

103 L-arginine modulates gut hormone release through the calcium sensing receptor in vitro and in vivo

Wang C¹, Kang C¹, Xian Y¹, Ding L¹, Zhu W¹, Hang S

¹Nanjing Agricultural University

L-arginine (L-Arg) is associated with regulation of food intake and blood glucose levels through stimulation of gut hormone release. Evidence suggests that calcium-sensing receptor (CaSR) is able to regulate gut hormone release by responding to amino acids in the gut.

Here, we tested the hypothesis that L-Arg induces gut hormone release and suppresses food intake through the activation of CaSR both in vitro and in vivo.

Porcine duodenal tissues were used to investigate the role of CaSR in the secretion of gut hormones stimulated by L-Arg using perfusing system in vitro. Rats were used to verify the results in vitro and the effects of L-Arg on food intake. In Vitro, porcine duodenum was perfused with 0-50 mM L-Arg to investigate the responses of cholecystokinin (CCK) and glucose-dependent insulinotropic peptide (GIP). Similarly, extracellular Ca²⁺, CaSR agonists, antagonists and its down-stream molecules inhibitors were employed to evaluate the involvement of CaSR and its signalling molecules. In Vivo, thirty rats were oral gavaged with L-Arg or saline for one week to investigate the role of CaSR in gut hormones secretion induced by L-Arg, and the effect on food intake. Statistical significance was assessed by Student's T-test or one-way ANOVA.

Results, 20 and 50 mM L-Arg induced the secretion of CCK and GIP, and the effect was enhanced by extracellular Ca²⁺ and CaSR agonist but reduced by inhibiting CaSR and its downstream signal molecules adenylate cyclase (AC) and phospholipase C (PLC) (P<0.05). Oral administration of L-Arg to rats activated *CaSR* genes in the gut and anorexic factor *POMC* in the hypothalamus, promoted secretion of CCK and ultimately reduced food intake and body weight (P<0.05). In conclusion, L-Arg induced CCK and GIP release and suppressed food intake was related to CaSR and its down-stream signal molecules AC and PLC.

104 Duodenum and ileum respond to amino acids with increased CCK and GLP-1 release using an ex-vivo model in weaned pigs

Müller M¹, Roura E¹, van Barneveld R¹

¹The University of Queensland

Abrupt environmental and dietary changes post-weaning are associated with low voluntary feed intake and growth rate in piglets. Hence, highly appetitive and digestible protein sources together with amino acid (AA) supplementation are generally used in starter diet formulas to stimulate feed consumption. However, dry matter intake seems to stagnate within few days following weaning resulting from impaired intestinal function. Little attention has been placed on the effect of dietary synthetic AA on the secretion of gut hormones involved in satiety regulation. Our objective was to test AA previously reported as potent gut hormone secretagogues in other animal species on the release of satiety hormones in weaned pigs by using a high throughput ex-vivo model. We hypothesised that Arg, Phe and Gln will strongly stimulate anorexigenic hormones CCK and GLP-1 in pigs. Twenty five day-old piglets were weaned and euthanized after a three day adaptation period, and duodenum, jejunum and ileum samples were collected to study the effect of Lys, Phe, Arg and Gln at one, 10 and 100 mmol on CCK and GLP-1 release. CCK secretion was highest in duodenum (46 ± 11.6 pmol/l) and lowest in ileum (14.9 ± 11.1 pmol/l) (P<0.05), whereas GLP-1 was greater on ileum (483.06 ± 193.84 pmol/l) and lower on proximal segments (31.05 ± 7.22 pmol/l) (P<0.05). In addition, ileum was the segment most responsive to the AA tested. Our results proof the concept that AA can trigger satiety hormone secretion from the intestine using an "ex-vivo" model in pigs. Duodenum was identified as the main organ regarding CCK release, while GLP-1 responses were higher in the ileum. The testing of other dietary AA warrants further investigation.

105 Minor dietary supplementation with a protein hydrolysate improves ileal mucosal integrity and nutrient transport, potentially mediated by increased GLP-1/GLP-2 secretion

Tedo G¹, Pastor J¹, Mereu A^{1,4}, Ipharraguerre I^{1,5}, Lierh M², Rimbach G², Muller C³, Reina M³, Morais S¹

¹Lucta SA - UAB Research Park, ²Institute of Human Nutrition and Food Science, University of Kiel, ³Celltec-UB, Department of Cell Biology, Physiology and Immunology, University of Barcelona, ⁴Current address: Yara International ASA, ⁵Current address: Institute of Human Nutrition and Food Science, University of Kiel

Gastrointestinal functionality is regulated by a complex network of receptors exposed to the luminal environment that sense the presence of nutrients and other chemicals, triggering endocrine and neural pathways that ultimately affect physiological and metabolic responses. This is commonly referred to as “gut chemosensing”. Some of these mechanisms have started being explored in pigs, which was the objective of the present work, focusing on a protein hydrolysate (PH) rich in free amino acids that potently activate the porcine umami receptor. With this aim, 96 ((LWxLD)xPietrain) 21d-old weaned piglets were divided into two groups and distributed into 16 pens with six animals each (eight pens/treatment), and offered *ad libitum* pre-starter/starter non-medicated diets supplemented (PH) or not (CTR) with 1000 ppm of the PH. Individual BW and feed intake per pen were registered weekly during 35 days. On day 35, segments of jejunum and mid-ileum were collected (eight pigs/treatment). Gene expression of nutrient transporters and components of tight junctions (TJ) was assessed in intestinal mucosa samples by RT-qPCR. Furthermore, *in vitro* assays were performed to evaluate transepithelial electrical resistance (TEER) in Caco-2 cells and to measure GLP-1 secretion in GLUTag cells. Performance data were analysed with a mixed-effect model with repeated measures, and gene expression and *in vitro* results were analysed with a Student T-Test (SAS, v.9.4). Final BW was improved in the PH group vs. CTR group, 18.5 vs 16.1 kg, respectively ($P<0.05$). An up-regulation ($P<0.05$) of TJ and amino acid transport genes was observed in the ileum of the PH group. *In vitro* data showed an increase in TEER and stimulation of GLP-1 secretion by PH treatment ($P<0.01$). In conclusion, the addition of minor amounts of a PH may increase piglet performance through the modulation of intestinal integrity and nutrient transport, likely partly associated to the stimulation of GLP-1/-2 secretion.

106 1 Maternal conditioning with monosodium glutamate increases innate umami and sweet taste sensitivity in piglets

Guzmán-Pino S¹, Lazcano C², Cortez M¹, Barros P¹, Díaz Í¹, Figueroa J³, Valenzuela C¹, Roura E⁴

¹Departamento de Fomento de la Producción Animal, Universidad de Chile, ²MaxAgro S.A., ³Departamento de Ciencias Animales, Pontificia Universidad Católica de Chile, ⁴Centre for Nutrition and Food Sciences, The University of Queensland

Dietary maternal conditioning has claimed ample attention in an attempt to find strategies to improve dietary habits in humans and in pigs related to the improvement of feed intake at weaning. However, most of the work in pigs has been around feed volatiles as opposed to taste active compounds. Following the findings with feed and food volatiles, we hypothesized that innate preference for umami (e.g. monosodium glutamate –MSG–) and sweet tastes would be amplified by an early exposure to MSG via maternal fluids. Twenty-two sows (LxLW) were selected based on similar gestation status (day 85), parity number, body condition and back fat depth and randomly assigned to standard commercial gestating and lactating diets without or with 50 g/kg of MSG. Two-hundred-and-eight of the piglets born were randomly allocated to 8 nursery pens. Piglets were then trained in pairs to perform double-choice (DC) tests by simultaneously offering 2 drinkers containing plain water or a 200 mM sucrose solution. The experimental testing consisted of 2-minute DC tests offering water and one of the six concentrations of MSG (0.1, 0.5, 1, 3, 9 and 27 mM) or sucrose (0.1, 0.5, 1, 6, 12 and 18 mM). Preference thresholds were identified based on the lowest concentration of MSG or sucrose showing significant ($P<0.05$) preference over water. Statistical analysis was based on comparing preference values to the neutral value of 50% by using a Student's t-test. MSG thresholds were observed at 1 mM (63.9%, $P=0.005$) and 0.1 mM (57.7%, $P=0.045$) in piglets born from control and MSG-fed sows, respectively. Similarly, sucrose thresholds of piglets born from control and treated sows were 12 mM (72.5%, $P=0.002$) and 1 mM (66.4%, $P=0.031$), respectively. We concluded that the inclusion of MSG in maternal gestating and lactating diets increases the sensitivity of piglets for umami and sweet solutions.

