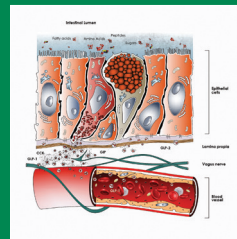


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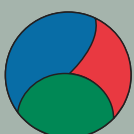
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Advances in Animal Biosciences

Proceedings of the 14th International Symposium on
Digestive Physiology of Pigs (DPP2018),
August 21-24, 2018,
Brisbane, Australia



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Subject areas can include aspects of Breeding and Genetics, Nutrition, Physiology and Functional Biology of Systems, Behaviour, Health and Welfare, Livestock Farming Systems, Human Health and Product Quality.

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Proceedings

of the 14th International Symposium
on Digestive Physiology of Pigs (DPP2018),
August 21-24, 2018, Brisbane, Australia

2018

Advances in Animal Biosciences

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Digestive Physiology of Pigs

The Proceedings of the 14th International Symposium on Digestive Physiology of Pigs (DPP2018), constitute summaries of papers presented as oral communication or posters at the DPP Triannual Conference 2018 held at the Brisbane Convention and Exhibition Centre, Brisbane, Australia, 21-24 August 2018.

The abstracts were peer-reviewed to guarantee the scientific standards set by the DPP program committee. Abstracts not meeting the minimum standards were rejected and have not been included in the proceedings. The abstracts accepted by the reviewers have been published as received. Views expressed in all contributions are those of the authors and not those of the DPP organizers or International Steering Committee.

Guest Editors

Eugeni Roura

Frank Dunshea

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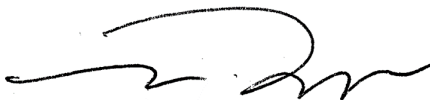
Preface

It is with great pleasure that I write this preface to the Proceedings of the 14th International Symposium on Digestive Physiology of Pigs (DPP), which took place at the Brisbane Convention and Exhibition Centre of the capital of Queensland (Australia) from the 21st to the 24th of August, 2018. This was the first time that a DPP conference was held outside Europe or North America. The DPP2018 attracted 483 delegates from 38 countries, of which 94 were students. This highly successful attendance was in good part thanks to the endless effort of the local organizing committee that I was honoured to chair. I would like to give special credit to the contributions of Expert Events (Lidia Dalton and Vicki Dwyer), Marta Navarro, Barbara Williams, Mike Gidley and Neil Gannon.

The scientific content of the conference was around 5 main sessions: 1) GIT nutrient sensing and the enteroendocrine system; 2) Gut health and intestinal immunity; 3) Rate and extent of ingredient digestion in the small intestine; 4) metabolic health and brain activity modulated by the gut; and 5) Microbiome development and barrier function. Each of the sessions consisted of a keynote speaker and six oral presentations selected from the abstracts submitted. The content of the proceedings in this special issue compile all the peer-reviewed abstracts presented at the DPP2018 after acceptance by the reviewers. More than 50 scientists helped reviewed the more than 320 abstracts submitted. I would like to acknowledge the contribution of each one of them. A full list of contributing reviewers has been added in the next pages. In addition, the excellent task of the Program Committee should also be commended (a list of the committee has been added in the back cover of this special issue).

One of the main successes of the conference was the sponsorship. The Sponsorship Committee did an outstanding job. Thanks to Prof. Rob van Barneveld –Chair-, David Cadogan, Heather Channon, Darryl D'Souza, Dave Henman, Robert Parkes and Eugeni Roura for their great contribution as part of this Committee. I would like to express as well my deepest gratitude to the 34 sponsors of the conference. A full list of the sponsors and logos can be found at the end, on the inside back cover of this publication. Special thanks to the main sponsors: Biomin (Diamond); Australian Pork Limited, DSM, EW Nutrition Australia, and Lucta (Platinum); and Agilia, Elanco, Norel, Pancosma and Zinpro (Gold). Not to forget the fundamental underwriting of the event by the DPP-North America group. Without the support of all of the above the DPP2018 would have never happened.

And my final sentence is an encouragement to the next DPP conference organizers. Good luck and all my support to the DPP2021. See you all in The Netherlands.



A/Prof Eugeni Roura

Co-Chair of the DPP2018

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Nutrient-specific appetites and the ecology of diet balance

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Appetite is not a unitary phenomenon, rather there are nutrient-specific appetite systems, which have evolved to cooperate to help animals achieve a balanced diet. These specific appetites manage the vastly multidimensional challenge of balancing the simultaneous requirements for dozens of different macro and micro-nutrients and other food components (e.g. fibre). By regulating a small number of key nutrient dimensions (protein, carbohydrate, fat, sodium, calcium) and relying on ecologically reliable correlations in natural foods, animals attain adequate intakes of the rest, which “come along for the ride”. However, these nutrient-specific appetites will compete rather than cooperate in an imbalanced nutritional environment. In addition, ancestrally reliable correlations among nutrients in natural foods can become broken, for instance by food processing. Competition among appetite systems for different nutrients results in expression of regulatory priorities for different nutrients, leading to imbalanced nutrient intakes with associated performance costs. A notable example is protein leverage, where the specific appetite for protein dominates total food and energy intakes in various species, including humans, leading to overconsumption of total energy and a predisposition to obesity on a diet in which protein is diluted by extraneous fats or carbohydrates. This narrative will be developed in the talk using data and examples from multiple species, from slime moulds to humans.

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Nutritional regulation of porcine intestinal development and weaning stress

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Piglet intestinal health is a major healthcare concern in swine production and plays a critical role in the whole growth and development. Numerous potential stress factors can induce intestinal dysfunction in piglets, which correlate with the modification of carbohydrate, proteins, lipid and nucleic acid metabolism, and they may further impair cellular function and result in clinical deterioration. To date, in our research group many experimental and clinical studies have been accomplished to solve the piglet intestinal health problems. Therefore, we summarize our own recent advances on nutritional regulation of porcine intestinal development and weaning stress, and we mainly focus on three parts: 1) The development of piglet intestine and alteration in weaning; 2) The protein metabolism for piglet intestinal crypt-villus axis; 3) Nutritional regulation of porcine intestinal development and weaning Stress. Briefly, by using the methods of nutrition, cell biology, molecular biological technologies, and omics technology, our studies were conducted to analyze the development changes of piglet intestine during the suckling and weaning periods, especially the alterations in intestinal crypt-villus axis, which involve intestinal absorption, epithelia proliferation, differentiation, barrier function, antioxidant capacity, protein metabolism, and microflora. Furthermore, under different stress or non-stress conditions, our studies aim to clarify the molecular mechanism of the effect of nutritional intervention on intestinal antioxidant system, immunity, microbial metabolism, mitochondrial function, proliferation and differentiation of enterocyte, as well as related signaling pathways in vivo and in vitro models. The ingredients of nutritional intervention include function amino acids (i.e., arginine, glutamine, glutamate, proline, γ -aminobutyric acid, aromatic amino acids, BCAA) and their metabolites (polyamines and α -ketoglutarate), as well as other functional active substances (melatonin and ethanalamine). Based on the all results, we will establish the nutritional strategies to prevent and alleviate intestinal injury in piglets, which further provide the theoretical and technical basis for improving intestinal health and growth performance in piglets.

Chemosensing of nutrients and non-nutrients in the human and porcine gutsI Depoortere¹ and E Roura²¹*Translational Research Center for Gastrointestinal Disorders, University of Leuven, Belgium;* ²*Centre for Nutrition and Food Sciences, The University of Queensland, Australia*

The gut is a large body interface between the external and internal milieus that requires continuous monitoring for nutrients and pathogens or toxic chemicals that need to be absorbed or eliminated, respectively. Several cell types of the gut epithelium such as Paneth, goblet, tuft and enteroendocrine cells contain chemosensory receptors, similar to those present on the tongue, which will elicit physiological responses relevant to the hunger-satiety cycle and/or defence mechanisms when activated. This talk will give an overview of the gut as a sensory organ and will further focus on how bitter and carbohydrates are sensed by enteroendocrine cells to control the release of the hunger hormone ghrelin involved in the regulation of energy and glucose homeostasis in isolated crypts of lean and obese subjects. In addition, an inside on the pig model will review the effect of dietary amino acids on gut anorexigenic peptide secretion, mainly CCK and GLP-1. Furthermore, the effects of intragastric administration of bitter compounds on gastrointestinal motility will be explained based on in vitro and in vivo studies in healthy human volunteers. An assessment of parallel discoveries on bitterant function in pigs will complement. These studies should help to provide insight on how targeting of extra-oral taste receptors in the gut with functional foods or specific agonists may open new clinical perspectives in humans and warrant high value for the pig model.

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

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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 Ca^{2+} , 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 Ca^{2+} 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.

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

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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.

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

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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.

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

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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 (L × LW) 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.

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

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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 RNeasy[®]. 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.

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Acceptability and palatability of garlic flavoured feed after social learning in nursery pigsJ Figueroa¹, L Salazar¹, C Valdivia², S Guzmán-Pino², C Valenzuela² and M Muller²¹Pontificia 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.

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Linking intestinal immunity to applied approaches in pig nutrition

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Gaining a deeper understanding into the underlying mechanisms associated with intestinal function and immunity during the weaning transition is critical to help shed new light into applied nutrition approaches to improve piglet performance and health during this critical life stage transition. The transient anorexia triggered at weaning leads to compromised intestinal barrier function and a localized inflammatory response. Considering barrier function, specific nutrient fractions appear to have a significant impact on the development and function of the immune and microbial systems around weaning. Understanding of the specific impacts of nutrient fractions in the small intestine and hindgut are important for helping to bring more focus and consistency to nutritional approaches to support health and immunity during the weaning transition period. The opportunity continues to be how to translate these modes of action into practical and scalable approaches for swine nutrition. We will focus specifically on practical nutritional approaches to influence intestinal immunity through lipid, protein and antioxidant nutrition.

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Impact of porcine reproductive and respiratory syndrome virus and reduced feed intake on jejunum function and integrity in pigsS Curry^{1,2}, E Helm¹, C De Mille¹, W Schweer¹, E Burrough¹ and N Gabler¹¹Iowa 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.

Abstract

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.

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

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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.

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

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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.

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

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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-1 α* gene expression in jejunum was significantly lower in LF piglets on the 8th day, and *IL-1 β* 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.

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

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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.

Abstract

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.

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Laminarin improves performance and intestinal health in post weaned pigs

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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.

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Rate and extent of macronutrient digestion in the small intestine: multiple mechanisms and consequences

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Rate and extent of macronutrient digestion in the small intestine: multiple mechanisms and consequences

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The small intestine is the primary site for enzyme digestion of macronutrients in pigs, as in humans. The rate and extent of this digestion has consequences for both animal productivity and long-term (human) health outcomes. For each macronutrient (protein, triglyceride, starch), digestion can start in the mouth (starch) or stomach (protein, triglyceride), but mostly occurs in the small intestine with catalysis by pancreatic enzyme secretions. The rate limiting steps in converting macronutrients into absorbable small molecules are likely to be one or more of (i) access of enzymes to macronutrient substrates, (ii) enzyme activity levels available for digestion, and (iii) uptake of products across the mucus layer that covers epithelial cells. From a combination of *in vitro* and *in vivo* results, factors affecting each of these processes will be discussed, in particular the roles of both soluble and insoluble dietary fibre components as modulators of each potential rate-limiting step.

The relative rate of enzymic digestion and digesta passage determines the site(s) of uptake of digested macronutrients. This has consequences for hormonal feedback processes of relevance to intake/satiety, as well as defining the residual digesta components that transit to the large intestine and therefore become available as feedstocks for the resident microflora. Dietary fibres can both accelerate passage and reduce digestion rates, so can play a key role here. In another example of digesta / animal interactions, reduced triglyceride hydrolysis in the presence of soluble cereal fibres is associated with lower levels of circulating bile and with reduced uptake of dietary cholesterol.

As the small intestine is the most difficult section of the digestive tract to access in humans, pigs have a major role to play in uncovering mechanisms of relevance to not only animal production but also human health.

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

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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. KL Christensen, MS Hedemann, HN Lærke, *et al.* 2013 *J Agric Food Chem* **61**, 7760–7768.

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

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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.

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

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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 4×4 Latin square, eight ileal-cannulated barrows (31.8 ± 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.

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

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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 ± 2.6 kg) were allotted to a 7 treatment × 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.

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

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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 (R^2) 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

Abstract

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.

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

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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.

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Impact of food, gut-brain signals, and metabolic status on brain activity in the pig model: 10 years of nutrition research using in vivo brain imaging

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The purpose of this presentation is to offer a panorama on 10 years of nutrition research using in vivo brain imaging in the pig model. After having presented the scientific rationale for exploring brain activity in the pig, as well as the advantages of this model for nutrition and neurosciences research with industrial, clinical, and translational applications, we will review four research topics in relation with gut-brain axis, nutrition and health. First, we will look at some work describing the brain responses to food signals including preferred and aversive flavors or basic tastants such as sweet and bitter at both oral and visceral levels. Second, we will review the impact of weight gain and obesity on the brain activity and metabolism, drawing the parallel with obese human patients. Third, we will evoke the concept of the developmental origins of health and diseases, and how the pig model can shed light on the importance of maternal nutrition during gestation and lactation for the development of the gut-brain axis and adaptation abilities of the progeny to nutritional environments. Finally, three examples of preventive or therapeutic strategies will be introduced: the use of sensory food ingredients or pre-, pro-, and post-biotics to improve metabolic and cognitive functions; the implementation of chronic vagus nerve stimulation to prevent weight gain and glucose metabolism alterations; and the development of bariatric surgery in the pig model, for the understanding of its complex mechanisms at the gut-brain level. A critical

conclusion will brush the limitations of neurocognitive studies in the pig model and suggest a roadmap for future research in nutrition.

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

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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.

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

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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

Abstract

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-Gene Project, funded from the European Union's H2020 Programme under grant agreement no 633531.

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

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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.

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

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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; $43 \pm 2.5\text{kg}$). Another 10 pigs/farm ($50 \pm 2.5\text{kg}$), received a bolus of uniformly-labelled- ^{13}C -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 $^{13}\text{C}:^{12}\text{C}$ 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 (11-25 $\mu\text{mol/kgBW}$, $P < 0.01$) and ILR (74-119 $\mu\text{mole/kgBW/h}$, $P < 0.01$) 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.

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

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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

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

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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.

Microbial endocrinology: why the integration of microbiology, neurobiology and nutrition matters to overall health and behavior in pigs

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The microbiota that inhabit the alimentary canal are a critical determinant of overall health, behavior and feed efficiency in the pig. The removal of antibiotics from feed has placed a renewed emphasis on understanding how digestive physiology and behavior may be dependent on the composition and activity of the trillions of bacteria that inhabit the porcine gut. This talk will utilize an evolutionary-based approach to show that one element unites all facets that contribute to porcine health; from nutrition to microbiota to behavior. That common element is a shared neurochemistry that has led to the development of the field of microbial endocrinology. The union of microbiology and neurobiology, termed microbial endocrinology, is the study of the ability of microorganisms to produce and respond to neurochemicals that originate either within the microorganisms themselves or within the host they inhabit. The perceived role of the microbiota as mainly concerned with digestion is changing to one which is highly interactive with components of the host not previously envisioned to be influenced by the gut bacteria. Neurochemicals, which are ubiquitous throughout nature, therefore serve as an evolutionary-based means of communication between host and microbiota. This means that nutrition, and the design of new feeds based on neurochemistry in addition to the more traditional concepts of energy, may represent new strategies that contribute to animal well-being. As will be discussed, the sensing and utilization of neurochemicals also enables bacteria to determine their physiology based upon their environment. This neurochemical sensing is particularly important during stress as it contributes to infectious disease pathogenesis. And critically, this neurochemical cross-talk includes the brain and behavior in what has become known as the microbiota-gut-brain axis. This talk will examine the new understanding of bacterial-host interaction mediated by neurochemicals in what has become known as the field of microbial endocrinology.

One of the central mechanisms by which this new understanding of the ability of bacteria to interact with the host has been the discovery that bacteria make many of the exact same neurochemicals that are made by the host including

those dealing with stress. Further, bacteria can also recognize and respond to these neurochemicals by altering their physiology and growth. As such, these neurochemicals become a common evolutionary language by which the bacteria communicate with the host and vice versa.

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

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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.

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

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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,

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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 LiBWP. Further analysis using the outputs from PICRUSt and iPath2 will test the hypothesis that the LiBWP microbiota has differing metabolic functions compared to HBiWP.

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Early-life feeding accelerates gut microbiota colonisation patterns in piglets

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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.

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

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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.

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

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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-finishing pigs would modulate intestinal microbial composition. A total of 36 pens of pigs (7 pigs/pen; ~ 33.4 kg) 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.

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

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Abstract

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 110 ppm zinc oxide (40ZnO, 110ZnO), 110 ppm 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 NH_4 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.

