA, Inc.*, *Alpharetta, GA*, [3]*Adisseo France, SAS*, *Commentry, France*.

The objective was to determine the requirement of in vitro produced embryos for the essential amino acid methionine. Oocytes were matured for 20-22 h and fertilized for 6-8 h. Embryos were cultured in groups of 15 in 25 µL microdrops of potassium simplex optimized medium - bovine embryo modification 2 (KSOM-BE2) at 38.5°C in 5% (v/v) oxygen. In Experiment 1 (n=963 putative zygotes in 4 replicates), embryos were cultured with 0, 35, 50, 100, 200 or 400 µmol/L L-methionine for 8 d. The percent of oocytes that cleaved was observed at Day 3 after insemination and blastocyst development at Day 7 and 8. At Day 7, a group of blastocysts was stained with Hoescht 33258 to determine total cell number. There was no effect of methionine concentration on cleavage rate. The percent of oocytes that developed to blastocyst was lower for embryos without methionine at Day 7 ($P<0.05$) and 8 ($P<0.01$) than other groups but was similar for embryos cultured with 35-400 µmol/L. Least-squares means were 4.2, 23.2, 18.2, 21.5, 16.3, and 21.3 for 0, 35, 50, 100, 200, or 400 µmol/L for Day 7 (SEM=3.4%) and 13.5, 36.1, 30.9, 33.6, 29.8 and 33.0 for Day 8 (SEM=3.4%). Total cell number was not affected by methionine concentration. In Experiment 2 (n=1,204 putative zygotes in 4 replicates), embryos were cultured with 0, 7, 14, 21, 28 or 35 µmol/L methionine. There was no effect of methionine concentration on cleavage rate. The percent of oocytes that developed to blastocyst was lower for embryos without methionine at Day 7 ($P<0.005$) and 8 ($P=0.01$). At Day 7, least-squares means were 8.2, 20.3, 27.2, 27.7, 24.3, and 21.2 for 0, 7, 14, 21, 28, or 35 µmol/L (SEM=2.5%). There was a tendency for 7 µmol/L to be lower than 14 ($P=0.07$) and 21 µmol/L ($P=0.06$). At Day 8, least-squares means were 17.8, 35.2, 37.8, 43.0, 37.9, and 33.6 for 0, 7, 14, 21, 28, or 35 µmol/L (SEM=3.5%). Means were similar for 7-35 µmol/L. In conclusion, methionine requirements for optimal blastocyst yield are between 7 and 21 µmol/L. Further studies to further define optimal concentration and to examine competence of embryos for development after transfer are warranted. *Support: Adisseo.*

Key Words: methionine, embryos, development

M215 Effect of exogenous insulin and fasting on estradiol production and growth hormone receptor (GHR) and insulin-like growth factor I (IGF-I) genes expression by the pre-ovulatory follicle of ewes. A. Schneider[1], L. F. M. Pfeifer[1], E. Schmitt[1], J. W. Silva Neto[1], L. T. Hax[1], M. M. Antunes[1], F. A. B. Del Pino[1], G. R. Paludo[2], and M. N. Corrêa*[1], [1]*Federal University of Pelotas*, *Brazil*, [2]*University of Brasilia*, *Brazil*.

The aim of this study was to investigate the effect of fasting and insulin injections for 96 hours on estradiol concentrations and expression of GHR and IGF-I mRNA in the pre-ovulatory follicle of ewes. In the eleventh day of the estrous cycle 15 ewes received an injection of $PGF_2\alpha$, 36 hours after a GnRH injection and 24 hours after a CIDR® was inserted and removed 6 days later together with an injection of $PGF_2\alpha$ (Day 0). In Day -2 the ewes were divided in: 1) control group (CG, n = 5) that received a maintenance diet; 2) insulin group (IG, n = 5) that received insulin injections (s.c., 0.25 IU/kg) every 12 hours