107 Upregulation of the GPR84 medium-chain fatty acid sensor in porcine ileum in pigs fed a high soluble fibre (inulin) diet.

De Jager N¹, Pluske J², Kim J², Roura E¹

¹Centre for Nutrition and Food Sciences, The University of Queensland, ²School of Veterinary and Life Sciences, Murdoch University

Dietary fibre is fermented mainly in the ileum, colon and caecum, producing short-chain fatty acids (SCFA) in pigs. In contrast, medium-chain (MC) and long-chain (LC) fatty acids (FA) in the gastrointestinal tract (GIT) are a product of the enzymatic digestion of dietary triglycerides requiring the interaction with bile acids for absorption. Recently, soluble fibre, such as inulin, was shown to bind bile acids partially preventing the absorption of FA. Pluske and co-workers (2013) found that the expression of FFAR2 (an SCFA sensor) increased in the mid-colon following high soluble fibre intake and subsequent increase in SCFA levels. However, a potential dietary effect on the expression of MC and LCFA sensors (FFAR1 and GPR84) was not studied. We hypothesised that FFAR1 and GPR84 would be downregulated in the GIT of pigs fed high soluble fibre diets due to the lack of substrate available in luminal contents. Pigs were fed for 21 days on either a rice-based diet, rice plus inulin and lupin hulls (R+I+L), or a wheat/barley based commercial diet (COM). Pigs were euthanised and ileum and mid-colon sections collected into RNAlater[®]. Standard qRT-PCR was performed to measure the gene expression levels of FFAR1, FFAR2, FFAR3, FFAR4, and GPR84. Expression levels were compared using the Pfaffl (2001) method and ANOVA and Tukey multiple comparison tests (SAS). The expression of GPR84 was significantly higher in the ileum of pigs fed the R+I+L diet compared to the rice and COM diets. In contrast, no dietary influences were observed ($P>0.05$) for the other genes and tissues studied. GPR84 has high affinity for capric and lauric acids. Since the R+I+L diet contained the highest amount of soluble fibre, we speculate that availability of MCFA at the site of absorption was particularly impaired causing an upregulation of the GPR84 as part of a compensatory mechanism.

108 Acceptability and palatability of garlic flavoured feed after social learning in nursery pigs.

Figueroa J¹, Salazar L¹, Valdivia C², Guzmán-Pino S², Valenzuela C², Muller M²

¹Pontificia Universidad Católica De Chile, ²Universidad de Chile

Pigs are able to acquire feed preferences through brief social interactions with experienced conspecifics that consumed that feed. However, no data exist about the acceptability or hedonism of learned feed. The hypothesis of the present experiment was that social learning of a flavoured feed in pigs could increase its intake and palatability. Sixty four weaned pigs (21 days old, 6.1 ± 0.22 kg) were allocated into 16 nursery pens (4 pigs/pen). After one week of adaptation, pigs were tested for social learning by moving two pigs per pen (demonstrators) to empty pens where they were offered a garlic (8 pig-pairs) or aniseed (8 pig-pairs) feed (Floramatic; Santiago, Chile) during 30 minutes. Demonstrators were returned to their original pens to interact with pigs that remained there (observers) for 30 minutes. Demonstrators were removed and observers were exposed to a garlic feed during 30 minutes. Garlic feed was exposed again to observers on day two and three to estimate the extinction of behaviours. Pig's acceptability was estimated by subtracting the final from initial weight of feeders. Palatability was estimated measuring observer's consumption patterns (consumption time/ number of approaches, 10 min) using video-records obtained from 16 video-cameras). Moreover, oro-nasal contact time between garlic demonstrators and their observers was measured. Data was analysed with an ANOVA procedure using the statistical software SAS[®]. Observers consumed more garlic feed when they previously interacted with demonstrators that had consumed garlic (206 vs. 162 g, $P=0.043$). However, no palatability differences were observed (5.36 vs. 4.13, $P=0.421$). Nevertheless, a positive correlation trend between consumption patterns and oro-nasal contact time of observers with garlic demonstrators were observed ($r=0.497$; $P=0.100$). The interaction with demonstrators previously exposed to a flavoured feed, increased observers acceptability for that feed, with a direct correlation between their oro-nasal interaction time and feed palatability after associative learning.

110 Impact of Porcine Reproductive and Respiratory Syndrome virus and reduced feed intake on jejunum function and integrity in pigs

Curry S^{1,2}, Helm E¹, De Mille C¹, Schweer W¹, Burrough E¹, Gabler N¹

¹Iowa State University, ²Oak Ridge Institute for Science and Education

Pathogen challenges are often accompanied by reduced feed intake making it difficult to differentiate between the direct impacts of pathogen versus attenuated nutrient and energy intake on various gastrointestinal response parameters. Therefore, our objective was to determine the impact of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)-challenge and reduced feed intake on jejunum markers of integrity and function of growing pigs using *ex vivo* Ussing chamber techniques. We hypothesized that at day post inoculation (dpi) 17, pigs that were either inoculated with PRRSV or negative for PRRSV and restricted fed (RF) to PRRSV level of feed intake would have reduced integrity and active nutrient uptake. Seventy-two pigs were randomly selected (BW = 11.34 ± 1.54 kg) and allotted to three treatments: 1) ad libitum-fed, PRRSV-naïve (Ad lib), 2) RF, PRRSV-naïve (RF), and 3) ad libitum-fed, PRRSV-inoculated (PRRSV). All pigs were housed in individual pens in a BSL2 facility and at dpi 0, treatment 3 was inoculated with a U.S. field strain of PRRSV. The RF pigs were pair-fed daily to the previous day's PRRSV-inoculated pigs' feed intake. At dpi 17, a subset of eight pigs per treatment were euthanized and fresh jejunum collected to measure *ex vivo* transepithelial resistance (TER), macromolecule (FD4) permeability, active glucose transport, and jejunal explant complete oxidation of C14-glucose. At dpi 17, PRRSV and RF pigs decreased ($P<0.05$) TER compared with the Ad lib pigs; whereas, FD4 permeability was not different among treatments. Complete oxidation of glucose in the jejunum was decreased ($P<0.05$) in PRRSV and RF pigs compared with Ad lib pigs. However, glucose transport was increased ($P<0.05$) in PRRSV pigs compared with Ad lib pigs, RF pigs being intermediate. Therefore, at dpi 17, alterations in mucosal integrity and glucose utilization of the jejunum in response to PRRSV may be partially explained by the reduction in feed intake.

111 Post-weaning diarrhoea in piglets in practice is associated with protein fermentation, but specific protein fermentation metabolites contribute differently

Gilbert M¹, van der Hee B², Gulersonmez M³, Stigter E³, Kies A⁴, Gerrits W¹

¹Animal Nutrition Group, Wageningen University and Research, ²Host-Microbe Interactomics, Wageningen University and Research, ³University Medical Center Utrecht, ⁴DSM Nutritional Products

It has been hypothesized that excess dietary protein underlies post-weaning diarrhoea (PWD) in piglets, potentially via protein fermentation in the hindgut and subsequent production of metabolites affecting gut wall integrity. Although a high protein diet reduces faecal consistency (Pieper et al., 2012; Wellock et al., 2006), it does not always result in diarrhoea (Htoo et al., 2007; Nyachoti et al., 2006). It remains unknown whether and which protein fermentation metabolites cause PWD under field conditions. To study this, rectal faecal samples were collected from weaned piglets with and without PWD, from seven farms in the Netherlands. Samples (n=82) were analysed for dry matter (DM) and ammonia-N. A targeted LC-MS platform was set-up for quantification of biogenic amines in faecal samples. Principal component analysis on ammonia-N and seven biogenic amines retained three principal components (PCs), in which PC1 had high, positive loadings for putrescine, cadaverine, spermidine and γ -aminobutyric acid; PC2 had high, positive loadings for ammonia-N and acetylated spermidine and PC3 had high, positive loadings for histamine and γ -aminobutyric acid. Using the GLM-procedure, PC1 ($P<0.001$) and PC2 ($P<0.001$) related negatively with faecal DM content, but no interactions with farm (included as fixed effect) were found. PC1 and PC2 together explained 52% of the variance in faecal DM content, with the largest part explained by PC2 (44%). Piglets were subsequently grouped into control (faecal DM>200g/kg) and PWD (faecal DM<150g/kg) and the mean+SD of PC1 or PC2 of the control group was used as a cut-off value for protein fermentation in the PWD group. PC2 (89%) but not PC1 (30%) appeared predictive for PWD cases. Overall, these data demonstrate a negative relation between protein fermentation and faecal dry matter, but highlight different contributions of specific protein fermentation metabolites to PWD in piglets in practice.