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Effect of dietary copper sources on animal performance, gut microbiota and ghrelin-growth hormone axis of piglets fed diets with antibiotics

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Two studies were conducted to investigate the effect of different Cu sources on performance, gut microbiota, ghrelin expression and serum growth hormone levels of pigs fed commercial diets. A total of 256 piglets weaned at 24 ± 2 d were randomly allocated to 4 treatments with 10 or 8 replicates of 4 or 3 piglets per pen in Trial 1 and 2, respectively. Experimental period (46 d) was divided in three feeding phases: Phase 1 (24 to 35 d), Phase 2 (36 to 49 d) and Phase 3 (50 to 70 d). Treatments included a Control T1 (with 10 mg/kg Cu from CuSO_4), additional supplementation from either CuSO_4 (T2) and tri-basic copper chloride (TBCC) (T3) at 160 mg/kg, or decremental levels of chelated Cu, (T4) (Cu-MHAC) at 150, 80, and 50 mg/kg in Phase 1, 2 and 3, respectively. Diets contained commercial levels of ZnO and antibiotics. Performance and microbiota ecological indexes in jejunum and cecum (richness, evenness and diversity) were calculated. In addition, stomach ghrelin relative mRNA expression and serum growth hormone levels were analysed for Trial 2. T4 pigs grew 4.2% heavier ($P < 0.01$), tended to increase feed intake ($P = 0.07$) and improved FCR ($P < 0.05$) than T1, T2 and T3 at 70 d. In experiment two, microbiota analysis showed significant differences only for cecum, where a more diverse microbial population (Shannon-Wiener index) was obtained for T4 in comparison to Control and T3 ($P < 0.05$). Copper supplementation increased cecum richness regardless the source, while evenness was lower to TBCC compared to Control and CuSO_4 . Ghrelin expression in the stomach increased in T2 and T4 ($P < 0.001$). However only T4 increased serum growth hormone over all other treatments ($P < 0.01$). In conclusion, the benefits of copper supplementation can be enhanced with more bioavailable sources such as Cu-MHAC even at lower doses and in diets with antibiotics.

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Carvone enantiomer specificity accounts for conformational changes in the porcine odorant-binding protein (p-OBP) bound state using a computational model

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Olfactory perception plays a fundamental role in animal behaviour, such as feed intake and social welfare. However, the mechanisms involved in odorant recognition are poorly understood at the molecular level. It has been hypothesized that odorant-binding proteins (OBP) play an important role in the events of odour detection by carrying, deactivating and/or selecting odorant molecules. Despite of the fact that the interaction between odorant and OBP is still unclear, different studies pointed out the high degree of specificity of these proteins, being able to discern between enantiomers. For example, the two enantiomers of carvone (a terpenoid found in several essential oils such as caraway and spearmint), the S(+) and R(-), have been reported to interact with the porcine-OBP (p-OBP). Nevertheless, the differential interaction between the two enantiomers and the p-OBP has not been determined to date. Thus, the aim of this study was to identify the molecular determinants that modulate the interactions between the two carvone enantiomers and the p-OBP. Docking assays coupled to Molecular Dynamics (MD) were carried out to compare the three structures: APO (unbound state) and HOLO-S(+) or HOLO-R(-) (bound states to the enantiomer S(+) and R(-), respectively). The analysis of the MD trajectories showed that the HOLO-S(+) structure presented a constant interaction pattern, while the HOLO-R(-) changed its interaction pattern from Trp88 and Asp86 to Asp102. The binding mode of the enantiomers changed the position of Phe55, fact that in the HOLO-S(+) structure was visible in the peptide skeleton as a conformational change. In conclusion, isomeric differences in odorant molecules result in significant conformational changes of the HOLO state of the p-OBP. Understanding the molecular mechanisms involved in environmental perception are a key step in designing the future husbandry strategies that will optimise productivity and welfare in pigs.

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Effect of fat source on gastric emptying, and blood cholecystokinin and triglycerides in pigs

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Gastric emptying can influence overall feed intake via changes in transit speed through the intestine or chemosensing within the intestine. The objective of this experiment was to test the effect of fat source on gastric emptying and blood CCK and triglyceride levels. It is hypothesized that the direct addition of dietary oil will decrease gastric emptying, and increase CCK and triglyceride appearance in the blood. To test this, ten duodenum-cannulated pigs (26.8 ± 2.9 kg) and twelve non-cannulated pigs (27.8 ± 2.5 kg) in a crossover design were given 20 minutes to consume a diet (470 ± 160 g) containing 5.8% soy oil, or of equal diet fat content from full-fat soy. Duodenal contents from cannulated pigs and blood from non-cannulated pigs were collected at multiple timepoints following the meal for measurement of duodenal fat content, blood CCK and triglycerides. Blood CCK and triglyceride concentration were plotted against time and different areas below the plotted data were investigated. Duodenal fat content was described using a power-exponential model and area under the curve (AUC) data were calculated. All collected model parameters and responses were analyzed using a linear mixed effects model to investigate differences between treatments using meal size as a covariate. No difference was detected for gastric emptying rate, AUC, or half-time gastric emptying using duodenal fat content. The addition of soy oil in the diet increased the initial slope of triglyceride appearance in the blood (4.7 vs 2.3 μM min⁻¹, *P* < 0.05) compared to full-fat soy, but did not affect the AUC up to 180 min. The initial slope of CCK was not changed by treatment, but the AUC was increased by the addition of soy oil (35.0 vs. 15.1 ng mL⁻¹ min, *P* < 0.01). While gastric emptying was not detected to be different, fat source affected both CCK and triglycerides.

Sucrose inclusion in gestating and lactating diets conditions preference thresholds of piglets for sucrose and monosodium glutamate

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Pigs show innate preference for sweet (e.g. sucrose) and umami (e.g. monosodium glutamate –MSG–) tastes. However, the influence of a prenatal sucrose inclusion on taste sensitivity of pigs remains unclear. We hypothesized that the inclusion of sucrose in maternal gestating and lactating diets would increase the preference thresholds for sucrose in post-weaning piglets. Twenty-two sows were selected on day 85 of gestation and randomly assigned to one of two gestating and lactating programs consisting of a standard commercial diet without or with 50g/kg of sucrose. Two-hundred-eight of the piglets born were randomly allocated to eight nursery pens at weaning (21 days). After an adaptation period of four days, piglets were trained in pairs to perform choice preference tests by simultaneously offering two drinkers containing 500 g of plain water or a 200 mM sucrose solution for 10 and five minutes in two consecutive days, respectively. The experimental testing consisted of offering a pair of piglets a choice between one of six different sucrose or MSG concentrations at or above the expected threshold values, for two minutes. Each pair of animals tested only one taste-concentration combination per day. Preference thresholds were calculated as the percentage of each taste solution consumed relative to the total fluid intake. Statistical analysis was based on comparing preference values to the neutral value of 50% by using a Student's t-test. The sucrose threshold was determined at 0.1 mM in piglets born from control sows (64.6%, $P=0.032$). However, piglets born from sucrose-fed sows showed a threshold of 15 mM (63.6%, $P=0.025$). In contrast, MSG thresholds of piglets born from control sows and treated sows were only two fold apart (6 and 3 mM, respectively). These results show that the sensitivity of piglets for sweet and umami solutions could be conditioned by maternal diets.

Piglet appetite for glutamate and sucrose solutions is not influenced by maternal conditioning with MSG

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Dietary maternal conditioning based on feed volatiles has gained attention related to the improvement of post-weaning feed intake in pigs. In contrast, there is no evidence that taste active compounds in maternal diets may similarly exert an influence on the hedonic value of the same taste in young animals. Contrary to smell, taste is believed to be a genetically hard-wired sense that innately drives consumption or rejection regardless of prior experiences. Thus, we hypothesized that piglets' appetite for umami (monosodium glutamate –MSG–) and sweet (sucrose) solutions would not be influenced by an early exposure to MSG. Twenty-two sows were selected on day 85 of gestation and randomly assigned to one of two gestating and lactating programs consisting of a standard commercial diet without or with 50 g/kg of MSG. One-hundred-and-four piglets were selected from each group and were randomly allocated to 4 nursery pens per group. The experimental testing consisted of 2-minute one-drinker test offering four different concentrations of MSG (1, 3, 9 and 27 mM) and sucrose (1, 6, 12 and 18 mM). Piglets' specific appetites were related to solution disappearance by weight and consumption patterns (consumption time/number of approaches) as measure of palatability. Data was analyzed by ANOVA. No differences ($P>0.05$) were observed in the consumption of MSG or sucrose solutions in piglets born from control or MSG-fed sows. Piglets born from MSG-fed sows showed a tendency to a lower MSG intake than did animals born from control sows at 1 (140.7 vs 216.5 g, $P=0.058$) and 3 (167.1 vs 245.7 g, $P=0.062$) mM. Similarly, no palatability differences ($P>0.05$) for MSG

or sucrose solutions were registered among animals coming from control or treated sows. We concluded that the appetite for umami and sweet is innate in piglets and does not change with MSG supplementation of maternal diets.

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Effects of *Lawsonia intracellularis* infection on number of enteroendocrine cells in mouse enteroids

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Enteroids, small intestinal organoids, possess all the cell types and a structural organization similar to crypts and villi found in the small intestine. Therefore, enteroids are a promising model to investigate host-pathogen interactions in enteric infections. *Lawsonia intracellularis* (*Lawsonia*) is the etiologic agent of proliferative enteropathy, an enteric disease that affects pigs and many other animal species. *Lawsonia* is an intracellular bacterium that causes proliferation of epithelial cells, accompanied by decreased number of goblet cells in affected intestines. The objective of this study was to evaluate the suitability of mouse enteroids as a model for *Lawsonia* infection. Mouse enteroids were divided in two groups for infection with *Lawsonia* by microinjection: microinjected with 60 – 120 nL of 8.5×10^5 bacteria/ml suspension (*Lawsonia*), and microinjected with sterile cell culture media (*Media*). Enteroids from all two groups were harvested at 1, 3 and 7 days post-infection. *Lawsonia* antigen was detected by immunofluorescence, and quantitative RT-PCR was used to analyze for enteroid cellular proliferation and epithelial composition. Preliminary analysis of gene expression indicates that *Lawsonia* infection decreased the expression of the goblet cell marker *Muc-2* by 20% compared with the *Media* group at 7dpi. Expression of the proliferation markers *Ki-67* and *Sox9* (marker for transit amplifying cells) increased by 5 and 23%, respectively, in the *Lawsonia* group compared with the *Media* group. The *Lawsonia* group also showed decreased expression of *CgA* (marker for endocrine cells) in relation to the *Media* group at 7dpi (approximately 80% lower). These preliminary results demonstrate that mouse enteroids are susceptible to *Lawsonia* infection and can reproduce the changes observed *in vivo*, making enteroids a suitable *in vitro* model for studying proliferative pathogenesis, and potentially, other host-pathogen interactions.

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Lawsonia intracellularis infection decreases the number of endocrine cells in pigs affected by proliferative enteropathy

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Lawsonia intracellularis is the causative agent of proliferative enteropathy in pigs and in other animal species. Intestinal mucosa infected by *L. intracellularis* present increased cellular proliferation accompanied by decreased numbers of goblet cells. It is still not well understood, however, the impact of *L. intracellularis* infection on other intestinal cell types, such as endocrine cells (ECs). Intestinal ECs are responsible for producing and releasing hormones and transmitters important to the intestinal homeostasis and the gut-brain communication. The objective of this study was to evaluate the effects of *L. intracellularis* infection on the number of ECs in intestines of pigs experimentally infected with *L. intracellularis*. Fifteen archival ileum samples from a previous infection challenge study were used. Formalin-fixed sections were processed for immunofluorescence staining targeting Chromogranin A (*CgA*), an EC marker, and *L. intracellularis* antigen. The number of ECs was counted in *L. intracellularis* positive and negative fields. The median of EC/field between the infected and non-infected groups was compared by

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Mann-Whitney test. The number of EC was lower in intestine sections where *L. intracellularis* was identified in comparison with non-infected intestines (9.5 ± 3.7 and 3.9 ± 2.4 cells/field respectively, $P < 0.05$). This result demonstrates that *L. intracellularis* infection diminishes the number of EC in the intestines affected by proliferative enteropathy. This finding supports our previous observation that *L. intracellularis* infection decreased expression of genes related to EC cells and expands the understanding of the intestinal epithelial modulation during the course of proliferative enteropathy.

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Dietary bitter compounds delayed gastric emptying and glucose uptake while increased plasma insulinotropic hormone GLP-1 in pigs

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Previous research from our group has shown that selected dietary bitter compounds decrease feed intake and fat deposition while improving feed efficiency in fattening pigs. However, the mechanism remains unclear. The discovery of the expression of bitter taste receptors in the gut epithelia, including stomach, as part of the enteroendocrine system, lead us to hypothesise that bitter agonists would slow down gastric emptying and induce plasma anorexigenic hormone secretion in pigs. Eighteen catheterised male pigs (BW = 21.5 ± 0.49 kg) were offered a control meal (containing the radioactive marker ⁹⁹mTc-sulfur colloid) or the same meal supplemented with caffeine or quinine at 2g/kg. Gastric emptying was measured using scintigraphy. Blood samples were collected 5 minutes prior to the test meal and every 5 minutes up to 30 min, and at 45, 60 and 120 min after the meal. Plasma hormones were determined using RIA and ELISA methods. The results showed that caffeine and quinine significantly ($P < 0.05$) delayed the passage of feed onto the duodenum. Compared to the control group caffeine and quinine resulted in 25.37 and 15.13 min delay in half-emptying time, respectively. In addition, quinine reduced ($P < 0.05$) post-prandial glucose uptake during the first 25 min, whereas both caffeine and quinine increased ($P < 0.05$) the insulinogenic index compared to the control group. Furthermore, 2 hours after feeding, plasma GLP-1 levels were higher ($P < 0.05$) in the caffeine than the control groups. Overall the results uncovered that dietary bitter compounds have the ability to mediate in gastric emptying rates and insulinotropic hormone secretion while delaying glucose uptake. Our results partially explain how bitterants may impact feed intake and decrease fat deposition in pigs.

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Selected bitter compounds may increase blood flow into the gastrointestinal tract by eliciting mesenteric artery smooth muscle relaxation in pigs

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Bitter taste perception has been related to the expression of the taste receptor sub-family T2Rs, in taste sensory cells of the oral cavity. The pattern of expression of T2Rs extends to extraoral tissues relevant to the digestive system where they have been related to the delay of gastric emptying and the relaxation of the smooth muscle tone in the small and large intestines. In addition, T2Rs in laboratory rodents and humans have also been found in the cardiovascular system (CVS), mainly heart. The current research tested the hypothesis that T2Rs are expressed in porcine mesenteric artery and that bitterant agonists may increase blood flow to the intestines by relaxing the smooth muscle of the vessel. Mesenteric artery samples were collected from six male Large White piglets at approximately 10 kg bodyweight. The T2Rs expression levels were determined by quantitative RT-PCR. The smooth muscle tone of mesenteric artery was tested using water baths with Tyrode's buffer maintained at 37°C and aerate with 95% O₂ and 5% CO₂. The mounted tissue samples were connected to an isometric transducer to record contractility from the

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smooth muscle layer. Bitter agonists caffeine, chloroquine, denatonium benzoate were added at 10 mM and positive controls acetylcholine and adrenaline were added at 1 mM to the water bath media. The 18 porcine T2Rs studied were expressed in the mesenteric artery. Caffeine, and denatonium benzoate at 10mM and adrenaline at 1 mM elicited immediate smooth muscle relaxation responses in mesenteric artery. Non-toxic bitter compounds are often abundant in plant tissues and they seem to account for low digestibility ratios. Thus, we speculate that the detection of non-toxic bitter compounds in the CVS relates to poor quality diets that require additional resources in terms of slowing the gastrointestinal motility and increasing the blood flow to support digestion.

