<sup>a</sup> Gembloux Agro-Bio Tech (GxABT), University of Liège, Gembloux, Belgium

<sup>b</sup> Productions in agriculture Department, Walloon Agricultural Research Centre, Gembloux, Belgium

<sup>c</sup> Fundamental and Applied Research for Animal & Health, Food Science Department, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

<sup>d</sup>Nutrition and Animal-Microbiota Ecosystems, Department of Biosystems, KU Leuven, Heverlee, Belgium

\*Corresponding author: Ester Arévalo Sureda. E-mail: ester.arevalosureda@uliege.be.

Apple pomace (AP), the remaining product after juice extraction, may have a potential health effect as it is rich in polyphenols and fibres. This study investigated if post-weaning diet supplementation with AP would be able to modulate microbiota in colon and induce beneficial effects on intestinal health. Antibiotic-free piglets were weaned at 28 days-of-age and were divided in three dietary groups: control diet, 2%AP and 4%AP supplemented with dried AP for 5-weeks until sample collection (n =4-6/group). Diets were iso-energetic and iso-nitrogenous. The colonic content was analysed for short-chain fatty-acids (SCFA) by HPLC, and microbiota composition was analysed by 16S rRNA sequencing using the bioinformatics tools QIIME and MicrobiomeAnalyst. Colon tissue was analysed for gene expression by high-throughput qPCR. Microbiota relative abundance was angular transformed for network analysis (WGCNA). AP treatment did not alter  $\alpha$ -diversity and tended to modify  $\beta$ -diversity (P = 0.086), especially the 2%AP group that significantly increased the abundance of the class Bacilli compared to controls (FDR = 0.01). SCFA showed no significant differences due to AP treatment. Gene expression of Interleukin-1 $\beta$ , Interferon- $\alpha$  and - $\beta$ , and Claudin-1 were significantly increased in the 2%AP group compared with controls (P < 0.05). Network analysis clustered microbial OTUs in 10 modules. The Magenta Module, including Paraprevotellaceae, Spirochaetaceae, Elusimicrobiaceae, and phyla TM7, showed a positive correlation with treatment (P = 0.02) and tended to correlate with acetate production (P = 0.09). The Brown Module, including the genera Bifidobacterium, Leuconostoc, Megamonas, Mitsuokella, Actinomyces, Ruminococcus, Pseudomonas, and Bacteroides, showed a correlation with acetate (r = -0.68; P = 0.007) and with expression of interleukin-1 $\beta$ , interferon- $\alpha$ and  $-\beta$ , and claudin-1 (r = 0.9; P < 0.0001). Blue-, Green- and Yellow Modules showed a tendency for negative correlation to SCFA production. Thus, AP dietary supplementation of piglets for 5 weeks post-weaning modulated microbiota and gene expression to a certain extent and correlations between bacteria and the host's response were observed.

## P148. Increased protein fermentation metabolites and pathogenic bacteria are linked to piglet post-weaning diarrhoea under practical conditions

Bart van der Hee <sup>a,b,\*</sup>, Myrthe Gilbert <sup>c</sup>, Ineke Heikamp-de Jong <sup>b</sup>, Merlijn van Gaal <sup>b</sup>, Boudewijn Burgering <sup>d</sup>, Walter Gerrits <sup>c</sup>, Jerry Wells <sup>a</sup>, Hauke Smidt <sup>b</sup>

<sup>a</sup> Host-Microbe Interactomics, Department of Animal Sciences, Wageningen University and Research, Wageningen, Netherlands <sup>b</sup> Laboratory of Microbiology, Wageningen University and Research, Wageningen, Netherlands,

<sup>c</sup> Animal Nutrition, Department of Animal Sciences, Wageningen University and Research, Wageningen, Netherlands

<sup>d</sup> Center for Molecular Medicine, Molecular Cancer Research Section, University Medical Center, Utrecht, Netherlands

