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Little is known about amino acid (AA) requirements of piglets right after weaning where piglets face multiple stressors. Stress level was hypothesized to affect AA requirements. To study this, piglets were exposed to a smooth transition onto solid feed by giving them regular meals until day 3 post weaning (C-d3, n = 8) or until day 10 (C-d10, n = 8). Another group of piglets were exposed to a higher stress level, i.e., withholding feed until 24 h after weaning (F-d3, n = 8). On day 3 and 10, piglets were fed ½ hourly test meals at a level of 2.4 × the energy requirements for maintenance. After 5.5 h, the test meal was mixed with U-13C labelled Spirulina hydrolysate and fed in the same way for another 6 h. Jugular and portal vein blood plasma samples were taken and analysed on tracer and tracee AA by LCMS and GC-C-IRMS. Differences in levels of jugular and portal blood were taken as a proxy for the arteriovenous difference over portal drained viscera (PDV). Net balances (NB) over the PDV were calculated for tracer and tracee, yielding an enteral and endogenous part of the NB. Data were analysed using a MIXED model.

F-d3 had a higher protein breakdown due to weaning stress as indicated by higher urea plasma levels compared to C-d3 (P = 0.09). F-d3 showed a negative NB for essential AA (EAA) from the feed (P = 0.05), resulting in a 19% lower flux of EAA from PDV (P = 0.05) compared to C-d3. The uptake of arginine by the PDV was 3x higher in F-d3 and C-d3 (P = 0.01) compared to C-d10 that showed a net release of arginine (P = 0.05). It can be concluded that a high level of nutritional stress influenced EAA flux from PDV and stimulates protein breakdown. A high arginine uptake by the PDV probably plays a role in this.

P216. Transcriptomic and DNA methylation response to feed intake in the duodenum in high- and low-feed efficiency pig lines

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Feed efficiency is a complex trait of interest in animal breeding to reduce environmental impact of livestock herding and the cost of animal feed. A divergent selection on feed efficiency was carried out in pigs for more than 10 generations at an INRAE experimental unit, establishing lines of relative high- and low-feed efficiency in large white pigs. The objective of this study was to identify the molecular mechanisms underlying the divergence in feed efficiency between the two pig lines in the duodenum. We focused on the duodenum because of its sensory role in the hunger satiety pathways.

Duodenum mucosal samples were collected in post-weaning pigs from the two divergent lines either after a 12 h period of feed restriction, or with a 10 h period of feed restriction followed by a 2 h period of ad libitum feed access (n = 6 per group and per line). Transcriptomes and methylomes were analysed by poly-A RNA sequencing (RNA-seq) and by methylated DNA precipitation followed by sequencing (MeDP-seq), respectively. Transcriptomic reads were analysed with *nf-core/rnaseq* and *limma-voom*. MeDP-seq reads were analysed with *nf-core/chipseq* and the BayMeth method.

A total of 962 genes were differentially expressed in the duodenum mucosa between the conditions (fasted vs fed), in contrast with 318 differentially expressed genes when comparing the divergent lines. The

DNA methylome of the duodenum mucosa was unaffected by short term feed intake, but was distinct between the lines. More transcripts were affected by short term feed intake in the feed-efficient line than in the other line, including genes of the incretine secretion pathways involved in the production of GIP and GLP-1.

Thus the duodenum transcriptomic response to short term feed intake was distinct in feed efficient and less efficient pig lines. These differences might in part be due to differences in DNA methylation profiles.

P217. Influence of n-3 fatty acids in sow and piglet diets on ileal transcriptome of piglets 28 days post-weaning

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Selection for hyperprolific sows has increased litter size and the frequency of piglets with low birth weight which are more vulnerable to the challenges of weaning. Due to their anti-inflammatory properties, there is growing interest in the use of n-3 fatty acids (FA) in post-weaning diets. However, very little is known about their mode of action. This study aims to determine changes in the ileal transcriptome of post-weaned piglets fed n-3 FA provided either through maternal diet (lactation) or directly in the post-weaning diet. We used piglets from six litters (3 control sows and 3 n-3 FA sows). From each litter, 2 piglets with low and 2 with large birth weight were selected at weaning. For each weight category one piglet was offered a control diet and the other one an n-3 FA diet. At day 28 post-weaning (± 56 days of age) ileal tissue samples were collected and total RNA was extracted. RNA-seq reads were mapped and quantified against reference genome Sscrofa11.1 using STAR and RSEM, respectively. Differential expression analysis was performed with DESeq2 using a Wald test to compare the different groups, adjusting for the factor sex in the model. Genes were considered differentially expressed with an adjusted P < 0.05 and absolute fold-change > 1.5. While n-3 FA in maternal diet modified the expression of only 8 genes, a total of 371 genes, 328 upregulated and 43 downregulated, were identified in n-3 FA piglets compared to control piglets. A functional categorization of the overexpressed genes revealed biological functions associated with intestinal and mineral absorption, and the metabolism of lipids. Furthermore, downregulated genes were related to gap junction channel activity and glutamate neurotransmitter release cycle. No effects of birth weight were observed. In conclusion, the inclusion of fish oil, mainly in piglet diet, modifies the ileal transcriptome regardless of piglet's birth weight.