112 FeedOmics, an approach towards understanding gut-health influenced by dietary protein source in pigs

Kar S¹, Smits M^{1,2}, Schokker D¹, Jansman A¹

¹Wageningen Livestock Research, Wageningen University & Research, ²Wageningen Bioveterinary Research

There is no clear definition of “gut-health” as it covers multiple aspects of the gastro-intestinal tract (GIT) that encompasses a number of physiological and functional features, including nutrient digestion and metabolism, a stable microbiome, mucosal functions and immune responses. A healthy gut negates severe (pathogenic) to mild (dietary) challenges for maintaining homeostasis and productivity in livestock. Unlike pathogens, measuring subtle responses induced by alterations in diet composition on physiological and functional features of the GIT remains challenging. We hypothesised that advancement of methods in molecular biology and physiology can enhance our understanding of the gut associated changes induced by dietary ingredient composition. In this study, pigs aged six weeks were fed for a period of four weeks with diets containing one of the following protein sources (dietary CP, 160 g/kg): soybean meal (SBM), wheat gluten meal, rapeseed meal, spray dried plasma protein, or black soldier fly (BSF). We measured blood amine metabolite profiles, intestinal microbiome composition (jejunum and ileum) and the genome-wide transcriptional responses of small intestinal mucosal tissue (jejunum and ileum) as response parameters. Metabolic and microbiota data showed most significant differences between dietary treatments. We observed distinct effects of BSF as protein source compared to other protein sources by principal component analysis (PCA) on the plasma amine metabolomics data. PCA and hierarchical clustering analysis performed on 16S rRNA gene sequencing data related to the microbial community structure in the small intestine showed distinct effects of BSF as protein source compared to other protein sources. Compared to SBM, lower (FDR < 0.05) expression of genes related to barrier function and immune signalling pathways in jejunal mucosal tissue of pigs fed with BSF were recorded. Here, we showed that employing a multi-omics (FeedOmics) approach enhances the resolution to understand the complexities of gut-health induced by dietary protein source in pigs.

113 Early-life Lactoferrin intervention alleviates the diarrhea by modulating intestinal permeability and changing the microbiota in suckling piglets

Hu P¹, Zhao F¹, **Jing W¹**, Zhu W¹

¹National Center for International Research on Animal Gut Nutrition, Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, Laboratory of Gastrointestinal Microbiology, College of Animal Science and Technology

Suckling period is characterized by intestinal dysplasia with the diarrhea in piglets. In this study, we investigated the effect of early-life lactoferrin (LF) intervention on the diarrhea by modulating intestinal permeability and changing the microbiota in suckling piglets. Sixty suckling piglets obtained from six sows (10 piglets per litter) were assigned to control group (CON) and LF group (LF) in each litter with breastfeeding. The piglets in the LF group were orally administrated with 8-12 ml LF solution three times per day during the age of 1-7 days (0.5 g/kg body weight per day); and the piglets in the LF group with the same dose of physiological saline. At the age of the 8th and 21th day, six piglets from each group were euthanized. Enzymatic technique, HE staining, and PCR experiments were used in present study. Independent samples t-test was performed using the SPSS software. Diarrhea incidence was significantly lower in LF piglets than in the CON piglets during 1-7 days (P<0.05), and LF piglets had a decreased trend on the diarrhea incidence during 1-21 days. Urinary lactulose to mannitol ratios were pronouncedly lower, whereas jejunal *occludin* and *Mucin-2* gene expressions were significantly higher in LF piglets compared with those in CON piglets on the 8th and 21st day (P<0.05). In addition, *IL-1α* gene expression in jejunum was significantly lower in LF piglets on the 8th day, and *IL-1β* and *IL-10* gene expressions were significantly higher in LF piglets compared with those in CON piglets on the 21st day (P<0.05). Notably, LF piglets had significantly lower *Escherichia coli* 16S rRNA copy number per gram of jejunum contents on the 8th day (P<0.05). In conclusion, our study shows that early-life LF intervention decreased the diarrhea incidence by modulating intestinal permeability and decreasing the number of *Escherichia coli* in jejunum.

114 Late gestation diet supplementation of resin acid-enriched composition increases sow colostrum IgG, piglet colostrum intake and modulates sow gut microbiota

Hasan S¹, Saha S¹, Junnikkala S¹, Orro T², Peltoniemi O¹, Oliviero C¹

¹University of Helsinki, ²Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences

Tall oil fatty acid (TOFA) and resin acid (RA) commonly termed resin acid-enriched composition (RAC) can modulate the microbial population in the gut, changes metabolism, and improve the feed conversion ratio. We investigated the effects of dietary supplementation of RAC on sow colostrum yield, colostrum composition and gut microbiota. The experiment was conducted in three trials in three respective herds. Sows were allocated either a control diet or a control diet supplemented with 5g RAC/day/sow during the last week of pregnancy. In one herd, faecal microbiota populations of sows at farrowing were assessed using 16S rRNA gene sequencing. Colostrum samples were examined for nutritional composition, acute phase proteins (APP) and immunoglobulin (Ig) content. All piglets were individually weighed at birth and 24 hours later in order to calculate colostrum yield (CY), and later at three to four weeks to calculate average daily gain (ADG). The RAC-fed sows had significantly higher IgG levels ($P < 0.05$) in all three herds but treatment did not influence colostrum IgA and IgM concentration. Protein, lactose and fat content of colostrum did not significantly differ between sows of the two diet groups ($P > 0.05$), but RAC fed sows had higher levels of colostrum serum amyloid A (SAA). CY was significantly higher in RAC-fed sows in herds 2 and 3 with heavier piglets between 3 and 4 weeks of age ($P < 0.05$), but not in herd 1 ($P > 0.05$). RAC supplementation significantly increased some beneficial and fermentative bacteria (*Romboutsia* and *Clostridium sensu stricto*) than the control diet group ($P < 0.01$) while some opportunistic pathogens (*Barnesiella*, *Sporobacter*, *Intestinimonas* and *Campylobacter*), including Proteobacteria, were suppressed. Therefore, RAC added to the sow diet at late pregnancy increases colostrum IgG, colostrum availability for neonate piglets, and seems to promote better maternal intestinal microbial sources.