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Effects of peptide and crystalline AA added into extremely low-crude protein diet on chemosensing of gastric acid secretion of pigs

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Previous study showed that low-crude protein (CP) diet supplemented with crystalline amino acids (AA) could increase nitrogen utilization efficiency and reduce nitrogen excrement. However, extremely low CP diet, even though balanced with full supplement of all AAs, reduced growth performance and adversely affected chemosensing of nitrogen nutrients along digestive tract, especially stomach. This study was conducted to compare effects of peptide and crystalline AA on growth performance and chemosensing of gastric acid secretion of pigs under the condition of extremely low-CP diet. A total of 24 barrows (17.95 ± 0.47 kg of initial BW and 63 ± 2 days of age) were assigned to one of three dietary treatments of 16% CP (Control, CON), 13% CP plus Crystalline AA (LAA), and 13% CP plus casein hydrolysate (mainly as peptide, LCH). The trial lasted 28 days. Results showed that LCH group had a greater ($P < 0.05$) average daily gain (ADG) than CON and LAA groups, and a higher ($P < 0.05$) average daily feed intake (ADFI) than LAA group. LCH group upregulated ($P < 0.05$) mRNA levels of the nitrogen nutrient sensor CaSR and GPR92 and the AA transporter B0AT1, which play an important role in AA pathway of gastric acid activation, in gastric mucosa relative to LAA. Compared with LAA, the activity of H^+ , K^+ -ATPase in parietal cells which mediated proton efflux was increased ($P < 0.05$) in LCH. Meanwhile, the concentrations of PKC and CaMK II, which were key intracellular substances of mediating activation of H^+ , K^+ -ATPase, were also enhanced ($P < 0.05$) in LCH relative to LAA. As a result, the pepsin activity in gastric lumen was significantly increased ($P < 0.05$) in LCH relative to LAA. Taken together, under extremely low CP-diet, supplementation of peptide outweighed that of crystalline AA in terms of the improvement of growth performance and chemosensing of gastric acid secretion of pigs.

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Effects of mix-fermented rapeseed meal by substituting soybean meal on growth performance, nutrient digestibility and blood indicators of growing pigs

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Abstract: The aim of this study was to develop a mix-fermentation of rapeseed meal, wheat bran and corn bran and investigate effects of feeding these mix-fermented feedstuffs to replace soybean meal on growth performance of growing pigs. In the feeding trial, 72 growing pigs (BW = 39 kg) were randomly assigned to one of three diets: corn-soybean meal (Con), 15% unfermented mixture (RM) or 15% mix-fermented feedstuff (FRM), which substituted 10.5% soybean meal, 2.25% wheat bran and 2.25% corn bran in diet. In the 28 day-trial, feed intake and growth were measured. Serum and feces were collected to determine serum biochemistry and apparent total tract digestibility

Abstract

(ATTD) of nutrients using acid-insoluble ash as indicator, respectively. The optimal ratio of rapeseed meal, wheat bran and corn bran was 70, 15 and 15% in the mix-fermentation using the combination of yeast, *Lactobacillus* and neutral protease. The optimal fermentation conditions were 55% moisture at 30°C for 60 hours. Mix-fermentation increased crude protein by 16% ($P < 0.01$) and three chloroacetic acid precipitated-nitrogen by 400% ($P < 0.01$) and decreased glucosinolate by 30% ($P < 0.01$), phytate by 38% ($P < 0.01$) and tannin by 21% ($P < 0.01$). Average daily gain was 626, 592 and 670 g/d for pigs fed Con, RM and FRM, respectively. Feed-to-gain ratio tended to be lower ($P = 0.07$) for pigs fed FRM (2.06) than pigs fed Con (2.26) and RM (2.35). Pigs fed FRM had greater ATTD of phosphorus than pigs fed RM ($P < 0.05$). Blood urine nitrogen was greater for pigs fed RM than pigs fed Con ($P < 0.05$) and did not differ between pigs fed FRM and Con. In conclusion, mix-fermentation may increase the nutrition value of rapeseed meal and mix-fermented rapeseed meal may replace some soybean meal in diets for growing pigs.

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Male grower pigs fed cereal soluble dietary fibres display reduced GIP response and delayed insulin peak following a feed challenge.

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Both acute and sustained soluble dietary fibre (SDF) consumption are associated with improved glucose tolerance in pigs and humans. However, the effects on glucose tolerance in grower pigs, adapted to diets with a combination of purified SDF have not been studied previously. It was hypothesized that pigs adapted to diets rich in cereal SDF would display improved glucose tolerance. In this experiment, cereal SDF wheat arabinoxylan (AX) and oat β -glucan (BG) were fed individually and in combination to determine the effect on glucose tolerance in jugular vein catheterized grower pigs.

Five groups of Large White male grower pigs were fed highly digestible diets containing either 10% AX, 10% BG, 5% AX with 5% BG, a model cereal whole wheat flour (WWF), or a control wheat starch diet (WS) with no SDF. Blood was collected over 240 minutes following a feed challenge and an oral glucose tolerance test (OGTT) on two separate days. Postprandial blood samples were used to determine plasma glucose, insulin, non-esterified fatty acids (NEFA), glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY), ghrelin, glucagon and cortisol concentrations.

No dietary effects on glycaemic response were observed following the feed challenge or the OGTT as determined by the area under the curve (AUC). A biphasic glucose and insulin response was detected for all pigs following the OGTT.

The current study showed male grower pigs have tight glycaemic control and glucose tolerance regardless of diet. In addition, pigs fed the combined SDF had a reduced GIP response and delayed insulin peak following the feed challenge. Incretin (GLP-1 and GIP) secretion appeared asynchronous reflecting their different enteroendocrine cell locations and response to nutrient absorption.

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Effects of dietary hyodeoxycholic acid on intestinal enteroendocrine cells differentiation and function, and serum biochemical indexes in weaning piglets

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Introduction Bile acids are metabolites of cholesterol in liver cells and have been considered to promote the metabolism of cholesterol, lipid and fat soluble vitamins in the gastrointestinal tract. Recent studies showed that

bile acids also functioned as signaling regulatory molecules that exert diverse endocrine and metabolic actions. Hyodeoxycholic acid is a secondary bile acid and it may involve in affecting the differentiation and function of enteroendocrine cells.

Material and Methods A total of 16 piglets (Duroc × [Landrace × York-shire]) weaned at 21 d of age were randomly assigned into 1 of 2 treatments, including a basal diet (control; n = 8) and the basal diet with 2 g/kg of hyodeoxycholic acid (HDCA; n = 8). On 28 d of age, serum, duodenum, jejunum and ileum samples were collected for analyzing serum biochemical indexes, immunohistochemistry, and mRNA expression. Statistical analysis was performed using Student's t-test with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

Results Compared with the control group, the number of chromogranin A positive cells was decreased ($P < 0.05$) in jejunal villi and tended to decrease ($P < 0.1$) in ileal villi in HDCA group. Dietary HDCA decreased serum GLP-2, and the mRNA expression of duodenal *Insm1*, jejunal *Pdx1*, and ileal *NeuroD1*, while increased the mRNA expression of *Insm1*, *Sst*, and *Gast* in jejunum ($P < 0.05$). Moreover, dietary supplementation with HDCA increased serum GLO, and IgA contents ($P < 0.05$), and tended to increase serum TP, and IgG contents ($P < 0.1$) in weaning piglets. In conclusion, the results of the present experiment indicate that dietary HDCA affects the differentiation and function of enteroendocrine cells and improves the immune function of weaning piglets.

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Early supplement of oat β -glucan influence the intestinal proteome of suckling pigs

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Pigs suffer from a broad spectrum of enteric diseases of which post weaning diarrhoea is most common and the major cause of morbidity and mortality in young animals. Early nutritional strategies is considered as important to accelerate development of gut physiology and immunology, which can contribute to reducing post-weaning enteric health problems in piglets. Dietary fiber is considered particularly interesting in this aspect, both due to their direct effect on the gut and via modulation of the gut microbiota. The aim of this project was to investigate if early supplement of oat β -glucan influence the protein profile in the intestinal mucosa. The study included ten piglets (Hampshire × Yorkshire) originating from five litters. Five of the piglets had been supplemented with oat β -glucan (40mg/kg body weight, dissolved in water), three times/week, from seven days age until weaning and the remaining five were handled in the same way but with supplement of water. The day before weaning, piglets were euthanized and tissue samples from distal ileum and middle colon was collected, immediately frozen in liquid nitrogen and stored at -80°C until analysis. A mass spectrometry-based shotgun proteomics approach was used to analyze the gut tissue samples. Proteins were extracted from the tissue using an optimized detergent n-octyl- β -D-glucoside extraction protocol and extracted proteins were tryptically digested on 3kDa spin filters. The samples were analyzed using a Q Exactive Plus mass spectrometer. Label-free quantification was performed using the MaxQuant software and treatment effects was evaluated using t-tests. The proteome was significantly different in ileal and colonic tissue samples. Several proteins were up or down regulated as a response of the β -glucan supplement but the treatment response was more profound in the colonic tissue, where several proteins linked with the energy metabolism were significantly upregulated.

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Phylogenics improve growth performance and intestinal barrier integrity in piglets

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Abstract

Phytogenic feed additives (PFA) are increasingly recognized for their potential to beneficially improve growth performance, nutrient digestibility and gut health in different livestock species. Phytogenics comprise a broad spectrum of plant-derived bioactive compounds, with a wide variety of physiological activities. However, the characterization of the underlying mechanisms of PFA to influence physiological pathways need more detailed investigation.

To study the influence of a PFA based on monocyclic monoterpenes, garlic-derived allyldisulfides and mucilages on growth performance and intestinal barrier integrity in post-weaning piglets the following 42-day study was performed: 132 Weaned piglets (Topigs40 x Piétrain, age 24-25 days) were allocated to 22 pens according to body weight and gender (50:50 m/f, two treatments with n=11 each). Pre-starter (day 0 to 14) and starter (day 14 to 42) diets were based on wheat, barley, corn and soybean meal. On days 14 and 42, one piglet per pen was slaughtered for sampling. Gut barrier integrity was measured by *ex vivo* FITC-4kDa permeability assay in distal small intestine samples.

Piglets receiving the PFA tended to show higher average daily gain (+5.6%, $P=0.052$) and daily feed intake (+4.7%, $P=0.078$) during the 42-days trial, as compared to control. In the small intestine of PFA fed piglets *ex vivo* FITC-dextran 4kDA permeability was reduced by 70% compared to unsupplemented control animals ($P=0.049$).

The results of this study clearly indicate the potential of PFA to beneficially influence growth performance of post-weaning piglets. Strengthening gut barrier integrity may result in a reduced transfer of harmful microbial metabolites and pathogens across the intestinal wall. Therefore, the observed effect of this PFA on permeability of the distal small intestine is probably one factor contributing to improved growth performance compared to the unsupplemented control.

In conclusion, the supplemented PFA in this trial improved growth performance and gut barrier integrity in post-weaning piglets.

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IPEC-J2 cells: a medium-throughput system for *in vitro* oxidative stress assessment in a porcine intestinal model

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The porcine small intestinal epithelial cell line IPEC-J2 has previously been used for *in vitro* oxidative stress assessment by flow cytometry. This method is accurate, but requires long analysis times and can only process a limited number of samples at a time. In this work, we describe a fast, medium-throughput method for oxidative stress analysis in IPEC-J2 cells, based on plate-reader analysis of fluorescence. Two different stressors (H_2O_2 and menadione) were tested at different concentrations (1, 2 and 3mM and 100, 200 and 300 μ M, respectively). Trolox (2mM) was used as the reference antioxidant and CM- H_2 DCFDA as the indicator of intracellular oxidative stress. The stressor agent and concentration were optimized in a 2-step test in IPEC-J2 cells grown in 96-wells culture plates (the number of cells seeded and growing time were kept constant to standardize the number of cells per well). First, we evaluated the oxidative capacity of H_2O_2 versus menadione at different concentrations and secondly, we checked which stressor's increase in ROS could be counteracted by the addition of 2mM Trolox. Three replicates were used and mixed models (fixed factors: stressor effect and antioxidant capacity of Trolox; random factor: plate nested within conditions) were fitted. Only 200 μ M menadione increased ROS levels in a reproducible manner, detectable with CM- H_2 DCFDA combined with fluorescence plate reading. Moreover, Trolox significantly decreased the production of intracellular ROS in non-stressed and stressed conditions (2mM H_2O_2 and 200 μ M menadione) ($P<0.05$). The coefficient of variation within group was similar among groups ($P=0.775$), although it was significantly higher in Trolox than in control cells (0.60 vs 1.13; $P=0.004$). In conclusion, the combination of CM- H_2 DCFDA fluorescence analysis by a plate-reader, Trolox as a reference antioxidant and 200 μ M menadione as a stressor agent, provide a replicable and reliable system for the evaluation of intracellular stress in IPEC-J2 cells.

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Effects of direct-fed microbial blends on immune response of weaned pigs challenged with F18 enterotoxigenic *Escherichia coli*

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The objective was to investigate the impact of an F18 ETEC challenge on systemic and localized immune responses of piglets, as well as to evaluate potential protective effects of direct-fed microbial blends (DFM1 and DFM2). The study tested the hypothesis that ETEC would elevate immune responses, while the DFMs would modulate this response, possibly by strengthening the intestinal barrier. Seventy-two 21-d old weaned piglets (BW = 6.42 ± 0.22 kg) were assigned to four treatments: 1) NC: Non-challenged (n = 10), 2) PC: F18 ETEC challenged (n = 10), 3) PC + DFM1 (n = 8) or 4) PC + DFM2 (n = 8). Pigs were housed two pigs per pen and either sham-infected with saline or orally challenged with ETEC on d 7 (0 d post-inoculation, dpi). Blood samples were collected on dpi 0, 1, 2, 4, 7, and 10 for analysis of cytokines and lipopolysaccharide bind-protein (LBP). Ileal mucosal samples were collected for cytokine and secretory immunoglobulin A (sIgA) analysis. Data were analyzed using PROC MIXED (SAS 9.4) with baseline as a covariate. Time course data were analyzed as repeated measures. Plasma LBP increased following ETEC infection ($P < 0.05$). IL-8 increased in the plasma of PC and DFM2 24 hours post-infection compared to NC ($P < 0.05$). On d 10, there were no differences in plasma IL-8 among treatments. Mucosal IL-8 increased in PC ($P < 0.05$). Challenged pigs had reduced sIgA, an anti-inflammatory mediator, compared to non-challenged pigs ($P < 0.05$), indicating a possible alteration of intestinal barrier function. These data provide insight into the role of IL-8 during an ETEC challenge, as it was localized in the intestine; however, there was no difference in systemic IL-8 at dpi 10. This localized pro-inflammatory response may be associated with negative impacts on the intestinal barrier.

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Effect of protein source on weaning diarrhea and performance in piglets

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The present study hypothesized that dietary protein sources with low anti-nutritional factors (ANF) and allergen concentration positively stimulate weaning piglets to higher growth due to lower inflammatory and diarrhea response. In total 216 weaned pigs (BW = 6.69kg) were randomly assigned to 5 dietary treatments with 6 replicates of 6 pigs per pen. Experimental period was d1-14 post weaning. Dietary treatments were control diet consisting of corn-wheat-soybean meal basal diet (CON). Test diets where CON were soybean meal was replaced with soy protein concentrate (SPC), fermented soybean meal (FSBM), fishmeal (FM), enzyme-treated soybean meal (ESBM). Diarrhea incidence was calculated based on daily feces scores from 1(hard) to 5(watery). Serum (d14) malonyldialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were measured. Three sections of duodenum were sampled to measure villus height and crypt depth. ANF and allergen concentrations in protein ingredients were: trypsin inhibitor (TIU/mg) 3.86 (SBM), 1.68 (SPC), 0.69 (FSBM), 0.75 (ESBM); Raffinose + Stachyose (mg/kg) 6.6×10^4 (SBM), 6.8×10^4 (SPC), 434 (FSBM), 1.9×10^3 (ESBM); Beta-conglycinin (mg/kg) 1.6×10^4 (SBM), 116 (SPC), 5.8×10^3 (FSBM), 147 (ESBM); Glycinin (mg/kg) 5.7×10^4 (SBM), 198 (SPC), 1.2×10^4 (FSBM), 299 (ESBM). Piglets fed ESBM had greater ($P < 0.05$) average daily gain (ADG) than CON, SPC or FSBM. Diarrhea incidence was lower ($P < 0.05$) in ESBM (1.79%) piglets than CON (6.15%). Piglets fed ESBM had higher ($P < 0.05$) SOD, and lower ($P < 0.05$) MDA concentration compared to CON. ESBM fed piglets had higher ($P < 0.01$) villi (472µm) than CON (450µm), SPC, FSBM and FM did not differ from CON. Crypt depth was not affected by treatment. In conclusion, protein sources with low ANF's and antigens leads to increased

Abstract

growth performance in weaning pigs supposedly due to reduced diarrhea, better gut development and oxidative status.

