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

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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

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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

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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

similar APEs compared with TFC samples. Therefore, dosing the reference marker for three times per day combined with pooled spot faecal sampling appeared the minimum requirement for obtaining acceptable estimates of FI. The dual marker technique is promising to estimate intake of multiple feeds in group-housing.

P123. Expression of DNA-repairing AlkBh proteins in the tissues of intrauterine growth retarded pig

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Intrauterine growth retardation (IUGR) is associated with reduced birth body mass, delayed gastrointestinal mucosa development, and impaired digestion and absorption of nutrients (mainly sugars). Studies showed that FTO protein, member of AlkBh family proteins which are responsible for DNA-damage repair, may be involved in creation of IUGR and further metabolic consequences of IUGR such as obesity and diabetes type 2 development. The aim of our study was to compare the expression of AlkbH family, namely AlkbH 1-8 and FTO proteins, in IUGR and normal birth weight (NBW) pig's tissues (n = 6 in each group) which are crucial for energy metabolism (i.e., liver, pancreas, skeletal muscles and adipose tissue) as well as to monitor pig performance and glucose tolerance from weaning to 9 months of life. In IUGR neonates we found revealed enhanced level of AlkBh 1,3,4,8 and FTO proteins in the liver ($P < 0.05$), and reduced level of AlkbH 4 protein in the muscles as compared to NBW neonates ($P < 0.05$). In adipose tissue the level of AlkBh 8 has tendency to increase as compared to NBW ($P < 0.06$) Interestingly, there were no differences (IUGR vs NBW) in oxidative stress in the examined tissues. The oral glucose load test in 2-month-old piglets showed significantly higher blood glucose response after 1 h in IUGR as compared to NBW (247 ± 58 vs. 142 ± 32 mg/dl; $P < 0.05$). Also in this age pigs showed the largest body weight difference between the IUGR and NBW. The body weight difference diminished starting from the 4th month of life. Concluding, we found changes in proteins expression in IUGR neonates which may be involved in creation of IUGR development during foetal development. Results also showed that in 2 month-old weaned IUGR piglets revealed functional alteration of carbohydrate metabolism is the highest. Further studies are needed to clarify specific controlling of the IUGR metabolism.

P124. An association of Curcuma and Scutellaria plant extracts protects against inflammation induced by LPS in intestinal porcine epithelial cells

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Weaning stress challenges the piglet's gastrointestinal tract compromising gut barrier function, disturbing digestive and absorptive capacity and increasing disease susceptibility. It is accompanied by local intestinal inflammation characterised by immune cell infiltration and increased pro-inflammatory cytokine gene expression. In the context of reducing antibiotic use in pig production, greater attention is given to natural alternatives to improve intestinal health and welfare. Relevant in vitro models, specific to livestock species, may be employed to better evaluate the biological properties and to better understand the mode of action of these natural alternatives. Furthermore, in vitro models allow the screening of a larger number of ingredients and doses, prior to their validation in in vivo trials. Our study aimed to investigate the ability of a plant extract association of Curcuma longa and Scutellaria baicalensis to modulate lipopolysaccharide (LPS)-induced inflammatory response in the porcine IPEC-J2 cell line. Cells were pre-treated, with Curcuma longa and Scutellaria baicalensis extract association (0.5 to 200 µg/mL) at 37°C for 1h and then co-incubated with LPS (10 ng/mL) for 1h. Data are expressed as means ± SEM. Analysis of variance were performed. mRNA levels for IL-8, TNFα and CCL20 were significantly increased by LPS (17, 82 and 40-fold respectively; $P < 0,01$) compared to control. The plant extract association alleviated inflammation induced by LPS by significantly reducing Il-8, TNFα and CCL20 gene expression compared to LPS group (3.6 to 20-fold depending on cytokines and doses considered; $P < 0.001$). Effects were more pronounced with 100 µg/mL of the association although significant effects were already obtained at the smallest dose tested (0.5 µg/mL; $P < 0.001$). Taken together, results suggest that this plant association can exert direct anti-inflammatory effects to protect piglet intestinal cells from LPS-induced inflammation. Results obtained in a specific intestinal porcine cell line are promising for future research in in vivo trials.

P125. Association of faecal consistency with myeloperoxidase as biomarker in weaned piglets

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Myeloperoxidase (MPO), a lysosomal protein related to neutrophil activity, is often suggested as a non-invasive biomarker for intestinal inflammation in humans and pigs. The current study evaluated the relationship between faecal MPO levels and growth, faecal consistency and faecal microbiota composition of weaned piglets subjected to different experimental diets. A total of 400 weaned piglets were assigned to 4 dietary treatments (identical basal diets supplemented with different feed additives) and were housed 10 per pen, in 2 consecutive batches on a commercial farm. Piglets and feeders were weighed at 0-, 14- and 42-days post-weaning. From d0 until d14, faecal consistency (FCS) was scored daily on pen level with a score between 1 (firm and shaped) and 4 (watery diarrhoea). On d5 and d28, a faecal sample was taken from one piglet per pen (median body weight). An individual FCS was attributed to these samples and they were analysed for MPO concentration by ELISA and for the main bacterial groups by qPCR. Statistical evaluation was done by ANOVA including effects of treatment, gender, and batch without interactions. Pearson and Spearman correlation coefficients were calculated for continuous and categorical variables, respectively. Although treatment significantly affected ADG and FCR in the first 2 weeks, no effects were observed on the mean FCS (2.14 ± 0.07, mean ± SE) or on MPO levels (d5: 1831 ± 129; d28: 1410 ± 103 pg/g). On d5, 25%, 22.5%, 30% and 22.5% of the faecal samples received scores 1, 2, 3 and 4, respectively. Moreover, no relevant correlations were found between the faecal MPO levels on the one hand and the FCS, diarrhoea incidence or ADG at individual or pen level. Neither did MPO correlate to faecal microbial composition, like gene copies of E. coli F4. It can be concluded from this experiment that faecal MPO is not associated with the visual assessment of diarrhoea.