115 Laminarin improves performance and intestinal health in post weaned pigs

Rattigan R¹, Sweeney T², Maher S¹, O'Doherty J¹

¹School of Agriculture and Food Science, University College Dublin, ²School of Veterinary Medicine, University College Dublin

Seaweed derived laminarin has shown potential for enhancing piglet growth. It was hypothesised that the optimum inclusion level of laminarin would enhance post-weaning performance by reducing *Enterobacteriaceae* populations and the incidence of post-weaning diarrhoea. At weaning, 96 pigs (3 pigs/pen, 8.39kg SD 1.09kg) blocked by live weight and litter were randomly assigned to 1 of 4 dietary treatments for 28 days: 1) basal diet; 2) basal +100ppm laminarin; 3) basal +200ppm laminarin; 4) basal +300ppm laminarin. Faecal scores (FS) were recorded daily on a scale of 1-5 with 1= hard, firm faeces and 5= watery, mucous like faeces. Average daily gain (ADG) and feed intake (ADFI) were determined weekly. On d14 post weaning, 1 pig/pen from the basal and best performing laminarin treatment (based on ADG and FS) were sacrificed and caecal and colonic digesta were collected and selected microbial populations were enumerated using 16s rRNA qPCR. The growth performance and faecal score data was analysed by repeated-measures using the PROC MIXED procedure of SAS while microbial populations were analysed using the GLM procedure of SAS. From d0-14, the 300ppm laminarin group had higher ADFI than the basal ($P < 0.001$), 100ppm laminarin ($P < 0.05$) and 200ppm laminarin ($P < 0.01$) groups. Similarly, the 300ppm laminarin group had a higher ADG than the basal group ($P < 0.05$) and tended to be higher than the 100 and 200 ppm laminarin groups ($P < 0.10$). From d0-28, the 200ppm and 300ppm laminarin groups had lower FS compared to the basal diet ($P < 0.05$ and $P < 0.01$ respectively). Supplementation of 300ppm laminarin reduced *Enterobacteriaceae* numbers ($P < 0.05$) in the caecum, increased Lactobacilli in the colon ($P < 0.05$) and increased the Lactobacilli: *Enterobacteriaceae* ratio in the caecum and colon ($P < 0.05$). In conclusion, 300ppm laminarin improved growth performance, faecal consistency and the Lactobacilli: *Enterobacteriaceae* ratio in the large intestine compared to the basal diet.

117 **In vitro and in vivo starch digestion kinetics of diets varying in dietary fibre content and composition**

Bonzi P¹, Nielsen K^{1,2}, Lærke H¹, Hedemann M¹, **Bach Knudsen K¹**

¹Department of Animal Science, Aarhus University, ²Department of Forensic Medicine, Forensic Chemistry, Aarhus University

The acute glycaemia is influenced by the rate of starch hydrolysis. In the current study, this aspect was investigated by comparing the relationship between *in vitro* starch digestion kinetics of diets varying in type and content of dietary fibre with the portal appearance of glucose in pigs ⁽¹⁾. Five experimental breads were used – a low dietary fibre white wheat bread (WWB), two high fibre whole grain rye breads provided without (WRB) and with whole kernels (WRBK) and two experimental breads based on wheat flour with added arabinoxylan concentrate (AXB) or oat β -glucan (BGB). The breads were provided in equal quantities of available carbohydrates to pigs equipped with catheters in the mesentery artery and the portal vein and with a flow probe attached to the portal vein for monitoring the blood flow rate. For *in vitro* measurements, samples were collected at 0, 5, 10, 15, 30, 60, 120 and 180 min., analysed for glucose and the cumulative hydrolysis curve modeled using a mechanistic growth model. From the catheterised pigs samples were taken every 15 min up to 60 min. and then every 30 min. up to 240 min and the cumulative absorption curve modelled with a sigmoid Gompertz model. The *in vitro* starch hydrolysis rate varied from 0.07 to 0.17 %/min with the lowest value for BGB and highest for WWB whereas the rate of glucose appearance in the portal vein was lower and more uniform ranging from 0.020 to 0.023 % absorbed starch/min but with the same diets being lowest and highest as *in vitro*. In conclusion, the differences between diets in *in vitro* hydrolysis rate were reflected *in vivo* although less pronounced.

1. Christensen KL, Hedemann MS, Lærke HN *et al.* (2013). *J Agric Food Chem* **61**, 7760-7768.

118 **Adaptation of feed intake behaviour of pigs to diets differing in resistant starch**

van Erp R¹, de Vries S¹, van Kempen T², Gerrits W¹

¹Wageningen University, ²Trouw Nutrition

End products from starch digestion and fermentation are important determinants of satiety regulation, and thus, feed intake behaviour. The aim of this study was to investigate the adaptation of feed intake behaviour of pigs, to diets differing in resistant starch (RS). We hypothesized that a greater level of RS (as % of total starch) increases meal size and lowers the number of meals per day, while ADFI remains unaffected.

Thirty-six groups of six pigs (25.4±2.8 kg), were allocated to one of two diets, containing either 50% high-amylose maize starch (High RS) or 50% waxy maize starch (Low RS), and TiO₂ as indigestible marker. During 28 days, groups were transferred to the other diet in steps of 25%, resulting in a 5-step titration, executed in upwards (LH) or downwards (HL) direction. Twelve groups received a control diet to correct for changes in digestion and feeding behaviour over time. Feed intake behaviour was recorded using electronic feedings stations. Grab faecal samples were collected to determine starch fermentation (Gerrits *et al.*, 2012). A mixed model was used to check if responses to RS intake differed from zero.

LH titration increased starch fermentation with 4.4 %-units (P<0.001) per step, and decreased ADFI (25 g; P=0.029) and meal size (3.1 g; P<0.001). HL titration decreased starch fermentation with 2.0 %-units per step (P=0.001), but did not affect feed intake behaviour. The high microbial activity due to a high RS intake at the start of HL titration remained increased during the whole titration period, possibly explaining the lack of effect of RS on feed intake behaviour. In conclusion, pigs increase their feed intake per meal when RS intake increases, whereas the opposite response was not observed.

119 Branched isomalto-oligosaccharides increase nutrient digestibility enhancing microbial activity in ileal-cannulated pigs

Heyer C¹, Hu Y, Wang W, Gänzle M, Zijlstra R

¹University of Alberta

Isomalto-oligosaccharides (IMO) may promote health by modulating intestinal microbiota depending on their degree of polymerisation (DP) and ratio of α -(1 $\rightarrow$ 4) to α -(1 $\rightarrow$ 6) linkages. However, *in vitro* digestibility results are contradictory depending on assay used and few *in vivo* digestibility data of α -(1 $\rightarrow$ 6)-linked tri- and tetrasaccharides exist. We hypothesized that IMO with α -(1 $\rightarrow$ 6) branches and consequent decreased IMO digestibility would increase hindgut substrate flow thereby shifting microbial and metabolite profiles in pigs. In a double 4x4 Latin square, eight ileal-cannulated barrows (31.8 $\pm$ 2.1 kg initial body weight) were fed corn starch-casein based diets containing 30 g/kg of one of four oligosaccharides: 1) linear IMO[I]; 2) IMO[II] with greater DP and more α -(1 $\rightarrow$ 4) linkages; 3) digestible maltodextrin; 4) resistant maltodextrin. Oligosaccharides were analysed by high-performance anion-exchange chromatography. Digesta were examined for apparent ileal digestibility (AID) of dry matter (DM), crude protein (CP) and gross energy (GE), microbial composition by 16S rRNA gene sequencing, and short-chain fatty acids (SCFA) by gas chromatography. Data were analysed using a MIXED model with diet as fixed effect, and pig and period as random effects. Compared to IMO[I], IMO[II] contained more panose (18.6 vs. 10.3%), less isomaltose (7.5 vs. 22.3%) and isomaltotriose (6.1 vs. 12.6%) and no maltose (0.0 vs. 3.6%). The AID of GE and DM were 3% greater ($P<0.05$) for IMO[II] and digestible maltodextrin than resistant maltodextrin. Apparent total tract digestibility of CP was 1% greater ($P<0.05$) for IMO[II] than resistant maltodextrin. Ileal propionate, isovalerate, and total SCFA was greater ($P<0.05$) for IMO[II] and digestible maltodextrin than IMO[I]. In conclusion, digestible maltodextrin and IMO[II] increased small intestine fermentation metabolites but did not influence faecal microbiota. Structural properties of IMO are important determinants of their functional properties within the porcine digestive tract.