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Porcine plasma protein supplementation attenuates intestinal inflammation in a mice model of both enteric and pulmonary challenge

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Introduction. Plasma proteins are recognized as a high-quality feed ingredient for farm animals. In these animals, dietary supplementation with spray-dried porcine plasma (SDP) increases growth rate and food intake. Previous experiments in rodents have shown that SDP attenuates intestinal inflammation induced by *S. aureus* enterotoxin B (SEB) and reduces the lung inflammatory response induced by LPS in mice. **Hypothesis.** The supplementation with SDP attenuates intestinal inflammation in animals exposed to multiple challenges induced by SEB and LPS. **Methods.** Male C57BL/6 mice were fed diets supplemented with 8% SDP (SDP group) or milk proteins (Control group) from day 19 (weaning) until day 33. Mice with multiple challenges were administered LPS from *E. coli* (12.5 µg; intranasal) at day 32 and 6 h later they were given SEB (25 µg; intraperitoneal). Single challenged mice were only given SEB administration and control groups received the corresponding vehicle. Twenty-four h after LPS, leukocyte populations in mesenteric lymph nodes (MLN) and cytokine expression in jejunum mucosa were analysed. **Results.** Single and double challenges augmented leukocyte recruitment into MLN, as well as the percentage of activated Th lymphocytes, monocytes and neutrophils (all $P < 0.05$). Gene expression of pro-inflammatory cytokines such as TNF- α , IFN- γ and IL-1 β in jejunum mucosa was also increased after single and double challenge ($P < 0.05$). These effects were ameliorated by SDP supplementation ($P < 0.05$) and accompanied by increased expression of IL-10, TGF- β as well as FoxP3 in jejunum ($P < 0.05$). **Conclusion.** Supplementation with animal plasma proteins mitigated intestinal inflammation by increasing the expression of anti-inflammatory cytokines. The anti-inflammatory effects of SDP are manifested also in animals with simultaneous challenges in both lung and intestine.

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Plants with antimicrobial effects to combat post-weaning diarrhea in piglets – allicin and acid-containing species

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Post-weaning diarrhea (PWD) is a major disease in pig production, and the main reason for a high use of antibiotics and zinc oxide. The use of zinc oxide will be phased out in Europe, and there is a worldwide agenda to reduce antibiotics in pig production, wherefore alternatives are needed.

We conducted *in vitro* tests to screen various plant species for bactericidal effects towards ETEC F4 and F18 (main PWD agents). A combination of ramsons (allicin) and lingonberries (acids) showed the strongest effect, and synergistically. The present study therefore aimed to investigate *in vivo*, if dietary supplementation of ramsons and lingonberries could reduce the number of coliform bacteria in the gastrointestinal tract (GI-tract) of weaners.

Weaners (2x8) were housed individually and allocated to two feeding treatments: a standard weaner diet (CONTROL); the same diet supplemented with 3% ramsons + 3% lingonberries as freeze dried powder (PLANTS). Rectal samples were taken on day 0, 7, and 13. On day, 14 and 15, the animals were killed, and

samples from the GI-tract collected. Data were analysed by Proc Mixed, SAS, and mean comparison by Tukey test.

The number of coliforms were similar in both groups on day 0, but on day 7 (6.88 vs. 4.89 log cfu/g) and day 13 (7.51 vs. 5.99 log cfu/g), they were significantly lowest ($P \leq 0.02$) in the PLANT group. Gastric pH was lowest in the PLANT group (4.99 vs. 4.19, $P < 0.001$). Coliform counts in stomach, distal small intestine, caecum, and colon digesta were lowest in the PLANT group ($P < 0.01$). The PLANT treatment did not affect lactic acid bacteria counts in faeces and the GI-tract, and feed intake was not affected either. The obtained results indicate a strong potential for using bioactive compounds in certain plants to combat post-weaning diarrhea in pigs.

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Weaning age affects intestinal health and performance in nursery piglets

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Early weaned piglets face environmental and nutritional changes which may modify the intestinal permeability followed by increased inflammatory response and diarrhea. The general hypothesis tested in this study is that early weaned piglets lose intestinal functions led by villous damage what results in low growth rate compared with late weaning. The objective of this experiment was to evaluate the piglets age at wean, challenged or not with *Escherichia coli* K88⁺ on intestinal morphometry, growth performance and diarrhea incidence. Forty two piglets (barrows) were weaned at 21 days (5.03 kg) and forty two at 25 days (6.88 kg). The piglets were assigned into four treatments (3 piglets/pen), seven replicates, in a factorial arrangement (2x2) with two ages at wean challenged or not with *E. coli* K88⁺ during twenty one days. All piglets were fed with the same diet *ad libitum*. At fourth and fifth days the challenged group was oral inoculated with 10^7 CFU/ml of *E. coli* K88⁺. One piglet per pen was euthanized at twelfth day of experiment and samples of jejunum and ileum were collected and fixed for intestinal morphology measurements. Diarrhea occurrences were recorded twice a day. The data were analyzed as a completely randomized design and effects of treatments were submitted to the PROC MIXED procedure. Differences were considered statistically significant when $P \leq 0.05$. No interaction was found between age and inoculation. Piglets weaned at 25 days had greater villous height in jejunum ($P=0.0345$) and ileum ($P=0.0421$) than 21 days piglets. Piglets weaned at 25 days had higher ADG ($P=0.0001$) and daily feed intake ($P<0.0001$) than 21 days piglets. Diarrhea incidence was increased in inoculated group ($P=0.0195$) but did not differ between age groups ($P=0.8992$). Weaning at 25 days of age improves the growth performance by promoting better intestinal health of piglets.

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Gut inflammatory and immune responses in a post weaning diarrhea model in early weaned piglets

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Weaning compromises gastrointestinal functionality, leading to inflammatory and immune disorders, which could result in post-weaning diarrhea (PWD). Both leguminous plant proteins and high levels of dietary proteins can influence gastrointestinal functionality in weaned piglets. The objective of the present study was to evaluate changes in gastrointestinal functionality, specifically inflammatory and immune responses of early weaned piglets fed a high protein diet containing soybean meal to induce PWD. A total of 32 male pigs weaned at 21 days with initial BW of 6.1 ± 0.6 kg, were fed either a low protein control diet (LP; 167 g of CP/kg) or high protein diet (HP, 210 g of CP/kg) for 19 days.

Abstract

Inflammatory response was evaluated after 12 and 19 days of experimental feeding in intestinal tissues and immune cells, mesenteric lymph nodes and feces collected from 8 to 10 individual piglets per group. Immune cells were isolated from ileal Peyer patches (PP) and mesenteric lymph nodes (MLN) for phenotyping of the major cell populations. Modulation of inflammatory gene expression was determined by PCR array targeting 84 genes at tissue levels including PP, MLN, ileum tissue and lamina propria. Differences between treatments were analyzed by t-test. Fecal samples were analyzed for the determination of lipocalin. An increase in fecal lipocalin was observed after 12 days of feeding the experimental diets. However, it was not significant due to a high individual variation. Phenotype of immune cells showed a variation in CD3 and SWC3 on days 12 and 19, respectively. An upregulation of inflammatory gene expression was more pronounced on day 12 compared to day 19. In conclusion, the high soy protein diet PWD model induces an inflammatory response at intestinal level allowing the identification of specific biomarkers of gastrointestinal functionality in early weaned piglets.

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Faecal volatile organic compounds as a non-invasive biomarker of gastrointestinal functionality in early weaned piglets

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The characterization of volatile organic compounds (VOCs) profiles in biological matrices such as faeces, could provide a non-invasive approach to evaluate gastrointestinal functionality (Celi et al., 2017). Currently, faecal consistency score is used to quantify the severity of diarrhoea in piglets, however, as this is a subjective test, more objective measures may be necessary. The purpose of this study was to evaluate the profiles of faecal VOCs in a post weaning diarrhea model in early weaned piglets. We hypothesised that faecal VOCs profiles would allow the separation of different faecal consistency scores. A total of 32 male pigs (BW 6.1 ± 0.6 kg) weaned at 21 d of age, were fed either a low protein diet (LP; 167 g of CP/kg) or a high protein diet (HP, 210 g of CP/kg) for 19 days. Individual piglet faecal samples were collected on Study Days 6, 12 and 19. Faecal scores were assessed visually using a scoring system from 0 to 3, where 0 = normal faeces, 1 = soft faeces, 2 = mild diarrhoea, and 3 = severe diarrhoea. Three different GC-IMS instruments (G.A.S. Dortmund, Germany) and a commercial FAIMS (Owlstone, Cambridge, UK) instrument were used to characterize VOCs. The data was processed using a well-established pipeline, which has been developed specifically for these types of studies (van Gaal et al., 2017). Analysis of faecal VOCs allowed us to identify piglets fed a HP or a LP diet with a good sensitivity and specificity. While the four instruments were not able to differentiate between faecal score 0 and 1 with adequate sensitivity and specificity, as the faecal score increased the differences between the groups become more pronounced. The characterization of VOCs in faecal samples may facilitate the development of a rapid non-invasive tool to monitor gastrointestinal functionality.

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Feeding CLA or MCFA to gilts and sows does not improve colostrum or milk composition or progeny blood lipid profiles

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Gilt progeny are lighter at birth and sale than sow progeny, maybe due to differences in colostrum and milk composition and volume between gilts and sows. Conjugated linoleic acid (CLA) and medium-chain triglycerides and their fatty acids (MCFA) are high energy feed ingredients that when fed to the dam could improve colostrum and milk composition, resulting in enhanced energy status of light birth weight progeny. We hypothesised that feeding these ingredients would improve colostrum and milk composition, increasing the blood lipid content of progeny, especially in

light weight gilt progeny. A subset of 30 gilts and 42 multiparous sows (parities two and three), and one male and one female piglet from each litter were selected from within a larger study. Four diets were fed from d107 of gestation until weaning (d27): a control diet (CON); 2.5% CLA diet (CLA); 0.1% MCFA diet (MCFA); and equal parts of the CLA and MCFA diets (BOTH). A serum sample was collected from piglets three days after birth and assayed for non-esterified fatty acids (NEFA) and triglyceride (TG) content. A colostrum (d0) and milk (d21) sample was collected from each dam and assayed for fat, protein and lactose content. Data were analysed as a linear mixed model in SPSS with diet, dam parity and their interaction as fixed factors. Diet and the interaction had no effect on any trait measured ($P \geq 0.10$). Gilts had higher ($P=0.007$) levels of fat in colostrum than sows, but there were no parity differences ($P \geq 0.10$) in protein, lactose levels or any milk composition traits measured. There were no differences in NEFA or TG concentration between gilt and sow progeny ($P \geq 0.10$). In conclusion, feeding CLA and (or) MCFA to gilts and sows did not improve their colostrum or milk composition or the blood lipid concentrations of their progeny.

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Citrus flavonoids supplementation in weanling diets may improve piglet intestinal health and performance allowing to reduce the use of antimicrobials

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It was hypothesized that flavonoids antimicrobial, antioxidant and anti-inflammatory properties may improve post-weaning (PW) piglet intestinal health. A total of 252 male and female 21-d old piglets ([LargeWhite x Landrace] x Pietrain) were selected at weaning and randomly distributed according to BW ($5.6\text{kgSD} = 0.86$) into 18 pens (9 pens per block of BW and 14 pigs/pen) where 3 treatments (same basal diet) were allotted. Treatments for PS (0-14d PW) and ST (15-35d PW) were , PS: T1) basal diet without antimicrobials (ZnO and AB), T2) T1 + ZnO (2.5kg/Tm) + Amoxicillin (300g/Tm) + Apramycin (100g/Tm), T3) T1 + Citrus flavonoids (300g/Tm) + Amoxicillin (300g/Tm); and ST: only T2 was adjusted as T1 + ZnO (1.5kg/Tm) + Amoxicillin (300g/Tm) + Neomicin (188g/Tm) + Tiamulin (100g/Tm). Piglet BW and feed disappearance were measured on d7, d14 and d35. ADFI, ADG and FGR were calculated. Intestinal tissue (jejunum and ileum) was collected on d7 (one pig/pen). Data was analysed with ANOVA by using the GLM procedure of SAS[®]. For the entire period, ADG and BW were greater for T3 than T1, being T2 intermediate ($P < 0.05$). Ileal VH, VH:CD and mitosis tended to be higher for T2 than T1 ($P < 0.10$). Genes related with barrier function (*TFF3*, *MUC2*, *OCL20*), nutrient transport (*SLC5A1.SGLT1*, *SLC13A1.NASI*, *SLC15A1.PEPT1*), digestive enzyme (*DAO*, *SI*), and digestive hormones (*GCG*) were overexpressed for T3 than T1 and T2 in jejunum ($P < 0.05$). However, barrier function (*TFF3* and *MUC2*), antioxidant enzymes (*GPX* and *SOD*) and immune response (*IFNGR1* and *IL8*) genes were under-expressed for T3 than T1 at ileum ($P < 0.05$). The improved barrier function, nutrient transport and antioxidant capacity in both jejunum and ileum by flavonoid supplementation may explain higher performance at the end of the nursery period. Flavonoid supplementation in weanling diets may support a reduction of antimicrobials by improving intestinal health and performance.

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Interrelationships between post-weaning body weight loss and gut health parameters

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Weaner piglets hardly consume enough feed and water to meet their physiological needs, leading to structural changes of the intestinal mucosa, diarrhea, dehydration and growth check. Weight loss is most often observed during the first five days post-weaning, and is a clear indicator for the well-being of the animal. In this study, we investigated which gut health parameters are related to the body weight change until d5 post-weaning. Therefore, 36 animals were

Abstract

selected at birth, with half of the animals having either a normal birth weight (1.37 ± 0.18 kg) or a low birth weight (0.84 ± 0.09 kg). Pigs were weaned at 21d or 28d of age, or were removed from the sow at 3d of age and raised on a milk replacer until weaning at 21d of age. This resulted in a 2x3 full factorial design ($n=6$). Small intestinal morphology, FD4 and HRP transmembrane flux, glutathione redox status, glutathione related enzyme activities, caspase-3, PCNA, occludin and claudin protein expression were determined in the duodenal and distal jejunal mucosa at d5 post-weaning. Pearson correlation analysis across treatments showed that the jejunal villus length ($r=0.622$), the duodenal FD4 permeability ($r=-0.571$), and both the duodenal ($r=-0.571$) and jejunal ($r=-0.501$) glutathione redox status were significantly ($P>0.05$) correlated with body weight change at d5 post-weaning. A stepwise multiple linear regression model ($R^2=0.766$) retained jejunal villus length, duodenal FD4 permeability, duodenal glutathione redox status and jejunal glutathione transferase activity as significant parameters in the model, explaining respectively 41, 17, 13 and 6% of the variation in body weight change (R^2 change). To conclude, this study confirms the importance of well-established gut health parameters such as villus length, but could also identify the glutathione redox status as a new parameter that relates to immediate post-weaning performance.

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Replacing antibiotics with 0.20% L-glutamine in swine diets: Impact on health and productivity following weaning and transport during different seasons

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Antibiotic use has been limited in United States swine production. Therefore, the objective was to determine whether supplementing L-glutamine at cost-effective levels can replace dietary antibiotics to improve piglet welfare and productivity following weaning and transport during different seasons. Based on previous research, we hypothesized that withholding dietary antibiotics would negatively affect pigs while diet supplementation with 0.20% L-glutamine (GLN) would have similar effects on pig performance and health as antibiotics. Mixed sex piglets [$N=480$; 5.6 ± 0.1 kg BW] were weaned (18.4 ± 0.2 d of age), and transported for 12 h during summer 2016 and spring 2017. Pigs were blocked by BW and allotted to 1 of 3 dietary treatments [$n=10$ pens/dietary treatment/season (8 pigs/pen)]; Antibiotics [A; chlortetracycline (441ppm) + tiamulin (38.6ppm)], No Antibiotics (NA), or GLN fed for 14 d. On d15-34, pigs were provided a common antibiotic free diet. Data were analyzed as a 2×3 factorial of season and diets using PROC MIXED in SAS 9.4. Day 14 BW and d0-14 ADG was greater ($P=0.01$) for A (5.6 and 18.5%, respectively) and GLN pigs (3.8 and 11.4%, respectively) compared to NA pigs, with no differences between A and GLN pigs. Day 0-14 ADFI increased for A ($P<0.04$; 9.3%) compared to NA pigs; however, no differences were detected when comparing GLN to A and NA pigs. Once dietary treatments ceased, no differences ($P>0.05$) in productivity between dietary treatments were detected. On d13, plasma TNF- α was reduced ($P=0.02$) in A (36.7 ± 6.9 pg/ml) and GLN pigs (40.9 ± 6.9 pg/ml) versus NA pigs (63.2 ± 6.9 pg/ml). On d33, villus height: crypt depth tended ($P=0.09$) to be greater in A (2.71 ± 0.09) and GLN (2.72 ± 0.09) compared to NA (2.54 ± 0.09) pigs. In conclusion, GLN supplementation improved pig performance and health after weaning and transport similarly to A across seasons; however, the positive effects of A and GLN were diminished when dietary treatments ceased.