P218. Dietary protein sources affect uremic toxin excretion in pigs

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Metabolites produced during protein fermentation by the microbiota in the hindgut, e.g. phenols and indoles, are known precursors of uremic

toxins. When absorbed over the colonic wall, these bacterial metabolites add to the pool of uremic toxins with potential deleterious health effects. Dietary interventions, including low protein diets, are used to reduce levels of uremic toxins from bacterial origin. However, as several bacterial uremic toxins (UT) arise from specific amino acids, we hypothesized that the composition of dietary proteins is a major influencer of UT produced. In this study, twelve pigs, equipped with a simple T-cannula at the distal ileum, were fed a variety of protein sources ($n = 10$) in diets containing 100 g CP/kg DM feed and a N-free diet, following a youden square design. Pigs received the diets for 7 days. Intestinal effluents were collected via the simple T-cannula at day 6 and 7 and analysed for their DM, N and AA composition. Urine spot samples were collected at day 5 and analysed for UT using GC-MS. With the use of principal component analysis we identified patterns in UT excretions as affected by dietary protein source. Preliminary data showed how groups of UT were similarly affected by dietary treatment. Hippuric acid concentrations correlated with 3-hydroxyhippuric acid and 3-hydroxyphenylpropanoic acid concentrations, these UT are bacterial breakdown products of polyphenols. 2-hydroxyhippuric acid concentrations, correlated with 4-hydroxyphenylpyruvic acid and 4-hydroxyphenylacetic acid, these UT are products of microbial tyrosine and phenylalanine metabolism. 4-hydroxyphenylpyruvic acid and 4-hydroxyphenylacetic acid were positively correlated with an increased flow of DM and with high N concentrations in the ileal digesta. Colonic tyrosine and phenylalanine flows (g/day) correlated positively to concentrations of 4-hydroxyphenylpyruvic- and 4-hydroxyphenylacetic acid. Preliminary observations show an effect of dietary protein source on UT concentrations and an influence on their production by the gut microbiota.

P219. Effects of dacitic tuff breccia and poultry by-products on jejunal morphology and gene expression of nursery pigs

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Dacitic tuff breccia (DTB) and poultry by-products (PBP) were added to nursery pig diets to evaluate their effects on jejunal morphology and gene expression in a 35d experiment. PBP were added as a replacement for fishmeal, and was expected to provide an increased immune challenge to the newly weaned pigs. DTB was added as a functional trace element source, and was hypothesized to ameliorate some of the negative impacts of PBP on the gut. A RCB design used 564 weaned pigs (20.1d of age, 6.2 ± 0.01 kg BW), blocked by BW and sex and allotted to four dietary treatments (15 replicates/treatment; 9–10 pigs/pen): 1) Negative control (NC), 2) NC + DTB (0.5%), 3) NC + PBP (4% chicken by-product meal + 2% feather meal – replacing fishmeal, corn, synthetic amino acids in NC), 4) NC + DTB + PBP. Phases 1–3 were each 7d and Phase 4 was 14d. One barrow/pen was harvested 11d post-weaning. Jejunal tissue and mucosa were collected for histological measures and gene expression. Jejunal villi height, crypt depth, goblet cell, lymphocyte, and enterocyte counts did not differ among treatments ($P > 0.10$). Pigs fed PBP had increased jejunal expression of interferon-alpha ($P = 0.041$) and interleukin-10 ($P = 0.037$), and tended to have increased expression of claudin-1 ($P = 0.076$). Pigs fed DTB tended to have increased jejunal expression of interferon-gamma ($P = 0.079$). Jejunal gene expression of tumour necrosis factor-alpha and nuclear factor kappa-beta did not differ among treatments. Previously reported improvements in nursery pig ADG and ADFI in DTB-fed pigs at this dose does not appear to be related to jejunal immune and villus structure measurements. Feeding PBP had minimal effects on jejunum architecture but increased the gut immune response. Further research is needed to understand the physiological mechanisms related to induced inflammatory aspects in the trade-off of using by-product feed ingredients.