from Day -2 to 2 and 3) fasting group (FG, n = 5), that was submitted to fasting from Day -2 to 2. Estradiol concentrations were evaluated on Day 2, when ovaries were also collected for evaluation of the follicular population and dissection of theca (TC) and granulosa (GC) cells of the pre-ovulatory follicle (> 4 mm). Expression of GHR and IGF-I mRNA was evaluated through real time RT-PCR. Data were compared among groups by one-way ANOVA using the Tukey-Kramer adjustment. The diameter of the pre-ovulatory follicle on Day 2 was not different among groups (7.60 ± 0.38 mm) neither the number of small (< 2 mm, 10.73 ± 2.19), medium (< 4 mm, 0.8 ± 0.2) and pre-ovulatory (1.13 ± 0.09) follicles per ewe was different. IG had higher (P<0.05) estradiol concentrations on Day 2 (53.70 ± 1.82 pg/mL) than FG (29.97 ± 6.96 pg/mL), but was not different from CG (35.92 ± 5.72 pg/mL). Although GHR or IGF-I mRNA expression was detected in GC and TC, no difference among groups was detected. For IG estradiol was positively correlated to follicular diameter (r=0.93, P<0.05), GC GHR (r=0.87, P<0.01) and IGF-I mRNA (r=0.79, P<0.1). In conclusion, insulin injection increased estradiol production without any change in the expression of GHR and IGF-I mRNA in the pre-ovulatory follicle.

Key Words: GHR, IGF, insulin

M216 TNFα and adipocyte-hepatic metabolism at drying off and during early lactation in dairy cows. H. A. van Dorland[1], H. Sadri[2], and R. M. Bruckmaier*[1], [1]*University of Bern, Vetsuisse Faculty, Veterinary Physiology*, *Bern, Switzerland*, [2]*Isfahan University of Technology, Department of Animal Science*, *Isfahan, Iran*.

Adipose tissue excretes components, such as TNFα, that play a role in the multifaceted regulation of lipolysis. This study investigated TNFα and other metabolic modulators in adipose tissue over time, and if TNFα is involved in interactions between adipose tissue and liver metabolism. Blood was sampled from 28 cows from week 10 ante partum (wk-10) up to week 4 post partum (wk4), and analyzed for concentrations of NEFA, glucose, insulin, and TNFα. Liver and adipose tissue biopsies were obtained in wk-10, on d1 post partum (d1), and in wk4. Adipose tissue was analyzed for mRNA levels by real-time RT-PCR of genes encoding for TNFα, peroxisome proliferator activated receptor γ (PPARγ), hormone-sensitive lipase (HSL), and fatty acid synthase (FASN). Liver was analyzed for mRNA levels of genes encoding enzymes of fatty acid β-oxidation (CPT1A CPT2, ACADVL), and of PPARγ. Data were evaluated by the Mixed procedure of SAS including biopsy time-point and parity as fixed effects with cow as repeated subject. Spearman Rank correlation coefficients were also calculated. Concentrations of NEFA were highest in wk-10, followed by d1, compared to wk4, suggesting adaptation to the reduction of nutrients at the start of the dry period. Plasma TNFα concentrations were very low (0.11±0.01 ng/ml) with no significant differences over time, suggesting that TNFα had a local effect. TNFα mRNA levels in adipose tissue were lowest (P<0.05) on d1 compared to the other time-points. HSL mRNA was highest (P<0.05) in wk-10, followed by d1, compared to wk4, which correspond to the high NEFA concentrations in wk-10. PPARγ mRNA levels were lowest in wk4 compared to the other time points. Levels of mRNA of FASN and liver parameters did not significantly change over time. Most significant correlations between adipose and liver tissue-related parameters were observed in wk4 compared to the other time-points. TNFα was not involved. In conclusion, TNFα is not a signaling factor that links adipose tissue and liver metabolism in healthy dairy cows. Other factors are responsible for the orchestrated regulation of adipose tissue and liver metabolism in wk4.

Key Words: dairy cow, adipose tissue, TNFα

M217 Early-weaning up-regulates the expression of sucrase-isomaltase in the jejunum of the piglet. D. Lackeyram*, T. Archbold, K. C. Swanson, and M. Z. Fan, *University of Guelph*, *Guelph, ON, Canada*.