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Effect of copper hydroxychloride and heat stress on growth performance, diarrhea incidence, and blood characteristics of weanling pigs

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An experiment was conducted to test the hypothesis that Cu hydroxychloride (Intellibond C, Micronutrients, LLC, Indianapolis, IN) improves growth performance and blood characteristics, and reduces diarrhea in weanling pigs without

or with exposure to heat stress. One hundred sixty pigs (6.14 ± 0.90 kg) were allotted to a 2×2 factorial design with two levels of choice white grease (CWG; 0 or 5%) and two levels of Cu from Cu hydroxychloride (0 or 100 mg/kg) with eight pens per diet. Diarrhea scores were assessed using a score from one to five (one = normal feces to five = watery diarrhea). From d 40 to 44, ambient temperature was increased from 24°C to 32°C to simulate heat stress. On d 14, d 28, d 40, and on d 44, blood samples were collected from 1 pig per pen and tumor necrosis factor- α (TNF- α), peptide YY, and blood proteins were analyzed. Results indicated no interactions between CWG and Cu hydroxychloride for overall growth performance. Average daily gain was greater ($P \leq 0.05$) from d 14 to 28 and also during exposure to heat stress, and fecal scores were reduced over the entire period ($P \leq 0.05$) for pigs fed Cu hydroxychloride diets compared with pigs fed diets without Cu hydroxychloride. There was also an increase ($P \leq 0.05$) in concentration of peptide YY and a reduction ($P \leq 0.05$) in TNF- α concentration on d 14 for pigs fed Cu hydroxychloride diets compared with pigs fed diets without Cu hydroxychloride. This may be attributed to the effect of Cu in enhancing the expression of hypothalamic appetite regulators and its bacteriostatic property in reducing inflammation caused by pathogens. In conclusion, supplementation of Cu hydroxychloride to diets fed to weaning pigs without or with addition of CWG reduces diarrhea incidence and improves growth performance and some blood characteristics.

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Effects of Oligosaccharide on Growth Performance, Blood Biochemical Indexes, Nutrient Apparent Digestibility, Intestinal Morphology and Disaccharidase Activity in Weaned Piglets

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Objective This study aimed to identify the effects of different oligosaccharide levels on growth performance, organ indices, blood biochemical indexes, nutrient apparent digestibility, intestinal morphology and disaccharidase activity of intestine in weaned piglets.

Methods Sixty weaned piglets at the age of 21 days with similar weight and growth conditions, were randomly divided into 3 groups with 5 replicates of 4 piglets. The supplemental levels of oligosaccharides in diets of 3 groups were 0 (control), 0.20% and 0.25%, respectively. The content of functional oligosaccharide in oligosaccharide was 35%, which was mainly composed of Xylo-oligosaccharide and Isomalto-oligosaccharide. The trial period was 28d. Statistical analysis was done using SPSS 17. The statistical significance of differences between means was assessed by One-Way ANOVA.

Result The results showed as follows: dietary supplementation of 0.20% oligosaccharides significantly improved the utilization rate of feed, the liver index, serum contents of triglyceride (TG), the apparent digestibility of dry matter (DM), crude protein (CP), crude fat, and gross energy (GE), lactase and sucrase activity of jejunum and ileum ($P < 0.05$), had a trend to increase heart index ($P = 0.086$), very significantly improved the apparent digestibility of calcium and phosphorus and lactase activity of duodenum ($P < 0.01$), significantly decreased the diarrhea rate ($P < 0.05$), very significantly decreased serum contents of alanine aminotransferase (ALT) ($P < 0.01$); dietary supplementation of 0.25% oligosaccharides significantly increased the content of serum glucose and the apparent digestibility of phosphorus ($P < 0.05$), very significantly improved the apparent digestibility of calcium ($P < 0.01$), significantly decreased serum contents of total protein ($P < 0.05$), significantly decreased the diarrhea rate ($P < 0.01$). In conclusion, the addition of oligosaccharide in the diet can improve the apparent digestibility of nutrients, promote liver metabolism, and decrease the stress and F/G of piglets, and the recommend supplemental level is 0.20% under the present experiment.

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Effects of epidermal growth factor on LPS-induced apoptosis in porcine intestinal epithelial cells

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Abstract

Epidermal growth factor (EGF) is a cytoprotective peptide that plays pivotal roles in cell growth, proliferation, regeneration, differentiation and maintenance of epithelial cell homeostasis. However, the effect of EGF on apoptosis in porcine intestinal epithelial cells under damage condition is still poorly understood. Hence, the aim of this study was to demonstrate the effects of EGF on apoptosis in porcine intestinal epithelial cells (IPEC-J2) challenged by lipopolysaccharides (LPS). Cell growth, LDH activity, apoptosis rate, apoptosis-related gene and protein expression were explored to support the protective effect of EGF on LPS induced apoptosis. Our results show that LPS treatment significantly inhibit cell growth and antiapoptotic protein (Bcl-2) expression, significantly increase LDH release, apoptosis rate, apoptosis-related gene (*Fas*, *Bax*, *Caspase-3*, *Caspase-8*, *Caspase-9*) and protein (P53, Fas, Bax, Caspase3) expression. While, EGF plus LPS treatment significantly increase cell growth and *Bcl-2* expression, significantly reduce LDH activity, apoptosis rate, *Fas*, *Bax*, *Caspase-3*, *Caspase-8*, *Caspase-9* gene expression, and P53, Fas, Bax, Caspase3 protein expression. Our results suggested that EGF through inhibiting proapoptotic genes expression, and promoting antiapoptotic genes expression to against LPS-induced cell damage.

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Effects of dietary supplementation with porous zinc oxide on the growth performance, intestinal barrier and immune function in weaning piglets

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This study aimed to investigate the effects of dietary supplementation with porous zinc oxide (P-ZnO) on growth performance, intestinal barrier and immune function in weaning piglets. A total of 192 piglets weaned at 21d were allocated into four treatments with six pens and eight piglets per pen, which were fed with negative control diet (NCD, without ZnO), positive control diet (PCD, NCD + 3000 mg/kg regular ZnO), low concentration P-ZnO diet (LP-ZnO, NCD + 750 mg/kg P-ZnO) and high concentration P-ZnO diet (HP-ZnO, NCD + 1500 mg/kg P-ZnO) for 2 weeks. The Results showed that piglets in PCD, LP-ZnO, and HP-ZnO groups had higher ADG ($P < 0.01$) and lower diarrhea rate ($P < 0.05$) than those in NCD group. There was no significant difference in ADFI, ADG and diarrhea rate among PCD, LP-ZnO, and HP-ZnO groups ($P > 0.05$). Compared with NCD, jejunum villus height and the ration of villus height/crypt depth in PCD and LP-ZnO were higher ($P < 0.05$) and had a tendency of increasing ($P = 0.07$). Furthermore, the mRNA relative expression of jejunum tight junction protein ZO-1 and occludin and cytokine TGF- β increased and the expression of jejunum IL-8, miR-122, and aquaporin 3 decreased in the piglets of PCD, LP-ZnO, and HP-ZnO when comparing with those in the piglet of NCD ($P < 0.01$). In addition, the HP-ZnO piglets had significantly higher serum IgG and IGF-1 level than NCD piglets ($P < 0.05$).

In conclusion, 750 and 1500 mg/kg P-ZnO were as effective as 3000 mg/kg regular ZnO in improving growth performance, decreasing post-weaning diarrhea, enhancing intestinal morphology and reinforcing intestinal mucosal integrity.

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Predictability of intestinal integrity based on body temperature modulation of pigs under heat stress and zinc supplementation

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Heat stress negatively impacts gut morphology and integrity but it is unclear how body and ambient temperature correspond to physiological changes in pigs receiving supplemental Zn. We hypothesized intestinal changes would be correlated with thermal shifts experienced by supplemented pigs. Gilts ($n = 80$; initially 72.0 kg) were housed (one of five pigs/pen) under thermoneutral (TN; 18.9–16.7°C) or cycling heat (HS) conditions simulating seasonal chronic heat (30°C/26.7°C for 12h:12h on days 24–63) with acute heat waves (32–33°C/29–30°C for 12h:12h on days 21–24,

42-45, and 63-65). Pigs were randomly allotted to one of eight temperature and diet treatments across 10 weight blocks. Treatments were arranged as a two × two × two factorial with main effects of environment (HS vs. TN), supplemented Zn (50 vs. 130 mg/kg available Zn), and Zn source (inorganic from ZnO vs. organic/inorganic-blend). Core body temperatures on days 42-45 were recorded via vaginal inserts and infrared thermal imaging was used to measure skin temperatures at 12-hour intervals. Jejunum and ileum samples were collected on day 65 for analysis of villus height, crypt depth, and jejunal gene expression of heat shock proteins (27, 70, 90), Occludin, and MUC2. Pearson correlation coefficients were generated using SAS 9.4. Day 42-45 ambient-temperature was negatively correlated with expression of HSP-27 ($r = -0.42$, $P = 0.047$), HSP-90 ($r = -0.49$, $P = 0.014$), and Occludin ($r = -0.69$, $P < 0.001$) in HS pigs. In organic Zn supplemented pigs, ambient-temperature was positively correlated with expression of HSP-27 ($r = 0.42$, $P = 0.034$) and MUC2 ($r = 0.45$, $P = 0.017$) and negatively correlated with villus height in jejunum ($r = -0.42$, $P = 0.027$) and ileum ($r = -0.38$, $P = 0.048$). Thermal Circulation Index (measure of heat dissipation) of HS pigs was negatively correlated with their ileum villus height ($r = -0.51$, $P = 0.015$) and positively correlated with HSP-70 expression ($r = 0.46$, $P = 0.041$). Core body temperature lacked correlation with most variables. In conclusion, thermal correlations with gut integrity characteristics existed for organic Zn supplemented and HS pigs.

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Changes in IUGR piglets gut and liver metabolism predisposing to preferential use of fatty acids as a source of energy

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Intrauterine growth retardation (IUGR) syndrome is defined as a neonate born in term but with lower birth mass and with characteristic shape of the head. IUGR occurs spontaneously in 6 to 10% of all pig neonates. IUGR is a real problem in the livestock due to the high mortality, decreased ability to grow, and reduced quality of fatted carcass. The aim of the present study was to find early causes of increased ability to adipose tissue commutation in IUGR piglets in gastrointestinal tract. Small intestine and liver tissue samples were investigated in 7 d-old spontaneously induced IUGR and normal body weight (NBW) littermate piglets using histometry, mass spectrometry and in-tissue cytometry analyses. The expression level of proteins involved in carbohydrate metabolism and glycolysis pathway in the liver (Dlat, HK1, Galk1, GPi, Aldob; fold change >1.4) and intestinal mucosa (Hk1; fold change = 1.52) were coincidentally shown to be down regulated. In the small intestinal mucosa lactase, key enzyme for digestion of lactose in sucklings, was also decreased. Increased expression of insulin receptor protein in the liver, which may result in block of proximal signaling in signal transduction pathway for insulin and decreased concentration of insulin on the blood. Increased expression of fatty acids binding proteins (FABP; $P < 0.05$) which indicate that in IUGR syndrome fatty acids can be used as preferential source of energy. In accordance, increased UCP3 expression ($P < 0.001$), in the liver might be the result of increased levels of fatty acid delivery to the mitochondria. Our data indicate that in IUGR piglets, carbohydrate metabolism especially glycolysis pathway is diminished, and in revenge preferential use of fatty acids as an energy source rather than carbohydrates is developed. If this pattern, if continued in the future life, is responsible for developing obesity, hyperlipidemia and cardiovascular diseases it needs further studies.

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Changes in the intestine and liver in intrauterine growth (IUGR) retarded neonatal piglets which may lead to their diminished performance

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Abstract

IUGR syndrome is defined as a neonate born in term but with low birth mass (<1.1 kg in Landrace pigs) and causes problems in livestock production due to high mortality in the early postnatal period and altered growth thereafter. Present study aimed to investigate some mechanisms of nutrient absorption and utilization in IUGR neonates which could be responsible for their diminished performance. Eight pairs of neonatal piglets, IUGR and normal body birth weight (NBW), birth body weight range 0.6-0.9 kg and 1.3-1.6 kg, respectively, were obtained from Polish Landrace × Pietrain crossbred multiparous sows. The piglets were killed at the postnatal day 7, and small intestine and liver tissue samples were subjected to microscopy and molecular analyses. Organometry showed reduced absolute weight of the liver and small intestine in IUGR as compared to NBW ($P < 0.001$). Light and scanning electron microscopy studies revealed in IUGR neonates decreased height of villi and muscularis thickness ($P < 0.001$), delayed disappearance of fetal-type enterocytes, decreased thickness of enterocyte microvilli as well as lack of enterocyte apical-canalicular system and as compared to NBW littermates. Mass spectrometry analysis showed decreased level of cathepsin and proteasomes in small intestinal mucosa (fold change > 1.4). The IUGR liver was characterized by increased level of insulin receptor, and high expression of UCP3 ($P < 0.001$), resistin ($P < 0.05$) and adiponectin ($P < 0.0001$) as compared to NBW. Metabolic function of the liver was diminished also by local inflammation manifested by increased Kupffer cells to hepatocytes ratio and elevated TNF- α expression ($P < 0.001$) in the Kupffer cells. Findings in IUGR small intestine may help to explain severely impaired digestive function even in the presence of nutrients in the gut as well as reduced ability to use pool of free amino acids by enterocytes and may directly predispose to high mortality and poor performance in the neonatal period.

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Effect of nutritional alternatives to limit the digestive disorder and performance impairment around weaning

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A total of 288 piglets (6.1 ± 1.0 kg) were randomly assigned to 1 of 4 treatments based on sex and body weight with 8 replicates per treatment and 9 piglets per replicates. Experimental design consists in a factorial design with 2 diets and 2 growing conditions (standard and degraded). Diets were iso net energy and iso digestible amino acid but were formulated to maximize the difference among ratio fermentable protein and NDF (0.82 vs. 0.26). Animal feed intake (FI), body weight (BW) and feed conversion ratio (FCR) were measured at the end of each dietary phase at 41 and 69 days of age. Diarrhea occurrence was recorded only during the prestarter phase. One animal per pen was slaughtered for dissection and organ weight measurement. Piglet performances were significantly affected by sanitary condition ($P < 0.001$) due to intake reduction. Animal growth was negatively impacted during the prestarter phase in degraded conditions ($- 21$ g/d; $P < 0.05$) but from starter phase and the whole period growth and FCR were better in degraded conditions ($+ 38$ g/d and -0.15 for starter phase and $+14$ g/d and -0.14 for global period, respectively; $P < 0.05$).

Feed conversion ratio were similar between dietary treatments in agreement with feed formulation. Therefore, FI was impaired by dietary fibers intake for low fermentable protein and NDF ratio (-57.3 g/d; $P < 0.01$) and associated with growth delays (-47.7 ; $P < 0.01$). No interaction was observed between diets and growing conditions.

Full gut tract weight was significantly affected by dietary treatment. Heaviest digestive tract observed for animals fed the high fiber diet (14.6 vs. 11.8 % of BW; $P < 0.01$). The differences were mainly related with highest development of caecum (0.97 vs. 0.55 % of BW; $P = 0.03$) and in a lesser extent of colon and small intestine (3.99 vs. 3.05 and 6.72 vs. 5.92 % of BW; $P = 0.07$ and 0.10 , respectively). Diarrhea occurrence was most pronounced for animal fed low fiber diet ($P > 0.001$).

Results suggest importance of environment and diet interaction for caecum and gut development. Anatomic changes might be associated with tremendous changes in growth, efficiency and allometry. Further data might be required for proper evaluation of this strategy on the following growing and finishing phases.

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The development of a *Streptococcus suis* serotype 9 challenge model in weaned piglets

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Streptococcus suis is an important bacterial pathogen in pigs and humans. The main colonization sites of *S. suis* are the upper respiratory tract and the gastrointestinal tract. *S. suis* serotype 9 (SS9) is frequently isolated from diseased pigs. This study aims to develop a SS9 colonization challenge model in weaned piglets. Forty 35-day-old piglets were randomly allocated to four treatments (2 pens/treatment; 5 piglets/pen). All piglets received one diet and those on treatments 2 and 4 were submitted to stress by mixing and 10 minutes' transport before challenge. On day 11, piglets on treatments 3 and 4 were nasally and orally inoculated with SS9 of 2.3×10^9 CFU, whereas PBS was provided to treatments 1 and 2. Tonsil-swabs on days 8, 12, 13, 15 and 18 and blood samples on days 8, 13 and 18 were collected. On day 18, ileal digesta were collected from euthanized piglets to quantify SS9 via qPCR. No clinical signs were observed. Blood samples and necropsy were negative for *S. suis*. On day 8, no difference of SS9 concentration in tonsils of piglets among the four treatments was found. From day 8 to 18, the evolution of SS9 colonization in tonsils differed among piglets from the four treatments ($P < 0.01$): the concentration of SS9 in tonsils of piglets from treatments 1 and 2 did not differ with time with average of 1.1 and 2.1 Log CFU/mL. However, the SS9 concentration in tonsils increased from 1.1 to 4.7 log CFU/mL, and increased from 2.2 to 4.8 Log CFU/mL from treatment 3 and 4, respectively. In the ileal digesta, piglets from treatment 3 and 4 showed higher concentrations SS9 than treatment 1 (4.6, 5.0 vs. 3.9 Log CFU/mL respectively, $P < 0.01$). The current SS9 challenge model is validated to study SS9 colonization in tonsil and ileum of post-weaning piglets.