120 Crystalline amino acids in diets do not influence calculated values for amino acid digestibility in feed ingredients fed to pigs

Oliveira S. F. M¹, Abelilla J. J¹, Htoo K. J², Stein H. H¹

¹University of Illinois at Urbana-Champaign, ²Evonik Nutrition & Care GmbH

An experiment was conducted to determine if addition of crystalline amino acids (AA) to diets during the adaptation period or during both adaptation and collection periods influence calculated values for apparent ileal digestibility (AID) or standardized ileal digestibility (SID) of AA in corn and soybean meal (SBM). Seven ileal-cannulated barrows (initial BW = 77.9 $\pm$ 2.6 kg) were allotted to a 7 treatment $\times$ 7 period Latin square design. Treatments included feeding diets containing corn or SBM without crystalline AA for the entire 7-days period, corn or SBM with crystalline AA for the entire 7-days period, or feeding corn or SBM with crystalline AA during the adaptation period (days 1 to 5) followed by corn or SBM without crystalline AA during the collection period (days 6-7). An N-free diet was also used. The AID and SID of CP and AA were calculated using values determined in corn or in SBM without or with crystalline AA to determine if crystalline AA influenced calculated values for AID or SID of CP and AA. Data were analyzed using the Mixed procedure by SAS. Results indicated that addition of crystalline AA in the adaptation period only or for all 7-days improved ($P < 0.05$) the AID and SID of some AA in corn but not in SBM. No differences in AID or SID of AA in corn or SBM diets were observed as a result of including or not including crystalline AA in the calculation indicating that crystalline AA are 100% absorbed and did not affect AID and SID of AA regardless of inclusion in the diet. Therefore, crystalline AA may be added to experimental diets in digestibility experiments before and during collection periods without affecting results, if crystalline AA are disregarded in the calculation of AID or SID of AA in ingredients.

121 Apparent amylase diffusion coefficient of milled grains determined *in vitro* is related to digestibility and the growth of pigs

Ratanpaul V¹, Williams B¹, Black J², Gidley M¹

¹CNAFS, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, ²John L Black Consulting

Grain digestibility in the small intestine (SI) affects the growth efficiency of pigs. However, there is no laboratory method for estimating SI digestibility of processed grain. The rate of grain starch digestion *in vitro* is directly related to the apparent amylase diffusion coefficient (AADC) of milled grains.

The hypothesis tested was that AADC determined *in vitro* is related to grain digestion in the SI and affects average-daily-gain (ADG) and feed-conversion-ratio (feed:gain, FCR). Milled sorghum (MS), steam-flaked sorghum (SFS), milled wheat (MW) and steam-flaked wheat (SFW) were digested *in vitro*, simulating porcine digestive processes to determine rate coefficients from first-order kinetics of starch digestion. AADC were determined from inverse square dependence of rate coefficients on the square of particle sizes. AADC of MS, SFS, MW and SFW were 0.014, 0.037, 0.06 and 0.12 mm²/hr respectively. AADC were analysed in a factorial design (ANOVA) with grain type (P<0.001), process-type (P<0.001), and interaction (P<0.01) of grain and process as factors.

Four nutritionally balanced diets, each containing one of the four grains, were randomly assigned to eight male Large White pigs (16.83 kg (mean); 0.640 kg (sd)) per diet. After acclimatisation for a week, the pigs were fed *ad libitum* for three weeks, and average-daily-feed-intake (ADFI), ADG and FCR were determined for periods 7-14, 14-21, 21-28 and 7-28d. For each period, a linear regression model was fitted between AADC and a) ADG, b) FCR. For period 14-21d, a positive-correlation (R²) of 0.96 (P=0.028) between AADC and ADG, and a negative-correlation of 0.82 (P<0.10) between AADC and FCR was found. For MS, SFS, MW and SFW at 14-21d - the ADG values were 0.869, 0.884, 0.914 and 0.941 kg, and the FCR values were 1.499, 1.487, 1.405 and 1.384. In conclusion, AADC of milled grains was closely related to the growth efficiency of pigs.

122 The influence of physical properties of feed and digesta on gastric emptying in pigs

Martens B^{1,2}, Schols H¹, Bruininx E^{1,2}, Gerrits W¹

¹Wageningen University and Research Centre, ²Agrifirm Innovation Centre

Nutrient digestion kinetics determine the rate of appearance of nutrients in the portal circulation. We studied the release of digesta into the small intestine in pigs fed diets which strongly differed in physical properties. We hypothesised that a higher apparent viscosity of the feed would reduce gastric emptying rates in both meal fed pigs and pigs in steady state. We assigned 90 pigs (23 kg BW) to one of nine treatments in a 3x3 factorial arrangement with starch source (barley, corn or high amylose corn) and form (as isolated starch, ground cereal, or extruded cereal) as factors. All test diets contained two inert markers to study liquid and solid digesta flow. After an adaptation period, ¹³CO₂ enrichment in the pigs breath was measured after meal feeding by following an oral dose of [1-¹³C] glycine and of [1-¹³C] octanoate, on two consecutive days. Following, pigs were hourly fed for 6h to reach a steady state, after which digesta were collected.

Based on rheological analysis, we found that gastric content of pigs that are fed in steady state behave like a (weak) gel and mean retention times in the stomach correlated negatively with this gels strength (r=0.47, p<0.0001). With regression analysis we identified significant positive correlations between gel strength and the fraction of large particles (r=0.73, p<0.0001), the dry matter content (r=0.35, p<0.0001), and water holding capacity (r=0.24, p=0.0001) of digesta. Furthermore, the ¹³C-breath test revealed biphasic gastric emptying patterns after meal feeding, but did not correlate with gastric retention times measured with inert markers after hourly feeding. Lastly, we found that gel strength, particle size and water holding capacity of diets were uncorrelated to stomach digesta, leading us to conclude that physical properties of diets change drastically upon digestion.

124 Insulin resistant pig as a model for type 2 diabetes (T2D) in humans

Ferenc K¹, Dib N², Bujok J³, Siewruk K¹, Matyba P¹, Sady M¹, Pawliński B¹, Wielgosz M¹, Gajewski Z¹, Grzesiuk E⁴, **Zabielski R¹**

¹Department of Large Animal Diseases with Clinic, Faculty of Veterinary Medicine, Veterinary Research Centre and Center for Biomedical Research, WULS – SGGW, ²European Health Centre, ³Department of General, Oncologic, Metabolic and Thoracic Surgery, Military Institute of Medicine in Warsaw, ⁴Department of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences

T2D associated with obesity is an increasing problem for human health worldwide, however, mechanisms of T2D development and regression still remain unclear. Pigs due to their similarities to humans, involving cardiovascular and digestive system structure and function and similar metabolism, are probably the best model for studying human diseases. However, previous reports claimed that insulin resistance in pigs is hard to obtain. The aim of present study was to develop insulin resistance in young pigs by nutritional manipulation. After obtaining insulin resistant pigs we checked if bariatric surgery can reverse insulin resistance. Weaned piglets (Polish Landrace x Pietrain crossbreed) were fed for 6 months with two different diets starting from the postnatal week 12: control group (C, fed according to NRC, n=6), and high energy group (HE, 150% energy intake, commercial feed from C group enriched with disaccharides (sugar) and fatty acids (rape oil), n=14). Every month, body weight gain, hematology, blood glucose and lipid profile were measured. Insulin resistance was monitored with fasting glucose tolerance test. Scopinaro bariatric surgery was performed under general anesthesia, with further antibiotic and nutritional treatment preformed according to procedures in human gastroenterology clinics. After 6 months, HE pigs gained >63% higher body mass as compared to control group. Monitored plasma glucose, triglycerides and cholesterol concentrations showed no changes in plasma cholesterol, and gradual and significant increase in plasma glucose and triglycerides concentrations (for both $P < 0.05$). Glucose toleration test after 6 months of HE indicated glucose intolerance in 10 out of 14 pigs. Bariatric surgery restored insulin sensitivity after 1 month in all of the operated pigs. In conclusion, high energy diet applied to young piglets resulted in development of insulin resistance which could be reversed by bariatric surgery. In the future, this nutritional model of insulin resistant pig may be useful in T2D studies.

