

# Nonruminant Nutrition: Nutrition and Physiology

**137 Effects of mesenteric infusion of 0, 61, and 120 mmol/h of volatile fatty acids on hepatic metabolism in fasted pigs.** U. Krogh\*, A. C. Storm, and P. K. Theil, Aarhus University, Department of Animal Science, Foulum, Tjele, Denmark.

The aim of the study was to quantify hepatic metabolism of precursors potentially used as fuels in pigs in a post absorptive phase. Four fasted (18 h) pigs (BW 58 kg  $\pm$  2; Mean  $\pm$  SD) were fitted with indwelling catheters in the portal vein, hepatic vein, mesenteric vein and -artery. VFA was infused into the mesenteric vein of anesthetized pigs to mimic effects of increased consumption of dietary fibers. Infusion 0 (INF0: 0 mmol/h VFA, 0.9% saline) was infused for 30 min followed by 45 min of infusion 61 (INF61: 61 mmol/h VFA) and 45 min of infusion 120 (INF120: 120 mmol/h VFA), with the consequence of a potential confounding effect of time. INF61 and INF120 contained 70, 20 and 5% of acetate, propionate and butyrate, respectively. Para-aminohippuric acid was infused to quantify blood flow and net hepatic flux of nutrients (NHF). Eight sets of blood samples were simultaneously drawn from the artery, portal-, and hepatic veins at 15 min intervals. Statistical analysis included fixed effects of infusion, sampling times (ST), random effect of pig, and interaction between infusion and ST, with ST included as repeated measure. Results of incremental change in NHF ( $\Delta$ NHF) (i.e., INF61- INF0 and INF120-INF0) are shown in Table 1. Glucose  $\Delta$ NHF tended to be higher at INF120 vs. INF61 ( $P = 0.07$ ), while no effect was observed for lactate. Urea NHF increased ( $P = 0.04$ ) from INF0 to INF61 and from INF0 to INF120, and  $\Delta$ NHF of urea at INF120 vs. INF61 did not differ ( $P = 0.74$ ). Oxygen  $\Delta$ NHF tended to be higher at INF120 vs. INF61 ( $P = 0.07$ ). The data indicate that VFA infusion levels influence hepatic glucose metabolism and oxidation pattern in the pig.

**Table 1.** Incremental change in net hepatic flux ( $\Delta$ NHF) of glucose, lactate, urea, oxygen and carbon dioxide (mmol/h) in fasted mesenteric-infused pigs at 61 (INF61) and 120 (INF120) mmol/h of volatile fatty acids

Metabolite	INF61	INF120	SEM	P-value
Glucose	5.1	28.2	16.5	0.07
Lactate	-3.8	-8.2	5.5	0.57
Urea	15.7	17.8	4.4	0.74
Oxygen	38.5	-66.9	67.7	0.07
Carbon dioxide	-97.9	-55.6	59.4	0.42

<sup>1</sup>INF61 = NHF61 - NHF0; INF120 = NHF120 - NHF0.

**Key Words:** gluconeogenesis, liver, VFA

**138 Leucine pulse stimulates protein synthesis and suppresses protein degradation pathways in muscle of neonatal pigs fed continuously.** C. Boutry\*, S. El-Kadi<sup>2</sup>, A. Suryawan<sup>1</sup>, S. Wheatley<sup>1</sup>, R. Orellana<sup>1</sup>, H. Nguyen<sup>1</sup>, and T. Davis<sup>1</sup>, <sup>1</sup>USDA/ARS Children's Nutrition Research Center, Houston, TX, <sup>2</sup>Virginia Tech, Blacksburg.

Using neonatal pigs as a dual-model for animal agriculture and biomedicine, we have shown that muscle protein synthesis is enhanced with intermittent bolus feeding (BOL) to a greater extent than continuous feeding (CON). Leucine can act as a nutrient signal to stimulate muscle protein synthesis; limited evidence suggests leucine suppresses protein degradation. To determine if leucine can enhance protein anabolism during CON feeding, pigs (n = 20; 8-d-old) received formula by orogastric tube for 24h CON or by BOL (every 4 h for 15 min). For the CON+LEU group, leucine was pulsed parenterally (800  $\mu$ mol $\cdot$ kg<sup>-1</sup> $\cdot$ h<sup>-1</sup>)