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Observation of stomach lesions in weaned piglets

D Guillou and N Lemoine

MIXSCIENCE

Stomach ulcers have long been described in pigs and sows, but etiology is not fully understood. Especially, ulcers are usually observed at slaughter time, which doesn't preclude hypothesis of development in earlier stages of life. Stomachs from 12 piglets were examined on 8th day post-weaning, originating from an experiment undertaken to study the effect of fibre source and particle size in feed on gut health and performance after weaning. Stomachs were separated from the intestine, opened, emptied and gently rinsed with saline water. Status of the internal face of the oesophageal area was scored according to the gastric ulcer index developed in Denmark for slaughter pigs (SEGES, 2011). The relationship between ulcer index and other measurements realised on same animals was studied (body weight, pH of gut contents, villi height and crypt depth in gut segments, myeloperoxidase activity in ileum content and faeces, faecal consistency score). Ulcer index was first considered numerical variable, then categorised per type of lesion. Linear correlation and analysis of variance were performed with R-software package. Lesions were observed in all 12 stomachs, ranging from light keratinisation (index 1) to substantial erosion (index 5). The severity of lesions didn't depend on experimental treatment. Linear correlation between ulcer index and other measurements was poor, except for pH in *colon ascendans* (-0.548) and duodenal crypt depth (0.534). Crypts were significantly ($P = 0.0305$) deeper in the duodenum of piglets showing gastric erosion compared to keratinisation, and a similar trend ($P = 0.064$) was noticed in the jejunum. No other parameter depended on stomach status. In conclusion, a few days after weaning

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stomach lesions were found associated to changes in intestinal histology but independent of intestinal inflammation biomarkers. In order to prevent further development of ulcers, early intervention should be considered to preserve pig's well-being.

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Dietary inclusion of essential oils and tributyrin mixtures selected as antibiotic alternatives from *in vitro* study improved piglet intestine health

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Development on antibiotic alternative feed additives is highly demanded by livestock industry. In current studies, firstly an *in vitro* study was designed to find potential antibiotic alternatives from various mixtures including oreganum plus tributyrin (OGT), methyl salicylate plus tributyrin (MST), nucleus garlic oil plus tributyrin (GOT), middle-chain fatty acids (MFA), and middle-chain fatty acids plus tributyrin (MFT). They were tested in a modified suspension bacteria culture system for growing *Escherichia coli*, *Salmonella* or *Staphylococcus aureus*. The tributyrin in the mixtures had been pre-incubated with pancreatic lipase before mixed and added in. At the end aliquots were taken for living bacteria counting after cultured in Petri dishes. From the study it was found OGT and MST inhibited all three pathogenic bacteria growth as well as MFA and MFT. Based on the results a feeding study was then designed to investigate effect of inclusion of the selected mixtures to piglet diet on gut health. Total 48 crossbred piglets averaged 8.8 kg were distributed in 12 pens and fed with one of 4 diets for 4 weeks: 1) a control diet (CON); 2) CON plus antibiotics (ATB); 3) CON plus 0.2% OGT; 4) CON plus 0.2% MST. At the end piglets from each group (n = 6) were slaughtered for sampling gut contents and tissues. From the study it was found MST tended to decrease (P = 0.10) pH in duodenum and resulted in lower (P < 0.01) pH in ileum. OGT as well as ATB increased (P < 0.05) villus length of duodenum, decreased (P < 0.01) crept depth and increased (P < 0.01) ratio of villus/crept of jejunum. MST increased (P < 0.01) villus length and ratio of villus/crept of both duodenum and jejunum. In conclusion, the studies suggest the oreganum/tributyrin (30/70) and methyl salicylate/tributyrin (30/70) are potential candidates of antibiotic alternatives for piglets by both inhibiting pathogenic bacteria growth and improving intestine health.

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***Lawsonia intracellularis*–*Mycoplasma hyopneumoniae* challenge alters intestinal function and integrity of grow-finish pigs**

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Lawsonia intracellularis (LI) and *Mycoplasma hyopneumoniae* (Mh) are two globally-distributed pathogens that cause significant morbidity and mortality in grow-finish pigs. However, mechanisms of reduced growth and feed efficiency are poorly defined. We hypothesized that reductions in performance are partially due to decreased intestinal function and integrity, thus this study aimed to evaluate intestinal function and integrity of pigs during a 21 day Mh and LI dual challenge (MhLI). Littermate pairs of barrows (50 ± 7 kg BW) were selected; one pig from each pair was assigned to either challenge or non-challenge treatments (n = 12). Pigs were individually housed, fed a corn-soybean diet, and allowed to acclimate for 21 days prior to inoculation. On days post inoculation (dpi) 0, MhLI pigs were dual inoculated with *Lawsonia intracellularis* and *Mycoplasma hyopneumoniae*. On dpi 21, all pigs were euthanized for ileal and colon tissue collection. Formalin fixed tissues were clinically scored and morphology analyzed, frozen tissues assayed for digestive enzyme activities, and fresh tissues mounted into modified Ussing Chambers to assess active nutrient transport, barrier integrity, and bacterial translocation. Data were analyzed using the Mixed Procedure of

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SAS with treatment as a fixed effect, age and start BW as covariates, and litter as a random effect. Compared with controls, MhLI pigs had decreased ADG (38%, $P < 0.001$), ADFI (25%, $P < 0.001$), and G:F (19%, $P = 0.012$). The MhLI dual challenge did not alter ileum morphology or transepithelial resistance ($P > 0.10$); however, *ex vivo* mucosal to serosal translocation of *Salmonella typhimurium* in the colon was increased (60%, $P = 0.003$) in MhLI pigs compared with controls. Additionally, MhLI pigs had increased ileal glucose transport (30%, $P = 0.05$) and decreased sucrase activity (30%, $P = 0.049$) compared with controls. This MhLI challenge antagonized intestinal function and integrity, and this may be a contributing factor to reduced pig performance.

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Galactosylated chitosan-oligosaccharides protect against enterotoxigenic *Escherichia coli* in piglets

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhoea in humans and piglets colonizing the small intestine and secreting toxins that cause fluid loss. *In vitro*, galactosylated chitosan-oligosaccharides (Gal-COS) reduced ETEC K88 adhesion to porcine erythrocytes. Therefore, we hypothesised that Gal-COS would reduce adhesion of ETEC and ETEC-induced diarrhoea in pigs. The small intestinal segment perfusion (SISP) model was used consisting of 10 jejunal segments in each of eight 5-week old piglets (body weight 10 ± 0.7 kg). In a 5×5 Latin square, five treatments – 1) α -Gal-COS; 2) β -Gal-COS; 3) Reuteran produced by *Lactobacillus reuteri*; 4) Raffinose; and 5) Saline – were allotted to five segments infected with ETEC expressing K88 fimbriae (ETEC K88), and five segments treated with saline (non-ETEC). Each pair of segments (ETEC and non-ETEC) were infused with 64 ml of 10 g L^{-1} of test substrate or saline for 8 h. Net fluid loss was calculated as difference of net fluid loss in ETEC segment and net fluid loss of paired non-ETEC segment used as indicator of diarrhoea and quantitative PCR determined ETEC K88 infection. Data were analyzed using the MIXED model with segment and test substrate as fixed effects, and pig as random effect. The β -Gal-COS tended to decrease ($P = 0.08$) net fluid loss caused by ETEC compared to other substrates. Number of eubacterial rRNA genes were 10-fold greater in ETEC segments than their paired non-ETEC segments, indicating that ETEC K88 accounted for $> 90\%$ of bacterial gene counts in ETEC-infected segments. Test substrates did not influence ($P > 0.10$) the number of ETEC bacteria in outflow fluid. In conclusion, the *in vivo* SISP model confirmed the anti-diarrheal effects of Gal-COS that were observed *in vitro* indicating that β -Gal-COS is a potential feed additive to reduce the impact of ETEC-induced diarrhoea in piglets.

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Egg-yolk antibodies supplementation protect weaned pigs against enterotoxigenic *Escherichia coli* induced diarrhea and improve gut health similar to antibiotics

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These studies evaluated egg-yolk antibody (IgY) as an effective diet additive for diarrhea in weaned piglets challenged with enterotoxigenic *Escherichia coli* (ETEC) K88 (Experiment 1, Exp. 1) and a potential substitute for antibiotics (AB) in relation to immune responses and gut health in weaned pigs (Experiment 2, Exp. 2). In Exp. 1, 45 weaned barrows (5.0 ± 0.3 kg initial BW) were randomly assigned to 1 of 3 treatments. The treatments consisted of pigs fed an unsupplemented corn-soybean meal basal diet and not challenged (NON-C) or challenged with ETEC K88 (CHA-C) on d 9 and pigs fed the same basal diet supplemented with IgY (500 mg/kg, CHA- IgY) and challenged with ETEC K88 on d 9. In Exp. 2, 222 weaned pigs (7.5 ± 0.6 kg initial BW) not challenged with ETEC K88 were randomly assigned to 1 of 3 treatments, including an AB-free basal diet (CON) and the basal diet with AB (ABD) or

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500 mg/kg IgY supplementation (IgYD). In Exp. 1, after challenge, CHA-C decreased ($P < 0.05$) ADG and ADFI, whereas CHA-IgY revealed no significant change compared with NON-C. Compared with CHA-C, CHA-IgY improved ($P < 0.05$) ADG and ADFI and decreased ($P < 0.05$) the diarrhea incidence in pigs. Inflammatory factor contents in the jejunum and ileum were greater in CHA-C than in NON-C ($P < 0.05$) and CHA-IgY ($P < 0.05$). The diet containing IgY alleviated the increase in the endotoxin and cecal *E. coli* count ($P < 0.05$). In Exp. 2, dietary supplementation with IgY had positive effects on the balance of intestinal flora ($P < 0.05$). Compared with CON, apparent intestine health in IgYD was improved ($P < 0.05$). Overall, IgY supplementation protects pigs against ETEC K88-induced diarrhea by reducing inflammatory responses and reducing intestinal damage and improving the balance of intestinal flora in weaned piglets. Therefore, IgY may be an AB alternative to improve diarrhea and intestinal health in weaned piglets and intestinal health.

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Dietary zinc and the development of immune competence in post weaning piglets

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Pharmaceutical levels of dietary zinc were shown to influence small intestinal microbiota and induce immune related transcriptomic changes in the intestinal mucosa in post-weaning (pw) piglets. To evaluate whether these changes induce an enhanced immune competence, a normal or high zinc diet (101 vs. 2508 mg/kg; $n = 32$ per group; NZn and HZn) was provided over d 0-14 pw on the clinical and immune responses followed by an oral challenge with an attenuated strain of *Salmonella typhimurium* STM on d 19 and 33 pw. The NZn group showed 53 and the HZn 47% anti-Salmonella antibody response in plasma on d 33 (14 d after first vaccination) and, respectively, 66 and 63% on day 40/41 (7/8 d post 2nd vaccination) ($p > 0.05$). The antibody concentrations (OD) were numerically higher in the HZn compared to the NZn piglets (OD values, 15 and 20 (d 33) and 19 and 26 (d 40/41) for NZn and HZn, respectively). On d 40/41 pw, ileal lymph nodes were free of live Salmonella in the NZn and HZn groups in 53 and 63% of the piglets ($P > 0.05$), respectively, with mean values of 16 and 24 cfu/g ileal lymph node tissue ($P > 0.05$), respectively. The expression of selected, in part immune related genes (MT1A, MUC20, SFTPD, TFF3, IL-6, IL-1- β , TNF- α , IL-8, HMOX1, and NFKB; $n = 8$) in the ileal mucosa tissue obtained on d 40/41 did not differ significantly between treatments. In conclusion, provision of the a high Zn diet in the post-weaning period did not change the immunological response and Salmonella translocation after an oral challenge with a Salmonella vaccine in the period of 3 to 5 weeks post weaning.

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Effects of dietary threonine:lysine ratio on performance and mucin gene expression in weaned pigs challenged with *Escherichia coli* K88

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A study was conducted to determine the optimal standardized ileal digestible (SID) threonine:lysine (Thr:Lys) ratio in weaned piglets challenged with *E. coli* K88. Thirty individually housed mixed-sex pigs (Duroc \times [Yorkshire \times Landrace]) with an initial BW of 6.6 ± 0.19 kg were randomly assigned to 5 dietary treatments with 6 replicates each. Dietary treatments consisted of 4 graded levels of SID Thr:Lys ratio (53, 59, 65, and 71%) and a control diet containing 59% SID Thr:Lys supplemented with antimicrobial growth promoter. Diets were corn-wheat-soybean meal-based with a constant SID Lys of 1.18% that was set to be the second limiting amino acid (AA) but adequate in other AA. On d 8, all piglets were orally challenged with 6 mL of *E. coli* K88 (10^{11} colony forming unit/mL). Body weights and pen feed disappearance were recorded weekly to determine ADG, ADFI, and G:F. On d 14, all pigs were

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ethanized to obtain ileal tissue samples to measure morphology, mRNA abundance of mucin (MUC) and cytokine genes. Growth performance was similar among dietary treatments. On d 14, increasing SID Thr:Lys decreased (linear; $P < 0.05$) plasma urea nitrogen (PUN) concentration. Using PUN as response criteria, the optimal SID Thr:Lys ratio was 60.3%. Dietary SID Thr:Lys ratio tended to increase (quadratic; $P = 0.07$) VH:CD and a SID Thr:Lys of 65.6% optimized ileal villous height : crypt depth ratio (VH:CD). Dietary SID Thr:Lys ratio tended to increase (linear; $P < 0.05$) the relative mRNA expression of MUC 20 (0.68, 1.05, 0.89, 1.37, 1.22), whereas MUC 1 and MUC 4 were not affected. In conclusion, the optimal SID Thr:Lys ratio based on ileal histomorphology and PUN in weaned pigs challenged with *E. coli* K88 was 65.6 and 60.3%, respectively. Collectively, an average SID Thr:Lys ratio of 63% optimized intestinal integrity in weaned piglets exposed to immune challenge.

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Effects of dietary antimicrobial peptides on growth performance, digestibility, intestinal morphology and digestibility in newly weaned piglets

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Introduction There is growing evidence that antimicrobial peptides could alleviate weaning stress, promote growth in early-weaning piglets. Therefore, the study was conducted to investigate the effect of AMP on the growth performance, nutrients digestibility, organs weights, blood biochemical profiles, intestinal morphology, and digestive enzymes in newly weaning piglets.

Materials and methods A total of 21 crossbred weaning piglets had initial body weight 7.11 ± 0.89 kg, were randomly allocated into three treatments: (i) basal diet, control; basal diet with antibiotics (ANT); basal diet with antimicrobial peptides cecropin and plectasin (AMP). The piglets were housed in individual pen for 14 days ($n = 7$ per treatment, each pig represents one replicate). At the second week of the experiment, pigs were measured apparent fecal digestibility of nutrients by using chromic oxide (Cr_2O_3) marker ($n = 7$). At the end of the trial, pigs were sacrificed for blood and tissue samples ($n = 7$).

Results and discussion No significant differences of treatments were observed on growth parameters, organ weights and digestibility of dry matter, gross energy and crude protein. Although AMP treatment had a slightly higher final body weight compared to the other two treatments. The inclusion of AMP significantly increased the serum insulin-like growth factor 1 level ($P < 0.01$), enhanced duodenal villus height ($P < 0.01$) and villus height to crypt depth ratio ($P < 0.01$), and elevated activities of jejunal maltase ($P = 0.03$) and sucrase ($P < 0.01$) in newly weaned piglets. In addition, dietary AMP tended to increase intestinal alkaline phosphatase ($P = 0.09$) and decrease duodenal crypt depth ($P < 0.10$) when compared with control group. To conclude, the addition of AMP displays positive effects in enhancing hormonal growth factor, alleviating structure damage and promoting digestive enzyme activities in the small intestine of weaned piglets. AMP maybe potentially serve as an effective alternative for antibiotics in the pork industry.

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Cecropin and plectasin as antibiotic alternatives affect blood biochemical profile, intestinal morphology, enzyme activity, and nutrient retention in nursery pigs

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Introduction With the increasing concern about antibiotic resistance, antimicrobial peptides are currently being evaluated as antibiotic alternatives in animal feed to promote growth and protect against infection. In the present study, we evaluated the feasibility of dietary cecropin and plectasin in nursery pigs on the growth performance, relative organs weight, blood biochemical profiles, intestinal morphology, digestive enzymes, and nutrients digestibility.

Materials and methods A total of 42 piglets (Duroc × Landrace × Large Yorkshire,) weaned at 21 days with an average body weight of 8.01 ± 0.64 kg, were randomly allocated into three treatments (n = 14 per treatment): basal diet, control; basal diet + antibiotics (ANT); basal diet + cecropin + plectasin at 800 mg/kg (AMP). The experiment lasted for 25 days. At the last week of the trial, seven pigs were fed chromic oxide (Cr_2O_3 , 2 g/kg) as an indigestible marker to determine apparent nutrient digestibility. At the end of the experiment, seven piglets were slaughtered and tissue samples were collected.

