In the present study we could demonstrate treatment differences in FI starting during week 3 PW. It is concluded that the presented model allows screening for feed preference in piglets. Further studies are needed to clarify whether habituation delay to a new diet, unfamiliar taste/smell of the PFA and/or potential PW stress factors caused the absence of effects of the PFA in FI directly PW.

P120. Generating piglet intestinal organoids to study the effects of luminal fermentation metabolites

Bart van der Hee [a,b,*], Myrthe Gilbert [c], Miranda van Triest [d], Boudewijn Burgering [d], Nico Taverne [a], Anja Taverne-Thiele [a], Hauke Smidt [b], Walter Gerrits [c], Jerry Wells [a]

[a] Host-Microbe Interactomics, Department of Animal Sciences, Wageningen University and Research, Wageningen, Netherlands
[b] Laboratory of Microbiology, Wageningen University and Research, Wageningen, Netherlands
[c] Animal Nutrition, Department of Animal Sciences, Wageningen University and Research, Wageningen, Netherlands
[d] 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.

Post-weaning diarrhoea (PWD) has been associated with maldigestion of dietary protein due to increased colonic protein fermentation. Fermentation-derived metabolites could affect the developing intestinal epithelium, which is an important barrier between luminal compounds and host circulation. Understanding the impact of individual metabolites on the piglet epithelium is difficult to study in vivo. Therefore, we generated a heterotypic cell culture using intestinal crypt-residing stem cells from piglets, which resemble piglets' epithelium and genetics. These intestinal organ-like structures (organoids) were subsequently grown as a planar monolayer, allowing luminal compound stimulation to study mechanistic responses. Organoid monolayers exposed to physiologically relevant levels of protein fermentation metabolites triggered distinctly different responses. For instance, tracking individual cells over time showed that ammonia inhibited cellular migration (50% reduction in monolayer repair rate and duration) whereas branched-chain fatty acids and hydrogen sulphide increased migration capacity but inhibited cell directionality. The lack of directionality indicated that hydrogen sulphide affects barrier capacity, which was verified using trans-epithelial resistance (40% loss of TER, $P < 0.01$) and small compound translocation (0.4 kDa) assays as well as transcriptome sequencing. Transcriptome sequencing also showed that hydrogen sulphide affects mitochondrial functioning, which was verified using a mitochondrial stress test that showed significantly lower basal respiration ($P < 0.001$), as well as reduced mitochondrial capacity ($P = 0.001$). This indicates that hydrogen sulphide not only affects mitochondrial activity and energy capacity, but also directly links to a decline in barrier functioning. Combining piglet-derived organoids that recapitulate epithelial diversity with individual protein fermentation compounds has therefore given insight into the effects of individual compounds on a cellular and molecular level.

P121. Validation of in vitro protein fermentation with faecal inoculum from pigs using the gas production technique

Hanlu Zhang *, John Cone, Arie Kies, Nikkie Wielen, Wouter Hendriks

Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands

*Corresponding author: Hanlu Zhang.
E-mail: hanlu.zhang@wur.nl.

To study the fermentation process in animal and its potential health effects, the in vitro gas production technique was developed by simulating the organic matter fermentation using a microbial inoculum. This technique can be adapted to study protein fermentation, using a nitrogen (N)-free but carbon-rich environment. In this way N from the substrate will be the limiting factor for microbial activity, reflected by the gas profile. The cumulative gas production should increase with the substrate N availability. Therefore, a fixed amount of N should be used but not a fixed amount of organic matter. To validate the current technique, the amount of N used, the carbohydrate concentration and repeatability were investigated based on previous pilot studies. Whey protein isolate and urea containing 5, 10, 20 and 40 mg N were used as substrates in two runs (duplicate in each run) and fermented with a faecal inoculum, prepared from twenty pigs. Easily fermentable carbohydrates (maltose, soluble potato starch, xylose and citric pectin; 10 or 20 g/L) were added in the buffer. Maximum gas production, obtained with the different samples, was compared by the MIXED model procedure in SAS, and repeatability was calculated using the coefficient of variation (CV) between and within runs. Results showed that compared to 10, using 20 g/L carbohydrate mixture in the buffer , N would become the limiting factor under the level of 10-20 mg, as no differences were found between 20 and 40 mg N. Using 20 g carbohydrate per litre buffer, the intra-run CV of most groups was below 10% and the inter-run CV of all groups was below 15%. In conclusion, a level of 10 mg N in the substrate and a concentration of 20 g carbohydrate mixture per litre buffer is a suitable system to evaluate the in vitro gas production of protein.

P122. Use of the Dual Marker Technique to Estimate Individual Feed Intake of Young Pigs

Tianyue Tang [a,*], Carola van der Peet-Schwering [c], Nicoline Soede [b], Bjorge Laurenssen [b], Erik Bruininx [a], Emilie-Julie Bos [d], Walter Gerrits [d]

[a] Animal Nutrition Group, Wageningen University & Research, Wageningen, the Netherlands
[b] Adaptation Physiology Group, Wageningen University & Research, Wageningen, the Netherlands
[c] Wageningen Livestock Research, Wageningen University & Research, Wageningen, the Netherlands
[d] Agrifirm Innovation Center, Apeldoorn, the Netherlands

*Corresponding author: Tianyue Tang.
E-mail: tianyue.tang@wur.nl.

We studied dual marker methods to estimate individual FI (feed intake) in pigs for use in group-housing. Twelve 6.5-week-old individually housed male pigs (18.8 ± 0.6 kg) were assigned to one of three oral dosing treatments supplying 180 mg of ytterbium chloride ($YbCl_3$)/day and 111 mg of dotriacontane (C32)/day as reference markers: once (R1), three times (R3) or five times (R5) daily. We hypothesized that R3 and R5 to be better than R1. Pigs were offered a diet containing 0.46 g/kg of chromium chloride ($CrCl_3$) and 0.15 g/kg of hexatriacontane (C36) as in-feed markers. The experiment lasted for 10 days: day -5-0: adaptation; day 1-3: dosing of reference marker; day 2-4: total faecal collection (TFC). Spot faecal samples were taken on day 3 at 1200 h, 1700 h, day 4 at 0700 h. Pigs were fed restrictedly three times daily at 133.6 g/kg BW0.60. Individual measured FI was recorded daily (0.78 kg/day per pig), and was compared to predicted FI using Yb:Cr and C32:C36. Due to unequal variance, R1 pigs were omitted from the statistical treatment comparison. When using TFC samples, the absolute prediction error (APE) (predicted FI-measured FI) in R3 and R5 pigs were numerically lower than in R1 pigs either predicted by Yb:Cr or C32:C36. The APE measured by C32:C36 was numerically lower than measured by Yb:Cr at all frequencies, and significantly ($P = 0.039$) in R3 pigs (C32:C36: 0.15 ± 0.02 kg/day; Yb:Cr: 0.29 ± 0.04 kg/day). When using C32:C36 to predict FI, pooled, but not single spot samples gave