every 4h. Plasma was collected at intervals during the last 4-h feeding cycle. At 25.25 h, muscle protein synthesis was measured by flooding dose methodology and intracellular signaling protein activation by Western blot. Data were analyzed by one-way ANOVA and mixed models for repeated-measures analysis. Plasma insulin increased 15min after a bolus meal and returned to baseline by 2h ( $P < 0.001$ ); insulin was unchanged in CON and CON+LEU. In CON+LEU, plasma leucine was higher after the leucine pulse (+192% 1 h after pulse,  $P < 0.0001$ ). LEU during CON feeding decreased plasma essential amino acid levels compared with CON ( $P < 0.0001$ ). Muscle protein synthesis was greater in CON+LEU (+24%) and BOL (+56%) than CON ( $P < 0.0001$ ). Muscle ribosomal protein S6 kinase 1 and 4E-binding protein 1 phosphorylation and eukaryotic initiation factor (eIF)4E/eIF4G formation were higher in CON+LEU and BOL than CON ( $P < 0.05$ ). AMP-activated protein kinase- $\alpha$ , eIF2- $\alpha$  and eukaryotic elongation factor 2 phosphorylation was unaffected by treatment. Microtubule-associated protein 1 light chain 3 (LC3)-II to total LC3 ratio was lower in CON+LEU and BOL than CON ( $P < 0.001$ ). There were no differences in Atrogin-1 and MURF-1 abundance and FoxO3 phosphorylation. These results suggest that administration of leucine pulses during continuous feeding increases skeletal muscle protein synthesis by stimulating translation initiation and suppressing autophagy-lysosome, but not the ubiquitin-proteasome, degradation pathways in neonatal pigs (NIH AR444474 and USDA/ARS 6250-51000-055).

**Key Words:** leucine pulse, protein synthesis, pig

**139 Temporal proteomic analysis reveals defects in small intestinal development of porcine fetuses with intrauterine growth restriction during gestation.** X. Wang<sup>1</sup>, C. Liu<sup>1</sup>, G. Lin<sup>1</sup>, C. Feng<sup>2</sup>, T. Wang<sup>1</sup>, D. Li<sup>1</sup>, G. Wu<sup>1,3</sup>, and J. Wang\*, <sup>1</sup>State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China, <sup>2</sup>Department of Obstetrics and Gynecology, China-Japan Friendship Hospital, Beijing, China, <sup>3</sup>Department of Animal Science, Texas A&M University, College Station.

The fetus/neonate with intrauterine growth restriction (IUGR) has a high perinatal mortality and morbidity rate, as well as reduced efficiency for nutrients utilization, which predisposes the offspring to malfunction and jeopardize the postnatal development of small intestine. Our previous studies showed a significant alteration of small intestinal proteome in IUGR piglets at birth and continuous impairment during nursing periods. With the consideration of fetal programming for intestinal development during gestation and the dramatically increase in volume and nutrients concentration of amniotic fluid from d 60 of gestation in pigs, the analysis is extended to IUGR porcine fetuses from d 60 to d 110 of gestation (mid- to late-gestation in pig) with a purpose of disclosing the mechanisms responsible for developmental programming of fetal gut. In total, 59 differentially expressed small intestinal proteins that are related to gut growth, development and reprogramming were identified. Results reveal increased levels of proteins and enzymes associated with oxidative stress, apoptosis and protein degradation, as well as decreased levels of proteins required for maintenance of cell structure and motility, absorption and transport of nutrients, energy metabolism and protein synthesis in fetal small intestine during mid- and late-gestation. Moreover, IUGR interfere with expression of fetal small intestinal proteins associated with gene regulation and signal transduction starting from middle to late gestation. Collectively, these changes in the proteome profile indicate alterations in the expression of some of the proteins in

the small intestinal tissue of IUGR porcine fetuses, which may predispose the gut to metabolic defects during gestation as well as disruption of fetal gut developmental programming.