Results and discussion Feeding the AMP diet had no effects on promoting final body weight gain, daily feed intake and relative organs weight in nursery pigs. However, piglets fed AMP showed improving jejunal villus height ($P = 0.01$), villus height to crypt depth ratio ($P < 0.01$) and tended to decrease crypt depth ($P = 0.08$) when compared to ANT. Furthermore, compared with control, AMP administration significantly affected blood biochemical parameters including creatinine, total protein, albumin, globulin and so on ($P < 0.05$), enhanced jejunal maltase activity ($P = 0.05$), and nutrient retention of dry matter ($P = 0.01$) in nursery pigs.

Conclusion Dietary AMP did not affect growth performance, but alleviated intestinal damage due to early weaning through promoting intestinal structure and enzyme, further to promote nutrient retention. This research provided the reference for the use of AMP as substitutes for antibiotics in the pork industry.

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Cecropin and plectasin as antibiotic alternatives affect blood indicators and nutrient digestibility in weaned pigs weighing 8 to 25 kg

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Introduction Antimicrobial peptides (AMP) as antibiotic alternatives have attracted considerable research interest. As evolutionarily conserved molecules of innate immune system, AMP cecropin and plectasin were primarily deployed as host defense peptides to protect against potential pathogenic microbes. In this research, we investigated the effects of cecropin and plectasin on the growth performance, nutrients digestibility, organs weights, blood biochemical profiles, intestinal morphology and digestive enzymes in weaning pigs weighing 8 to 25 kg.

Materials and methods The pigs with initial body weight 8.05 ± 0.65 kg were randomly assigned to three groups (7 per group): (i) basal diet, without any antibiotics or AMP, control (CON); basal diet + 1200 mg/kg antibiotics (ANT); basal diet + 800 mg/kg cecropin and plectasin (AMP). The cecropin and plectasin were economically produced through recombinant DNA technology in *Pichia pastoris* strain GS115 expressing system. After 39 d of treatment, the piglets were sacrificed and tissue samples were collected. At the last week of the experiment, apparent nutrient digestibility was measured using chromic oxide as an indigestible marker.

Results and discussion No significant differences in ADFI, ADG, or G:F were noted between AMP and ANT. Dietary AMP decreased ($P < 0.05$) the levels of aminotransferase, D-lactate as well as IgG in the serum, enhanced ($P < 0.05$) the digestibility of crude protein when compared with dietary ANT. Furthermore, the addition of AMP also tended to increase ($P < 0.10$) jejunal villus height, serum IGF-1 level and relative weight of heart.

Conclusion In summary, compared with ANT, dietary AMP showed more effective in decreasing intestinal and liver cell damage, maintaining the stable of immune system, promoting nutrient digestion, elevating serum growth hormonal factors in weaned pigs weighing 8 to 25 kg. The result suggested cecropin and plectasin may be an effective alternative for antibiotics in the pork industry.

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Vitamin B6 alters diarrhea rate, intestinal morphology and inflammation response in weaned piglets fed a high-protein diet

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As an important coenzyme factor, vitamin B6 participates in many metabolic reactions, especially the metabolism of amino acids. However, there were few studies reported how vitamin B6 mediates intestinal health in animal. This study was conducted to investigate the effects of dietary vitamin B6 on the growth performance, diarrhea rate, intestinal morphology and the mRNA expression of inflammatory cytokines in weaned piglets fed a high-protein diet (22% crude protein). Twenty-four piglets (Landrace × Large White × Duroc) were weaned at 21 d of age (7.03 ± 0.15 kg body weight) and randomly assigned into 4 dietary treatments, consisting of dietary supplementation with 0 mg/kg (group A), 2 mg/kg (group B), 4 mg/kg (group C), or 7 mg/kg (group D) vitamin B6, for a 14 d period. The results showed that dietary vitamin B6 had no significant differences in growth performance of weaned piglets, while the diarrhea rate in group C was increased ($P < 0.05$) compared with group D. No differences in jejunal villus height (VH), crypt depth (CD), and VH/CD ratio, as well as ileal CD and VH/CD ratio were observed among dietary treatments. However, ileal VH in group D was greater ($P < 0.05$) than that in group A. In jejunum, no significant differences in IL-1 β , TNF- α , IL-10, and TGF- β mRNA expression were detected among dietary treatments. In ileum, the mRNA expression of IL-1 β and TGF- β in group D was significantly increased ($P < 0.05$) compared with group A. In conclusion, the results of the present study indicate that including vitamin B6 in high-protein diet alters diarrhea rate, ileal morphology and inflammation response in weaned piglets.

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Protein degradation in intestinal crypt epithelial cells of weaning piglets

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Introduction The intestinal crypt is responsible for the continual renew of epithelial cells along the crypt-villus axis. However, weaning stress reduced feed intake and affected crypt depth in piglets. The reduction in feed intake may result in protein degradation and therefore affects crypt depth in weaning piglets. The objective of this study was to determine protein degradation in crypt epithelial cells of weaning piglets.

Material and methods A total of 30 piglets were weaned at 21 d of age and 6 piglets were randomly slaughtered at 0 d, 1 d, 3 d, 7 d, and 14 d post-weaning. The crypt epithelial cells in mid-jejunum were isolated using a sequential isolation method. The synthesis of proteins related to protein degradation was analyzed by the isobaric tags for relative and absolute quantification method or Western blotting. The mRNA expression of genes involved in protein degradation was determined using real-time qPCR.

Results Protein involved in protein catabolic process were mainly up-regulated in jejunal crypt epithelial cells during the post-weaning period. Moreover, the abundance of proteasome 20s in crypt epithelial cells was

Abstract

significantly increased ($P < 0.05$) during the post-weaning period. However, the mRNA expression of *cathepsin C*, *PSMA2*, *PSMA3*, and *PSMA4* were gradually decreased ($P < 0.05$) from d 0 to d 14 post-weaning.

In conclusion, the results of the present study indicate that weaning stress affects protein degradation in jejunal crypt epithelial cells of piglets.

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Effects of micro-encapsulated organic acids and essential oils on performance and gut integrity of weaned piglets challenged with ETEC K88

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Control of post-weaning scours is a major concern in pig production. This study intended to evaluate the effects of a selected formula of micro-encapsulated organic acids and essential oils [P(OA + EO)] on performance and intestinal integrity of weaned piglets challenged with enterotoxigenic *Escherichia coli* (ETEC-K88). A three weeks cage study was conducted using 30 crossbred (Duroc × Landrace × Large-White) 28d old weaned barrows with initial body weight of 7.41 kg individually housed in metabolic cages with six replications randomly assigned to one of the following five treatments: PC) non-challenged control; NC) ETEC-K88-challenged control; OA) ETEC-K88 + 50 mg/kg kitasamycin + 100 ppm olaquinox + 5 kg/t Free organic acids; P1) ETEC-K88 + 50 mg/kg kitasamycin + 100 ppm olaquinox + 1 kg/t P(OA + EO) (Jefo, Canada); and P2) ETEC-K88 + 50 mg/kg kitasamycin + 100 ppm olaquinox + 2 kg/t P(OA + EO). Pigs of groups NC, OA, P1 and P2 received an oral dose of 10 mL of ETEC-K88 culture (1×10^9 CFU/mL) at d7. Data were subjected to statistical analyses (GLM procedure, SAS). Differences among treatments were separated using Student-Newman-Keul's range test with a level of significance at $P \leq 0.05$. Supplementation of OA and P(OA + EO) numerically improved ADG over NC in the two weeks after challenge (424, 486 and 529g for OA, P1 and P2 respectively vs 390g for NC) with P2 being significantly higher ($P < 0.05$) than NC. P1 and P2 numerically improved GF over NC and OA with P1 being significantly higher ($P < 0.05$) than NC and OA. One week after challenge, P1 had significantly ($P < 0.05$) higher ileal occludin protein expression compared to NC, OA and P2. Ileal Zonula occludens-1 expression was decreased ($P < 0.05$) in NC while there was no difference between PC and P1. In conclusion, the formula of [P(OA + EO)] could improve growth performance and intestinal integrity of weaned piglets facing *E. coli* challenge.

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Redox balance and immunity of piglets pre- and post weaning after treatment with hemp and fish oil, and vitamin E

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Enteric infection in piglets is associated with oxidative stress and inflammatory reactions. Polyunsaturated fatty acids (PUFA) and lack of antioxidants enhance the reactive reactions. We hypothesized that fat source and vitamin E to piglets influence their immune responses pre- and post weaning after *E. Coli* challenge. From day 10 after birth, an oral supplement (1 ML/day) of fish oil or hemp oil were provided suckling pigs ($N = 24$). Half of the piglets on each treatment received additional 60 mg natural vitamin E/kg feed. Pigs were blood sampled weekly from day 10 until the end of the experiment (day 35 of age). After weaning (day 28), pigs were challenged individually by inoculating an equal mixture of F4 and F18 *E. Coli* (10^8 CFU), and were killed 5 days after. Statistical analyses of data were performed according to a model including effect of fat, vitamin E, and time (blood sampling). Hemp oil was rich in C18:2n-6 (53.8%), and C18:3n-3 (16.3%), but without C20:5, C22:5 and C22:6. Fish oil contained C20:5n-3 (20.2%),

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C22:5n-3 (2.2%), C22:6n-3 (12.7%), C18:2n-6 (1.5 %), and C18:3n-3 (0.8 %) fatty acids. All these PUFA were present in plasma of all piglets, and the proportion increased with age, and was affected by treatments. Plasma α -tocopherol concentration decreased from d 28 of age to the end of experiment, and the concentration was affected by vitamin E ($P=0.006$) and fat ($P=0.05$) treatments. Treatments influenced plasma immune responses (measured as immunoglobulins, and cytokines after in vitro stimulation at weaning). However, fat source had no influence ($P>0.05$) on ileal fatty acid concentrations, nor immune responses obtained after in vivo *E. Coli* challenge measured as IgA and expression of inflammatory gene expression in mucosa. In conclusion, piglets' plasma rather than intestinal redox status and immunity was affected by oral treatments of fat source and vitamin E.

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Validation of a quantitative biomarker of gut inflammation in weaned piglets

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Weaning is a critical period in pig's life, often associated with digestive infections by opportunistic pathogens. Therefore, weaning diet design aims at reducing digestive tract inflammation to minimise scours. A trial involving 240 weaned piglets (21 days of age) was carried out to measure the effect of 5% of coarsely ground fibre-rich raw materials (sunflower seed by-product or barley) added to an antibiotic-free basal weaning diet, on post-weaning performance, faecal consistency and records of sanitary interventions. In order to assess the digestive tract inflammatory status, four piglets per treatment were sacrificed on 8th day post-weaning, and their digestive tract removed. A 5 cm segment of the ileum was taken 0.8 m before the ileo-caecal valve, rinsed with Phosphate Buffer Saline, fixed in formaldehyde and embedded in paraffin. Segments were cut into 4 μ m thick sections to measure villi height and crypt depth and to count the number of neutrophils in the mucosa after immuno-histochemical anti-myeloperoxidase (MPO) labelling. Ileum and rectal contents were sampled and assayed for MPO activity (measured by spectrophotometric method based on oxidation of O-dianisidine). Consistency of rectal samples was scored according to Bristol stool scale. Analysis of variance was performed to describe the effect of treatment or stool consistency on the measurements. MPO level in gut contents did not differ statistically between treatments. Higher MPO activity was found in pasty or liquid faeces than in regular-shaped stools ($P=0.004$). Neutrophils counts in the ileum mucosa were also higher in piglets showing degraded faecal consistency ($P=0.022$). These results confirm that faecal consistency is related to gut inflammation after weaning. To preclude drawbacks inherent to visual scoring, faecal MPO seems to be a relevant non-invasive quantitative marker of the inflammatory status of piglet's gut.

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Dietary corn bran increased intestinal cellulolytic bacteria and alleviated inflammatory response in weaned pigs

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Corn bran (CB) as a fiber ingredient derived from agricultural by-products can improve growth performance and gut health in pigs. However, the interplay between CB and evolution of gut microbiota in weaned pigs is less clear. The hypothesis of this study was that dietary CB can affect bacterial community composition and antioxidant/anti-inflammatory activities. A total of 24 weaned pigs were randomly allocated to 2 treatment groups fed control (CON) diet based on corn starch without fiber components and 5% CB diet for 28 d. Gut microbiota composition was analyzed by 16S rRNA gene sequencing. Characteristics of antioxidant and cytokines in serum were measured by spectrophotometric and ELISA assays. Statistical analyses were performed in R version 3.2.2. Differences in growth performance and

Abstract

biochemical characteristics were assessed using student's t-test ($P < 0.05$). On d 14, the higher abundances of Bacteroidetes and Proteobacteria were observed in CB treatment ($P < 0.05$). At the genus level, the population of Lachnospiraceae and Prevotellaceae_UCG-001 were increased in CB group on d 14 and d 28 ($P < 0.05$). The presence of Ruminococcaceae_UCG-002, Lachnospiraceae_XPB1014_group, and Fibrobacter were distinct bacteria in CB group. Compared with CON treatment, piglets fed dietary CB showed a higher level of interleukin-10 production ($P < 0.05$) and a significant decrease in the concentration of malondialdehyde ($P < 0.05$). In conclusion, a diet containing 5% CB increased the abundance of bacterial community related to cellulolytic fermentation and utilization and improved host response to biological stress of weaned pigs. These changes might contribute to the beneficial effects of CB on growth performance.

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Efficacy of medium-chain fatty-acid salts alone or protecting sodium heptanoate in front of an ETEC K88 oral challenge in piglets

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Medium-chain fatty-acids (MCFA) with antimicrobial properties have gained interest as in-feed additive alternative to antibiotics. This study evaluated the efficacy of distilled coconut fatty-acid sodium salts (DICOSAN[®], minimum laureate 32%) or protected sodium heptanoate (HEPT'ON PLUS[®], 40% sodium heptanoate protected with the same distilled coconut fatty-acid sodium salts) against an enterotoxigenic *Escherichia coli* (ETEC) K88 challenge in weanlings. Ninety-six 3-week-old piglets (5.6 ± 0.94 kg) were distributed into 32 pens and four experimental groups: three challenged and receiving different experimental diets: a plain diet (CTR); or supplemented with DICOSAN[®] (DIC); or HEPT'ON PLUS[®] (HPP) at 3 kg/t; and a non-challenged fourth group receiving the plain diet (PLB). Intake and weight were monitored along 15-days. After one week, animals were orally inoculated with ETEC K88 (1.5×10^9 cfu) or placebo, and fecal consistency and rectal temperature evaluated afterwards. On days 4 and 8 post-inoculation (PI), one animal per pen was euthanized to evaluate enterobacteria and coliforms counts in ileal-colonic contents and inflammatory response (TNF α and PigMAP). The oral challenge promoted a mild diarrhea with incidences of 9.5% in PLB compared to 41.2% in CTR. The additives did not modified performance, clinical outcomes nor inflammatory markers. Regarding microbial analysis, DIC showed a numerical reduction in ileal microbial counts at day 8 PI ($P < 0.20$) compared to CTR (5.64 vs. 6.62-log cfu enterobacteria/g and 5.59 vs. 6.42-log cfu coliforms/g). Microbial reductions with DIC reached a statistical trend ($P < 0.10$) in colon (9.9 vs. 11.0-log cfu enterobacteria/g, and 9.8 vs. 11.0-log cfu coliforms/g). Regarding HPP, this treatment also showed reductions of enterobacteria in colon at day 4 PI ($P = 0.12$) (9.86 vs. 10.70-log cfu/g). In summary, although we were not able to demonstrate benefits on performance or clinical response under our experimental conditions, both additives were shown to control the growth of enterobacteria in the gut.