Sucrase-isomaltase (SIM) is a small intestinal apical membrane disaccharidase that hydrolyses both sucrose and maltose. The objectives of this study were to examine the responses of SIM activity and protein abundances associated with the mucosal homogenate (H), intracellular soluble (S), and the apical membrane (M) fractions as well as SIM mRNA abundance and its regulation during early-weaning in comparison with suckling pigs. A total of 20 Yorkshire piglets, 10 suckling (SU) and 10 early-weaning (WN) with an average BW of 3 kg at the age of 10 d, were used in this study. Weanling piglets were fed a corn and SBM-based diet for 12 d. Proximal jejunal samples from both SU and WN groups were collected. Sucrose (0-25 mM) and maltose (0-60mM) were used in the enzymatic kinetic experiments. Abundances of SIM protein and mRNA were analyzed by Western blot and the real time RT-PCR using β-actin as the housekeeping gene. The jejunal SIM maximal specific activity (μmol/mg protein.min) for sucrose was increased (P<0.05) in weaning piglets (H: WN, 159.02±3.25 vs. SU, 76.72±2.89; S: WN, 16.42±1.28 vs. SU, 5.85±1.01; and M: WN, 141.39±3.84 vs. SU, 69.47±4.73). Similar increases (P<0.05) were observed for maltose (H: WN, 55.11±0.29 vs. SU, 40.79±0.81; S: WN, 17.81±0.24 vs. SU, 12.70±0.61; and M: WN, 516.51±1.05 vs. SU, 389.92±1.18). Corresponding increases (P<0.05) in the SIM protein abundance for the WN group was also observed in all of the jejunal fractions, H, 34%; S, 84%; M, 61%, respectively. Furthermore, early weaning increased (P<0.05) the relative abundance of SIM mRNA by 1.9 fold (WN, 0.306±0.03 vs. SU, 0.105±0.01). The increase in SIM mRNA could be accounted for by an increase (P<0.05) in the abundance (arbitrary units) of a key intestinal homeodomain transcription factor Cdx2 (WN, 2.365±0.01 vs. SU, 1.073±0.03). In conclusion, early-weaning increases small intestinal SIM activity at transcriptional, translational and post-translational levels.

Key Words: gene expression, sucrase-isomaltase, weanling pigs

M218 Effect of propionate infusion on hepatic PEPCK and glucose-6-phosphatase expression in neonatal Holstein calves. S. S. Donkin*, E. Cedeño, and S. L. Koser, *Purdue University*, *West Lafayette, IN*.

Cytosolic phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme for gluconeogenesis in liver, is sensitive to nutritional and hormonal cues. The objective of this experiment was to determine the effects of in vivo propionate supply on hepatic PEPCK mRNA expression. Sixteen male Holstein calves were blocked by birth date, and assigned to either: saline infusion (4 ml/min), propionate infusion (2 mmol/h/kg $BW^{.75}$), acetate infusion (3.5 mmol/h/kg $BW^{.75}$), or pre-treatment with phlorizin (100 mg at 8 h intervals for 24h) followed by propionate infusion. Blood samples were collected immediately prior to the initiation of infusion via indwelling jugular vein catheters and at hourly intervals during the 8 h infusion period. Liver biopsy samples were obtained immediately after the end of the 8 h infusion period and analyzed by real time PCR for pyruvate carboxylase (PC), PEPCK, glucose-6-phosphatase (G–6–Pase) and 18S mRNA. Abundance of PEPCK mRNA, relative to 18S was increased (P < 0.05) in response to propionate and acetate infusion (0.34 vs. 1.89 ± 0.24, arbitrary units). Propionate with phlorizin pretreatment did not alter PEPCK mRNA (0.34 vs. 0.69 ± 0.24, arbitrary units). Expression of PC mRNA was similar among all treatments. Abundance of G–6–Pase followed a pattern similar to PEPCK mRNA and was elevated (P < 0.05) for calves given acetate and propionate relative to saline control (0.82 vs. 2.70