125 Plasma metabolites related to nitrogen efficiency in low and high birth weight pigs

Verschuren L^{1,2}, Jansman A², Calus M², Bergsma R¹, Hedemann M³

¹Topigs Norsvin Research Center B.V., ²Wageningen University & Research, Livestock Research, ³Aarhus University, department of Animal Health

Birthweight of piglets has a major effect on average daily gain later in life, and therefore possibly influences nitrogen efficiency of pigs. Concentrations of particular plasma metabolites might be markers for nitrogen efficiency. The present study investigated differences in nitrogen metabolism between low and high birthweight pigs at grower-finisher age (between 98-126 days of age) using untargeted metabolomics. Plasma samples were collected of 40 grower-finisher pigs (three-way crossbreeds, low or high birthweight) at onset of the experiment (D0), and after the first (D17) and second (D28) experimental period. In a change-over design, pigs were either fed a protein adequate (100%) or a protein restricted (70%) diet. Plasma metabolites were characterized by untargeted liquid chromatography–mass spectrometry, and results were subjected to a discriminant approach combined with principal component analysis to discriminate pigs based on birthweight, diet fed, and diet fed within birthweight groups. Low vs. high birthweight pigs could be distinguished based on a limited number of metabolites. Pigs fed a protein adequate or restricted diet also had very distinct metabolite profiles. However, different metabolites were important for distinguishing the effect of diet in the low compared to the high birthweight piglets. Further identification of the metabolites linked several metabolites to possible differences in nitrogen metabolism between pigs with different birthweight. In conclusion, our results show a clear effect of birthweight and dietary protein restriction on plasma metabolites, the effect of dietary protein restriction being birthweight dependent. This study is part of the Feed-a-Gen Project, funded from the European Union's H2020 Programme under grant agreement no 633531.

126 FTO protein expression in gastrointestinal tract as a marker of nutritional status

Sady M¹, Ferenc K¹, Pilżys T², Dylewska M², Skorobogatov O², Matyba P¹, Gajewski Z¹, Grzesiuk E², Zabielski R¹

¹Department of Large Animal Diseases with Clinic, Faculty of Veterinary Medicine, Veterinary Research Centre and Center for Biomedical Research, WULS – SGGW, ²Department of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences

Genome-wide association studies linked single nucleotide polymorphisms (SNPs) within introns of Fat mass- and obesity-associated gene (*FTO*) with obesity and type 2 diabetes (DT2) but the role of *FTO*, product of this gene, in this association is unclear. The aim of present study was to localize *FTO* expression in the tissues relevant for obesity and DT2 development. Porcine tissues were sampled after 6 months of three different dietary treatments: control (C, according to NRC dietary requirements, n=7), low energy (LE, 50% energy intake, n=6) and high energy (HE, 150% energy intake, n=6). We also sampled tissues from 7 d-old intrauterine growth retarded (IUGR) piglets (n=7) as a model of early predisposition to obesity and DT2 development, and normal body weight (NBW) littermate piglets as a control (n=7). *FTO* expression level was measured by Western blotting, mapping by in-tissue cytometry and visualized in confocal microscopy. Western-blot analysis revealed high *FTO* expression level in the cerebellum, hypothalamus and kidney, regardless of energy intake. In-tissue cytometry showed high *FTO* expression in specific tissue areas or in particular types of cells. Namely, in the pancreas *FTO* expression was very high in insulin producing β -cells and its level was related to nutritional status. In the liver, *FTO* expression was particularly abundant near the intralobular bile ductuli, and in the gut mucosa it was expressed mostly in the apical part of the villi and in the crypts. In neonatal IUGR piglets, compared to NBW, higher expression was found in cerebellum, adipose tissue and spleen, and lower expression was found in salivary glands, liver, duodenum, thyroid, kidney and muscle. In conclusions, in tissues where the level of *FTO* is low, it occurs in the specific type of cells and its presence may be of help to better understand its influence on obesity and diabetes development.

127 Relation between farm health status, immune stimulation, amino acid metabolism, and N-efficiency in growing pigs

de Vries S¹, Sakkas P¹, van der Peet-Schwering C², Kampman- van de Hoek E^{1,2}, van den Borne J¹, Jansman A², Gerrits W¹

¹Animal Nutrition Group, Wageningen University and Research, ²Wageningen Livestock Research, Wageningen University and Research

Current nutrient recommendations for pigs are based on requirements measured under experimental, high sanitary, conditions. Under practical sanitary conditions, however, immune system of pigs may be stimulated, and nutritional requirements may be affected. Several studies have shown increased requirements for specific amino acids (AA) in case of chronic immune stimulation, but the requirements under various on-farm conditions, are unclear. To investigate the relation between immune stimulation, AA-metabolism, and N-efficiency, under practical sanitary conditions, growing pigs at six farms, varying in sanitary status, were compared. Farm management procedures were aligned, the same diets were used, and pigs were of the same crossbreed. N-retention at adequate- or low-protein diets was evaluated (8 pigs/farm; 43±2.5kg). Another 10 pigs/farm (50±2.5kg), received a bolus of uniformly-labelled-¹³C-AA in the ear vein, under steady-state-conditions. Plasma pool sizes (PPS) and irreversible loss rates (ILR) of AA were calculated based on changes in ¹³C:¹²C of AA in blood during 2h post-administration. Blood acute phase proteins (APP), white blood cells, anti-KLH-IgG, and anti-KLH-IgM were measured. These immune system parameters (ISP) were clustered using Principal Component Analysis and correlations with PPS and ILR of AA (n=46 pigs) and marginal N-efficiency (n=33 pigs) were tested. N-retention (64.6-71.9% of digestible-N-intake for adequate-protein diet; 67.5-71.0 for low-protein diet, P=0.07), marginal N-efficiency (0.56-0.73, P=0.14), PPS (11-25µmol/kgBW, P<0.01) and ILR (74-119µmole/kgBW/h, P<0.01) of tryptophan, and ISP varied among farms, but were not related to health classification. Parameters of the innate immune system (neutrophils, anti-KLH-IgG) positively correlated with ILR of tryptophan and phenylalanine, and PPS of phenylalanine and isoleucine. Otherwise, no associations between ISP and AA-metabolism nor N-efficiency were observed.

This indicates that AA-requirements may differ between farms, but seem unrelated to AA needed for the production of APP. Instead, AA-requirements seem to be more driven by indirect costs (e.g. tissue repair, increased protein-turnover) for subclinical infections.

128 Antibiotics-induced modulations of large intestinal microbiota altered bile acid profile and host lipid metabolism of piglets

Pi Y¹, Gao K¹, Peng Y¹, Mu C¹, Zhu W¹

¹Laboratory of Gastrointestinal Microbiology, College of Animal Science and Technology, Nanjing Agricultural University

Abstract: Gut microbiota plays important roles in bile acid (BA) metabolism. The BA can regulate host metabolism. However, the contribution of microbiota in large intestine to host metabolism remains unclear. To investigate the effects of antibiotics induced microbiota alteration in the large intestine on microbial BA metabolism, and host metabolism, 14 distal ileal cannulated piglets were randomly assigned into two groups and infused either sterile saline or saline with mixture antibiotics (ampicillin 150 mg/kg/day; gentamicin 4 mg/kg/day; and metronidazole 30 mg/kg/day). After 25 days' infusion, pigs were euthanized and the serum, urine, colonic digesta, and liver samples were collected. The main bacteria in colon and the BA profiles in colonic digesta, serum, and liver were determined. The metabolites in serum and urine were investigated by metabolome. Liver transcriptomics were investigated by RNA-sequencing. Results showed that antibiotics could modulate the populations of main bacteria in colon by decreasing Bacteroidetes, Actinobacteria, *Bifidobacterium*, *Prevotella*, *Clostridium* cluster XIVa, and *Clostridium* cluster IV, while increasing Firmicutes, *Lactobacillus*, and *E. coli* ($P < 0.05$). In addition, antibiotics infusion also changed the secondary BA profiles (deoxycholic acid, and lithocholic acid) in colonic digesta and this change was paralleled with the changes of these BA in serum and liver ($P < 0.05$). Further correlation analysis showed that the markedly changed gut bacteria in colon were strongly correlated with the change in BA profile. Moreover, antibiotics infusion markedly affected host fatty acid (FA) biosynthesis revealed by the results of both metabolomics in serum and urine and transcriptome in liver. These results indicate that antibiotics-induced modulations of main bacteria in large intestine can change host BA metabolism and lipid metabolism. These findings may provide a new insight into that the contributions of microbiota in large intestine to gut BA metabolism and host metabolism.