\*Corresponding author: Bart van der Hee. E-mail: bart.vanderhee@wur.nl. Piglets are commonly weaned abruptly, and a shift in diet and early stage of intestinal development is often concurrent with post-weaning diarrhoea (PWD). Previous studies have shown that decreasing dietary crude protein content in piglet feed could alleviate PWD, but there are contradicting results in literature. Furthermore, tested concentrations (17 vs. 25%) are generally far above protein levels in current practice (14-16%). Therefore, the objective of our study was to identify associations between PWD and protein fermentation under practical conditions. We sampled piglets (n = 7 control and 13 diarrhoea per farm, total 80 piglets) on 4 farms prospectively pre- (-14 days) and post (2, 4, and 5 days) weaning (PW). Piglets with or without diarrhoea were selected on day 4 PW and dissected for intestinal and physiological measurements on day 5 PW. We found an increase of heat-stable toxin (ST)A and STb-producing E. coli in piglets suffering from diarrhoea 4 days PW (n=52) compared to controls (n=28) in all 4 separate farms (P < 0.001), and a concurrent significant increase in protein fermentation metabolites ammonia, hydrogen sulphide, and branchedchain fatty acids (all P < 0.05). This suggests that even at dietary protein concentrations of 14-16%, there is an association between protein fermentation and PWD. However, metagenomic analysis of the faecal material 4 days PW showed that not all diarrhoeic animals have high levels of STa, STb, or a panel of other toxins. This suggests PWD is associated with increased protein fermentation and pathogenic bacterial species, but that there is no direct causal link that induce PWD for individual piglets.

## P149. The influence of dietary ingredient quality and protein level on gut microbiota composition and microbial metabolites in weanling pigs

Nuria Canibe <sup>a,\*</sup>, Anna Schönherz<sup>a</sup>, Helle Nygaard Lærke<sup>a</sup>, Mette S Hedemann<sup>a</sup>

<sup>a</sup> Aarhus University, Tjele, Denmark

\*Corresponding author: Nuria Canibe. E-mail: Nuria.Canibe@anis.au.dk.

Gut microbiota composition and function, influenced by dietary substrates, can affect post-weaning diarrhoea risk in piglets. We hypothesized that feeding piglets with varying starch- and protein quality, and protein level, starting during suckling, affects gut microbiota composition and metabolism. A standard diet based on enzyme-treated soybean meal, barley and wheat (STD) or an optimized diet with puffed barley and wheat and enzyme-treated, easy digestible soy protein (TEST), at two protein levels (19.5 vs. 18.2% CP), was fed (another group was not offered creep feed) (5 treatments) to 40 litters starting from day 3 of age. The same four diets were fed after weaning, piglets fed no creep feed were fed high-protein diets. One pig per treatment (N = 8) was sacrificed on day -7, 0 (weaning), 5 and 14. Digesta from distal-small intestine (SI) and proximal-colon were collected to investigate microbial- and metabolite profiles. Data were analysed using a mixed model in SAS, and alpha- and beta diversities in R. No effect of diet was detected before weaning. Compared to STD, the TEST group had lower concentrations of SCFA (98 vs. 107mM) and branched chain fatty acids (day 5) (1.1 vs. 1.7 mM) in colon; of lactic acid in SI (7 vs. 26 mM); and of biogenic amines in SI (40 vs. 87 mM) and colon (117 vs. 174 mM). Total lactobacilli and bacteria (day 5) numbers in SI were lowest in the TEST group, and lactobacilli lowest in the 18.2% CP group on day 5. Enterobacteria numbers were highest in the TEST group on day 14. No effect on ammonia concentration was detected. Microbial alphadiversity was highest in the TEST group, and beta-diversity differed between the TEST and STD group (P=0.001). In conclusion, improved quality of protein and cereal sources (puffed) seemed to reduce gut microbial activity. The tested protein level had only small effects.