**Key Words:** intrauterine growth restriction, intestine, pig

**140 Identification of porcine short-chain fatty acid receptors, GPR41 and GPR43, and their expression pattern in different development stages.** G. Li\*, H. Su, Z. Zhou, and W. Yao, *Laboratory of Gastrointestinal Microbiology, Nanjing Agricultural University, Nanjing, Jiangsu, China.*

In addition to supplying energy, short chain fatty acids also play a regulatory role in various physiological processes. Current researches revealed that SCFAs functioned as natural ligands for GPR41 and 43. To date, there is no systemic research about porcine GPR41 and 43. In this study, we tried to identify porcine GPR41 and 43 and determine their tissue distribution. Each 3 of Duroc × Landrace × Yorkshire pigs were slaughtered when 1, 25, 35, 70, 115 and 160 d old. Various tissues (liver, spleen, ileum, colon, heart, kidney, adipose tissue and skeletal muscle) were collected for RNA extraction. RT-PCR, Western blot and immunohistochemistry was used to detect GPR41 and 43 expressions in different tissues. The rapid amplification of cDNA ends was used to amplify the 3' and 5' end regions of porcine GPR41 and 43 mRNA. The qPCR was carried out to investigate expression pattern of GPR41 and 43 in different tissues and different development stages. A search of the pig genome database in GenBank revealed pig genome contains GPR41 and 43 genes, located on chromosome 6, highly similar to these genes in human and rodents. The RT-PCR, Western blot and immunohistochemistry analysis indicated that both GPR41 and 43 mRNA were detected in the tested tissues. A 2219bp and 1908bp nucleotide sequences representing the full-length cDNA sequence of porcine GPR41 and GPR43 was obtained and encoded 335 and 329 amino acid sequence, respectively. The qPCR results showed that GPR43 was most highly expressed in spleen, while GPR41 was in ileum. The highest expression level of GPR41 in ileum and colon was on 70d and 1d, respectively. GPR43 expression in ileum and colon was on 25d and 70d, respectively. Furthermore, porcine GPR41 and 43 was expressed in adipose tissue with a significant time-dependent manner. Their expression level was upregulated after birth with a peak at 70d, and then decreased, suggesting that these 2 receptors may exert function mainly in early stage.

However, further studies are essential to clarify their function in different development stages and different physiological processes.

**Key Words:** GPR41 and 43, short-chain fatty acids, pig

**141 Effect of breed on the expression of sirtuins (Sirt1-7) and antioxidant capacity in porcine brain.** Y. Ren\*, T. Shan, L. Zhu, J. Huang, and Y. Wang, *Institute of Feed Science, Zhejiang University, Hangzhou, Zhejiang Province, China.*

Sirtuins, nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase (HDAC), play important roles in a variety of biological processes including metabolism, apoptosis, oxidative stress, cytokine responses and further influencing aging. In brain, aging increased the risk of psychiatric disorders and vascular diseases caused by age-related oxidative damage. Sirtuins are highly expressed in brain, indicating that they may play critical roles in cerebral development and nervous system. Sirt1 and Sirt3 have been proved to protect brain from oxidative stress. Furthermore, obesity may lead to oxidative stress and the plasma total antioxidative capacity (T-AOC) is different between obese and healthy people. Therefore, the aim of this study was to determine breed differences of porcine sirtuins expression and antioxidant capacity in brain between Jinhua pig (a fatty breed of China) and Landrace pig (a lean breed). The effect of age on sirtuins expression was also investigated. At the age of 180 d, the mRNA levels of Sirt1 ( $P < 0.05$ ) as well as Sirt2 ( $P < 0.01$ ) and Sirt4 ( $P < 0.05$ ) were greater in Jinhua pig, but the mRNA levels of Sirt3, Sirt5, Sirt6 and Sirt7 were lower ( $P < 0.01$ ) compared with Landrace pig. Likewise, the mRNA levels of sirtuins were significantly greater in Jinhua pig except for Sirt5 and Sirt7 at the same BW of 64 kg. Meanwhile, Jinhua pig had higher antioxidants activity than Landrace pig either at the same age ( $P < 0.05$ ) or at the same BW ( $P < 0.05$ ). In addition, mRNA levels of sirtuins were decreased with age in brain in both breeds from 30 d to 120 d. These results indicated that sirtuins expression was different between fatty and lean pigs in brain and may correlate to antioxidant capacity. Sirtuins expression could be also downregulated by age in porcine brain. These results may provide useful information for better understanding of the physiological roles of sirtuins and for further regulating metabolism and antioxidant stress in pig.

**Key Words:** antioxidant, brain, sirtuin