Keywords: antibiotic; bile acid; lipid; metabolome; microbiota; metabolism

129 Carryover effect of prior fiber type consumed on metabolic markers and fecal microbiome in Ossabaw pigs

Ajuwon K¹, Almeida V¹, Yan H¹, Nakatsu C¹

¹Purdue University

Carryover effect of prior fiber consumption on metabolic markers and fecal microbial composition was investigated in Ossabaw pigs. Treatments were arranged in 2×2 factorial with 2 fiber sources, 4% inulin or cellulose (Solka-Floc®) and fat levels (5 or 15%) for the low-fat diet (LFD) and high-fat diet (HFD), respectively. Pigs were fed the two fiber diets for the first 56d (nursery phase), and thereafter fed either the LFD or HFD containing no added fiber source from day 56 to 140 (growing phase). Pigs on the HFD were heavier ($P = 0.05$) than those on LF (64.61 vs. 68.38kg), regardless of prior fiber type consumed. Pigs that were fed cellulose during the nursery and later fed the HFD had the highest ADG ($P < 0.05$). Feeding the HFD resulted in higher back fat (BF) (13.41 and 18.18 ± 0.12 mm for LFD and HFD, respectively; $P < 0.01$). The HFD resulted in higher ($P < 0.01$) serum insulin (0.014 and 0.016 ± 0.001 mg/L for LF and HF, respectively) and glucose (100.89 and 125.03 ± 4.39 mg/dL for LF and HF, respectively) concentrations. Inulin increased ($P \leq 0.02$) jejunal expression of SREBP-1c and CL-4, but reduced ($P < 0.05$) TNF α and IL-6 expression in the ileum. Fecal microbial alpha diversity was significantly different ($P < 0.05$) between the inulin and cellulose fed pigs at the end of the nursery and finishing phases. Beta diversity (Jaccard, Bray Curtis or unweighted Unifrac analysis) was only different at the end of the nursery phase, but not the growing phase, indicating that effect of prior fiber consumption during the nursery phase did not carry through to the end of the growing phase. Results indicate that continuous fiber consumption might be more effective at maintaining the microflora and metabolic health than a discontinuous feeding.

131 The effect of environmental challenge on gut microbiota composition in growing pigs

Guan X¹, Schot C², Ramayo Caldas Y³, van Baarlen P², Molist F¹

¹Schothorst Feed Research, ²Host-Microbe Interactomics Group, Wageningen UR, ³Animal Breeding and Genetics Program, IRTA

Stress may negatively affect performance and welfare of pigs, partially via adverse effects on gut microbiota. In this study, we investigated the microbial composition of feces via 16S rDNA profiling during a mild stress period. Pigs were housed with ten per pen in rooms containing 8 pens. During a 68-day experiment, 160 pigs were housed in 4 normal rooms as control, and 160 pigs in 4 other rooms were exposed to three types of challenges (cold environment, regrouping and fasting). On 8 different days, the temperature was dropped by 10 °C. On days 8, 25 and 41, 2 pigs from each pen were exchanged with 2 pigs from another pen in the same room. Fasting was induced on 2 days. On day 42, from 32 boars (control) and 32 boars (challenge), faecal samples were collected for the microbiota profiling. Qiime software was used to detect Operational Taxonomical Unit (OTU) and the taxonomy classification was determined using the Greengenes database. The effect of challenge was evaluated at OTU, genus and functional predictions (KO) levels. Diversity indexes (α -diversity, β -diversity and richness), sparse partial least squares discriminant analysis (sPLS-DA) and differential abundance analysis (DA) were performed based on sample classification (control vs. challenge). Control pigs showed higher α -diversity and richness than challenge pigs ($P < 0.05$), suggesting a significant effect of challenge on the gut microbiota diversity. To be noted, 93% (14/15) of the most discriminant OTUs identified in the sPLS-DA were confirmed as DA between control and challenge groups. The taxonomic classification of these OTUs include biologically relevant genera such as *Clostridium*, *Coprococcus*, *Dorea*, *Treponema*, *Lachnospira* and *Prevotella*. We also identified discriminant and DA metabolic pathways that were more abundant in control than challenged pigs. Collectively, a strong effect of the challenge on the microbiota composition and functional level of growing pigs was observed.

132 Associations between birthweight, performance and faecal microbiota of piglets pre and post-weaning

Gaukroger C¹, Edwards S¹, Adams I², Rushton S¹, Kyriazakis I¹

¹Newcastle University, ²Fera Science

Low birthweight pigs (LBiWP <1.25kg) who are growth restricted have immature gut physiology, thus potentially creating a different environment to support bacterial growth compared to heavy birthweight pigs (HBiWP). Some LBiWP seem able to catch up to their heavier conspecifics, although the mechanism behind this is still uncertain. The study hypothesised that microbiota richness, diversity and profiles will differ between LBiWP and HBiWP, identifying potential microbiota markers of performance.

Faecal samples were collected from sibling pairs of heavy (BiW, 1.5-2.0kg) and light (BiW, 0.8 -1.25kg) piglets on days four, eight, 14, 21, 27, 32, 35, 42, 49 and 56 of age from 13 litters. Samples were snap frozen and stored at -80°C until pyrosequencing of the 16S rRNA gene, using the Illumina MiSeq system. Data were analysed in QIIME and R using principal component analysis and linear mixed effect models.

Bacteroidetes and Proteobacteria were the most abundant phyla pre-weaning. Post-weaning the Bacteroidetes phyla predominated, with lower levels of Actinobacteria, Cyanobacteria, Fibrobacteres, Firmicutes, Fusobacteria, Tenericutes and Verrucomicrobia. Bacterial richness and microbiota diversity increased with increasing piglet age ($P < 0.001$), but no significant differences were observed between LBiWP and HBiWP.

There was no effect of BiW on relative abundance of bacteria, but there was a significant interactive effect between BiW and average daily gain on the relative abundance of two bacterial taxa pre-weaning and 14 taxa post-weaning ($P < 0.05$). Increased abundance of Bacteroidales and Clostridiales was associated with higher growth rates pre-weaning ($P < 0.01$). Of the low abundance taxa post-weaning, Lactobacillales and unclassified Bacteroidales were lower in LBiWP with lower growth rates ($P < 0.01$).

Lower abundance of unclassified Bacteroides spp. and Lactobacillus could act as biomarkers of poorer performance post-weaning in LBiWP. Further analysis using the outputs from PICRUSt and iPath2 will test the hypothesis that the LBiWP microbiota has differing metabolic functions compared to HBiWP.

133 Early-life feeding accelerates gut microbiota colonisation patterns in piglets

Choudhury R^{1,2}, Middelkoop A², Gerrits W³, Kemp B², Bolhuis J², Kleerebezem M¹

¹Host-Microbe Interactomics, Wageningen University and Research, ²Adaptation Physiology Group, Wageningen University and Research, ³Animal Nutrition Group, Wageningen University and Research

Early-life microbiome perturbations have been suggested to affect host development, physiology and behaviour, which can persist throughout life. We hypothesise that early-life feeding (pre-weaning access to solid feed) can affect gut microbiome colonisation patterns and corresponding intestinal development in piglets. The experiment was conducted in two batches where the control group exclusively consumed sow's milk (n=24 selected from 10 litters), whereas the intervention group had access to customised fibre-rich early-feeding diet from two days after birth (n=24 selected from 12 litters) in addition to sow's milk. Rectal swabs were collected until six weeks of age to investigate gut colonisation patterns by 16S rRNA sequencing. Additionally, a subset of piglets from both groups (n=14 each) were sacrificed at weaning (four weeks of age) and intestinal tissue samples were collected to evaluate the molecular effects of early-life feeding. Independent of early feeding intervention, dynamic patterns of gut microbiota colonisation were observed. Age (or developmental programming) and batch effects were found to be dominant factors in explaining microbiota variation in the samples. Early-life feeding increased microbial diversity from two weeks of age onwards ($P<0.05$) and led to significant differences in microbiota composition from three weeks of age (PERMANOVA test on UniFrac Distance, $P<0.05$). Multivariate analyses using Canoco 5 package, indicated that early feeding induced changes included the expansion of typical fibre-degrading microbes like *Prevotella*, as well as several anaerobic Firmicutes genera like *Faecalibacterium*, *Megasphaera*, and *Coprococcus* that are commonly associated with post-weaning microbiota adaptations. The results show that early-life feeding can accelerate pre-weaning colonization of microbial groups that commonly appear in the piglet intestine after weaning. The mucosal consequences of this accelerated intestinal microbiota development are currently studied using the tissue samples obtained from the subset of animals sacrificed at weaning.

134 Impacts of dietary fiber and carbohydrases on intestinal microbiota in ETEC challenged pigs

Li Q¹, Burrough E², Peng X³, Dorman K³, Patience J¹

¹Department of Animal Science, Iowa State University, ²Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, ³Department of Statistics, Iowa State University

Enterotoxigenic *Escherichia coli* (ETEC) is one of the major causes for diarrhea in young piglets, with potentially serious economic impact. This study was conducted to test the hypothesis that dietary soluble (10% sugar beet pulp) and insoluble fiber (15% corn DDGS) with or without exogenous carbohydrases will regulate intestinal microbiota, thereby modulating the microbial response to an ETEC challenge in pigs. Sixty piglets (6.9 ± 0.07 kg) were randomly assigned to six treatments (n=10) including a non-challenged control (NC), F18 ETEC-challenged positive control (PC), soluble fiber+ETEC (SF), insoluble fiber+ETEC (IF), and SF or IF with carbohydrases (SFE or IFE). Pigs were individually housed and received ETEC inoculant or PBS on day seven post-weaning. Intestinal contents were collected on day 14 or 15 for total DNA extraction. The V4 region of the 16S rDNA was amplified and sequenced. High-quality reads (total 6,671,739) were selected and clustered into 3,330 OTUs based on 97% sequence similarity. No differences were observed in α -diversity among treatments ($P>0.10$). The ileal microbiota in NC and PC had modest separation in the weighted PCoA plot ($P<0.05$). The PC increased *Proteobacteria* (45.57 vs. 1.44%) and decreased *Firmicutes* phyla relative abundance (53.76 vs. 97.40%) compared to NC in the ileum; SFE, IF, and IFE reduced the magnitude of those bacterial changes. At the genus level, *Lactobacillus* dominated the ileal microbiota in NC, representing 91.99% of the detected bacterial population; PC numerically decreased its abundance to 50.85%. The PC increased ileal *Escherichia-Shigella* relative abundance compared with NC (35.86 vs. 0.47%; $P<0.01$); SFE (4.97%), IF (4.38%), and IFE (13.96%) numerically reduced its abundance. In conclusion, ETEC challenge increased *Escherichia-Shigella* and decreased *Lactobacillus* in the ileum. The magnitude of these bacterial changes was reduced by SFE, IF, and IFE, thus better maintaining microbial homeostasis.

135 Effect of dietary carbohydrase supplementation and soaking of cereals on intestinal microbiota in grow-finisher pigs

Torres-Pitarch A^{1,2}, Cormican P¹, Rea MC^{1,3}, Crispie F^{1,3}, Gardiner GE⁴, O'Doherty JV², Lawlor PG¹

¹Teagasc, Animal and Grassland Research and Innovation Centre, ²School of Agriculture and Food Science, University College of Dublin, ³APC Microbiome Ireland, University College Cork, ⁴Department of Science, Waterford Institute of Technology

Soaking the cereal fraction of liquid diets (cSLF) prior to feeding may improve its nutritional value. Xylanase and β -glucanase (XB) are carbohydrases commonly used to improve nutrient digestibility in pig diets. Both strategies may result in the release of substrates for use by intestinal microbes. It was therefore hypothesized that feeding cSLF $\pm$ XB to grow-finisher pigs would modulate intestinal microbial composition. A total of 36 pens of pigs (7 pigs/pen; ~33.4kg) were allocated to 1 of 4 treatments: (1) fresh liquid feed (LF); (2) cSLF; (3) LF+XB and (4) cSLF+XB. The cereal fraction of cSLF diets was soaked with water for 3h prior to feeding. Pigs were fed the liquid diets (28.6% DM) for 71 days and then slaughtered. Digesta was collected from the terminal ileum and caecum of pigs for microbiota profiling using 16S rRNA gene sequencing and for volatile fatty acid (VFA) analysis. VFA data were analysed by the MIXED procedure of SAS. Differential relative abundance (RA) profiles of the intestinal microbiota at genus and exact amplicon sequence variant (ASV) level were contrasted between treatments and correlated with pig growth and VFA concentrations. In the ileum, *Lactococcus* was more abundant in cSLF+XB than in LF pigs ($P<0.01$) and *Raoultella* was more abundant in cSLF pigs than in LF+XB pigs ($P<0.01$). In the caecum, *Lachnospiraceae_AC2044_group* was less abundant in cSLF than LF+XB pigs ($P<0.01$), and *Escherichia/Shigella* was lower in cSLF and cSLF+XB compared to LF+XB pigs. A number of differences were also observed between dietary treatments at ASV level. *Raoultella* RA was positively correlated with ileal butyrate concentration while *Lachnospiraceae_AC2044_group* RA was negatively correlated with caecal butyrate concentration. In conclusion, feeding cSLF diets to grow-finisher pigs enhanced beneficial bacteria in the ileum and LF+XB increased the abundance of potentially pathogenic bacteria in the caecum compared to SLF diets.

136 Dietary zinc oxide or Zn-Lysinate differentially modulate the colon microbiome, microbial activity and antibiotic resistance genes in weaned piglets

Pieper R¹, Dadi T², Pieper L³, Vahjen W¹, Franke A⁴, Reinert K², **Zentek J¹**

¹Institute of Animal Nutrition, Freie Universität Berlin, ²Department of Mathematics and Computer Science, Institute of Computer Science, Freie Universität Berlin, ³Institute for Veterinary Epidemiology and Biostatistics, Department of Veterinary Medicine, Freie Universität Berlin, ⁴Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel

We studied the influence of high or low dietary ZnO with Zn-Lysinate on the intestinal microbial metagenome, metabolic activity, and occurrence of antibiotic resistance genes in the colon of weaned piglets. A total of n=40 piglets were blocked into four groups and fed either 40 or 110ppm zinc oxide (40ZnO, 110ZnO), 110ppm Zn-Lysinate (110Zn-Lys), or 2500 ppm ZnO (2500ZnO). After three weeks, colon digesta samples analyzed for bacterial metabolites (D-/L-lactate, short chain fatty acids (SCFA), ammonia). Metagenomic sequencing of colon microbiota was performed by Illumina NextSeq500 sequencing. Bacterial taxa at species level were identified from quality-checked metagenomic reads using a new Species Level Identification of Microbiota from Metagenomes tool. Assignment of metagenomic reads into Gene Ontologies (GO) was done using the EBI metagenomics database. Differential abundance of bacterial taxa and functional genes was analyzed using partial least squares discriminant analysis (PLS-DA) and VIP scoring in R. Metagenomic reads were also checked for presence of antibiotic (AR) resistance genes. Performance and health status did not differ significantly. Lowest concentration of total and individual SCFA as well as NH₄ was determined in 2500ZnO group as compared with 40 ZnO, 110ZnO and 110 ZnLys ($P<0.05$), whereas highest concentration of total SCFA, propionate and n-butyrate was found in the 110ZnLys group ($P<0.05$). At genus level, no differences between 40ZnO, 110ZnO and 110 ZnLys were observed, whereas 2500ZnO showed a strong influence on many genera. However, at species level, a clear grouping according to dietary zinc concentration and source was observed, indicating different effects for ZnO or Zn-lysinate as zinc source. GO analysis revealed significant differences in metabolic function related to sporulation, stress response, and carbohydrate metabolism. Finally, the abundance of AR genes was only higher in the 2500ZnO group ($P<0.05$) but not with the other zinc sources.