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Impact of medium-chain fatty-acid salts alone or protecting sodium heptanoate on weanlings colonic microbiota after an ETEC K88 oral challenge

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Medium-chain fatty acids (MCFA) alone or protecting sodium heptanoate were tested as antibiotic alternatives against post-weaning colibacillosis. The impact of distilled coconut fatty-acid sodium salts (DICOSAN[®],

minimum laureate 32%) or protected sodium heptanoate (HEPT'ON PLUS[®], 40% sodium heptanoate protected with the same distilled coconut fatty-acid sodium salts) were evaluated on colonic microbiota after an enterotoxigenic *Escherichia coli* (ETEC) K88 challenge in weanlings. The study consisted of a randomized design (n = 8 pens; three piglets/pen) as follows: a plain diet (CTR); or supplemented with DICOSAN (DIC); or HEPT'ON PLUS (HPP) at 3kg/t. After one week, animals were orally inoculated with ETEC K88 (1.5x10⁹ cfu) or a placebo. On days 4 and 8 post-inoculation (PI), one animal per pen was euthanized to sample colonic contents. Based on results on microbiological plate counts (enterobacteria and coliforms), samples from day 4PI of HPP and day 8PI of DIC and their respective CTR were submitted to microbiota analysis by 16S rRNA gene sequencing using MiSeq[®] technology. Bioinformatics analysis followed QIIME pipeline and R software packages. Regarding HPP supplementation, CTR and HPP clusters were overlapped in non-metric multidimensional scaling (NMDS) (envfit P = 0.310) with no differences in alpha or beta diversities (P > 0.10). Despite no structural changes were detected, HPP was able to reduce Enterobacteriaceae (P = 0.071) beyond other minor families (Anaeroplasmataceae, Elusimicrobiaceae and RF16). In the case of DIC supplementation, this additive promoted structural changes in the microbiota being CTR and DIC clustered separately in NMDS (envfit P = 0.034). However, no differences were found in alpha or beta diversity and neither in abundances at specific taxonomic levels. To summarize, both additives were able to modify colonic microbiota. Whereas DIC was able to induce structural changes in the colonic ecosystem, but not in specific microbial groups, HPP was shown to selectively reduce members of the Enterobacteriaceae family.

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Dietary raw potato starch modulates intestinal immune status and beneficial host-microbe interactions

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With recent limitations to antibiotic use in livestock species, alternatives are sought to maintain swine health and production. Fiber-type prebiotics can support bacterial fermentation in the hind gut and beneficial metabolites, including butyrate, can affect host immune status. Dietary raw potato starch (RPS) is a potential prebiotic and we hypothesized that inclusion of RPS in a pig nursery diet would enhance cecal butyrate-producing bacterial populations and induce a more tolerogenic immune state. Three-week old pigs (n = 14/group) were fed a basic corn-soy diet or same diet amended with reduced corn plus 5% RPS mixed in (all other components equal, no plasma included) for 3 weeks immediately post-weaning. Feces, cecal contents and mucosa, blood and lymph nodes were collected at necropsy to evaluate microbial populations, butyrate and IgA levels, immune cell phenotype and gene expression. Wilcoxin tests were used for statistical analysis between groups. Correlations for network analysis were calculated with CCREPE for compositional data or the rcorr function from the hmsic R package. RPS intake increased abundances of anaerobic *Clostridial* species and increased cecal and fecal butyrate levels (p = 0.05). Flow cytometric analysis revealed no differences in the percentage of cecal T cells, but piglets fed RPS had a lower ratio of CD8 α ⁺ to CD4⁺ single-positive T cells, and a greater percentage of regulatory T cells (CD25⁺/FoxP3⁺) within the CD8 α ⁺ T cell population (p < 0.05). However, there were no significant changes in regulatory T cell populations in the blood or intestinal lymph nodes (p > 0.05). Immunohistochemical staining of the cecum substantiated no overall difference in T cell abundance, and no difference in the number of IgA-secreting cells. However, IgA levels in the feces were increased in RPS-fed pigs (p = 0.01). Real-time PCR analysis revealed evidence of increased barrier function as expression of MUC2 (p = 0.05) and IL6 (p = 0.05) mRNA was increased in the cecum of pigs fed RPS, but no significant differences in cecal levels of DEF1B, IL17A, IL-22, IL-10 or TGFB mRNA across groups (p > 0.05). A network analysis encompassing host and microbial changes in the cecum revealed regulatory T-cells correlated positively with butyrate levels, luminal IgA concentration, DEF1B expression levels, and mucosa-associated anaerobic bacterial species. Overall, these data indicate that the prebiotic RPS can modulate pig intestinal immune status and may serve as a method to enhance swine enteric health towards a more tolerogenic phenotype.

Dietary sodium diformate and monolaurate affect faecal pathogen load in lactating sowsC Lückstädt and C Hutter
ADDCON GmbH

Creating and maintaining a healthy intestinal environment has become essential to productivity and food safety programmes alike. This study tested the efficacy of a blend of sodium diformate (80%) with the monoglyceride of lauric acid – monolaurate (20%), on decontamination of Gram-negative and Gram-positive bacteria in sow faeces. Forty multiparous sows on a research farm in Saxony-Anhalt, Germany, were allocated to two equal groups and fed a commercial lactation diet from one week before farrowing until the piglets were weaned at 26 days. The test diet contained 1.0% of the sodium diformate – monolaurate mixture. Water was available *ad libitum* throughout the trial. The lactation diet was fed according to a feeding curve, from the last week before farrowing to day 13 of lactation and thereafter *ad libitum*. On the 21st day of lactation, freshly excreted faecal matter was collected from all sows and analysed for *E. coli*, enterococci and streptococci counts. Data were analysed using the t-test and a significance level of 0.05 was used in all tests. The *E. coli* count in the faeces differed significantly ($P=0.0012$) between the control and treatment groups. Faeces of treated sows had a 98% lower *E. coli* count (CFU/g). Similar results were measured for enterococci ($P=0.027$) and streptococci ($P=0.014$), which were reduced by 99% and 75%, respectively. In general it can therefore be stated that the use of dietary sodium diformate and monolaurate is an effective and sustainable tool in improving gut health in sows, by reducing pathogen load at intestinal level and decontaminating the faeces of the sow and thus reducing the risk of cross-infection to the piglets.

Ex-vivo investigation of the anti-inflammatory effects of pomegranate peel extracts on a porcine colonic tissue explantF Mastrogiovanni^{1,2}, A Mukhopadhyaya², N Lacetera¹, R Bernini¹, A Romani³ and T Sweeney²¹Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia; ²School of Veterinary Medicine, University College Dublin; ³Department of Statistics, Computing, Applications “G. Parenti” (DISIA), Phytolab, University of Florence

Bioactive compounds extracted from food byproducts have wide application in the animal feed industry. Pomegranate peel extract (PPE), obtained by hydroalcoholic treatment of peel and seeds, has previously shown anti-inflammatory activity *in-vitro*. Therefore, the objective of this study was to investigate the anti-inflammatory effects of varying concentrations of PPE on a lipopolysaccharide (LPS) challenged porcine colonic tissue explant. We hypothesised that the higher concentrations of PPE would suppress the LPS induced inflammation of colonic explants. Porcine colonic tissue explants were surgically removed from six animals immediately post-slaughter and incubated with 1 ml of culture media with 10 µg/mL of LPS with PPE at 0 (control), 1, 2, 5, 10 or 25 µg/ml. The tissues were incubated at 37°C and 5% CO₂ for 3 hours and then transferred into RNeasy Lysis Buffer. Supernatants from the tissue culture plates were collected and stored at -80°C until used for ELISA. IL-8 concentrations in the supernatant were measured using a Porcine IL-8 ELISA kit. The tissues were processed for RNA extraction and expression of a panel pro-inflammatory cytokines was measured using qPCR. All data were normalised and analysed as a complete randomised design experiment using one way ANOVA function of GraphPad PRISM software. Exposure of the LPS challenged colonic explants with 5 and 25 µg/ml of PPE suppressed IL-8 production ($P<0.01$) compared to LPS challenged controls. The 5 µg/ml of PPE also suppressed *IL1β*, *IL6* and *IL8* gene expression levels ($P<0.05$) in LPS challenged colonic tissues compared to controls. Therefore, to conclude, the 5 µg/ml of PPE elicits the highest anti-inflammatory activity in the LPS challenged colonic explants. These results warrant an in-depth analysis of the PPE as a potential anti-inflammatory ingredient that can be used for pigs undergoing inflammation of gut, especially during the post-weaning period.

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Effects of continuously infusing glucose or casein at the terminal ileum on systemic inflammation

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Rapid carbohydrate and protein fermentation may compromise the large intestine's barrier function and cause an immune response. We hypothesized that continuously infusing glucose or casein at the large intestine would alter circulating leukocyte dynamics and inflammatory biomarkers. Crossbred gilts (81 ± 1 kg BW) fitted with ileocecal cannulas and jugular catheters were enrolled in two experimental periods (P). Period 1 (four days) was used for collection of baseline measurements. At the beginning of P2 (four days), pigs were assigned to one of three infusion treatments: 1) control (CTL; water; $n=7$), 2) glucose (GLU; 500 g/d; $n=6$), and 3) casein (CAS; 300 g/d; $n=6$). Water, GLU, and CAS solutions were continuously infused through the ileocecal cannula at a rate of 125 mL/h for the entirety of P2. Data were analyzed using the MIXED procedure of SAS. During P2, feed intake decreased in GLU relative to CTL pigs (14%; $P=0.02$); however, it did not differ between CTL and CAS treatments ($P=0.12$). Mild hyperthermia occurred with both GLU and CAS relative to CTL pigs (0.3 and 0.1°C, respectively; $P<0.01$). Circulating neutrophils increased in CAS relative to CTL pigs (26%; $P=0.01$) but were similar between CTL and GLU treatments ($P=0.27$). Blood monocytes decreased in GLU (26%) and tended to be decreased in CAS (16%) relative to CTL pigs ($P=0.01$ and $P=0.06$, respectively). Circulating lipopolysaccharide binding protein decreased in GLU relative to CTL pigs ($P=0.04$) but was similar between CTL and CAS treatments ($P=0.45$). Additionally, no differences in tumor necrosis factor alpha were observed across treatments ($P=0.52$). In conclusion, continuously infusing GLU or CAS into the terminal ileum appeared to mildly stimulate the immune system but had little or no effects on the circulating inflammatory markers we measured.

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Increasing intestinal protection with specific algae extracts

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In the small intestine the mucus layer and transmembrane proteins act as the first line of defense against pathogens and toxins present in the lumen. The mucus layer protects the epithelium from dehydration, mechanical damage, and autodigestion, as well as preventing pathogen absorption and systemic dissemination. The various transmembrane proteins control the paracellular space in the mucosa. Both the mucus layer and the transmembrane proteins play important roles in the maintenance of gut health. Specific algal extracts have shown the capacity to stimulate the excretion of mucin proteins by colonic goblet cells (Barcelo *et al.*, 2000). Olmix has isolated two different algae extracts (from *Ulva* sp. and *Solieria chordalis*) and tested their effect on the intestinal barrier.

MSP[®]_{MUCIN} addition to mucus-secreting HT-29 MTX cells induced early gene expression (after 1h) of MUC1 and MUC5AC (from 52 to 115 times and from 104 to 687 times higher than the control respectively) at 0.1 and 1mg/ml. After 4 hours, this effect was maintained for MUC1, and enhanced for MUC5AC. MUC2 gene expression was also increased, to a lesser extent, after 4 hours of stimulation with 0.1 and 1 mg/ml of MSP[®]_{MUCIN} (2 to 7 times higher than the control). The gene expression of tight junction proteins was moderately stimulated by the MSP[®]_{MUCIN}. MSP[®]_{BARRIER} induced early ZO-2 gene activation (up to 24 times higher than the control) followed by ZO-1 expression after 4h (from 29 to 47 times higher than the control) in inflamed conditions. In standard conditions, MSP[®]_{BARRIER} induced an early and transitory expression of claudin 2 (CLDN2) (7 times higher than the control). MSP[®]_{BARRIER} also induces mucin protein gene expression but to a lesser extent than MSP[®]_{MUCIN}.

Both algae extracts tested showed a capacity to protect the intestinal barrier. MSP[®]_{MUCIN} by high stimulation of mucin secretion and MSP[®]_{BARRIER} by increasing the tight junction expression.

Pre-weaning feeding stimulates gut development of piglets at weaningA Middelkoop¹, R Choudhury², W Gerrits³, B Kemp¹, M Kleerebezem² and L Bolhuis¹¹*Adaptation Physiology Group, Wageningen University & Research;* ²*Host-Microbe Interactomics Group, Wageningen University & Research;* ³*Animal Nutrition Group, Wageningen University & Research*

Piglets weaned early are challenged by deprivation of sow's milk and an abrupt change to a post-weaning diet, which usually consists of solid feed. This sudden shift in diet physical form and composition, in combination with stress and acute fasting, results in undesirable changes in gut morphology and microbiota. We hypothesized that pre-weaning feeding can be a tool to prepare the gastro-intestinal tract of suckling piglets for the post-weaning diet. Therefore we studied the gut development of early-fed (EF, i.e. piglets provided with fibrous solid feed from two days of age, n = 12 litters) and non-fed piglets (NF, i.e. no solid feed before weaning, n = 10 litters). At weaning (i.e. four weeks of age) a subset of piglets (n = 14 per treatment, seven males and seven females) was sacrificed to measure organ weights, intestine length and intestinal pH. Data were analysed using GLM. Models included treatment as fixed effect and bodyweight at slaughter as covariate. At slaughter, EF-piglets weighed 8.5 ± 0.4 kg and NF-piglets weighed 8.2 ± 0.3 kg. EF-piglets tended to have a heavier pancreas compared to NF-piglets (EF: 15.5 ± 0.8 vs. NF: 13.5 ± 0.7 gram; $P = 0.05$). Full ($P = 0.02$) as well as empty ($P = 0.03$) small intestine (including and excluding digesta respectively) were heavier in EF-piglets (Full, EF: 482.5 ± 31.3 vs. NF: 421.7 ± 17.8 gram; Empty, EF: 370.3 ± 20.3 vs. NF: 328.5 ± 10.5 gram). In addition, the full colon tended to be heavier for EF-piglets (96.8 ± 7.5 gram) compared to NF-piglets (80.4 ± 4.2 gram; $P = 0.08$). Moreover, the small intestine tended to be longer (EF: 8.3 ± 0.2 vs. NF: 7.8 ± 0.2 meter; $P < 0.10$) and the cecum plus colon were longer (EF: 1.3 ± 0.03 vs. NF: 1.2 ± 0.02 meter; $P < 0.05$) for EF-piglets. Lastly, the intestinal pH was lower in cecum (EF: 6.3 ± 0.1 vs. NF: 6.7 ± 0.1 ; $P < 0.001$) and colon (EF: 6.9 ± 0.1 vs. NF: 7.2 ± 0.1 ; $P < 0.01$) for EF- compared to NF-piglets. In conclusion, pre-weaning feeding stimulates gut growth and function at weaning.

Effect of cyclic heat stress and supplemented inorganic and organic zinc source levels on intestinal gene expression and morphologyK Mills¹, J Feldpausch¹, A Duttlinger¹, S Elefson¹, J Radcliffe¹, Z Rambo² and B Richert¹¹*Purdue University;* ²*Zinpro Corporation*

Heat stress can negatively impact gut morphology and integrity, but it is unclear if supplemental zinc could reverse any of these negative effects. Therefore, our objective was to determine if zinc source and concentration improves performance and gut integrity of heat stressed finishing pigs. Crossbred gilts (n = 400; initially 72.0 kg) were housed (5 pigs/pen) under thermoneutral (TN; 18.9–16.7°C) or cycling heat (HS) conditions simulating seasonal chronic heat (30°C/26.7°C for 12h:12h on days 24–72) with acute heat waves (32–33°C/29–30°C for 12h:12h on days 21–24, 42–45, and 63–65). Treatments were arranged as a 2 × 2 × 2 factorial with main effects of environment (HS vs. TN), supplemental Zn (50 vs. 130 mg/kg), and Zn source (ZnO or organic/inorganic blend: Availa[®] Zn/ZnO). Pigs (5/pen) were randomly allotted to treatments (10 rep/trt). On day 65, one pig/pen was harvested, intestinal tissue collected and analyzed for gene expression (heat shock proteins (HSP) 70, 27, and 90, occludin, and mucin-2) and morphology (n = 80). Data were analyzed using SAS 9.4. Ileal villi were shorter in HS pigs ($P = 0.020$). A 3-way interaction (Environment × Zn Source × Zn Level) was observed for villus height ($P < 0.02$) and HSP27 ($P = 0.05$) expression in the jejunum. The villus height 3-way interaction was primarily caused by the 50 mg/kg inorganic Zn treatment increasing villus height under HS and all other treatments decreasing villus height under HS. A tendency ($P \leq 0.10$) for a Zn Source × Zn Level was observed for Jejunal HSP70 and HSP27 expression with 130 mg/kg of inorganic Zn causing an increased expression and 130 mg/kg of organic Zn causing a decreased expression relative to 50 mg/kg of each source. HS tended to increase HSP70 ($P < 0.08$). Finally, HSP90 expression was greater ($P < 0.05$) in pigs supplemented organic Zn compared to pigs supplemented with inorganic Zn. Our data suggests both environment and supplemental Zn impact intestinal function.

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Sainfoin (*Onobrychis viciifolia*) tannins reduce enterotoxigenic *Escherichia coli* infection symptoms in weaning piglets

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Infection with enterotoxigenic *Escherichia coli* (ETEC) is an important health threat for weaning piglets. As tannins limit *E. coli* infection, a study was set up to assess the efficacy of Sainfoin (*Onobrychis viciifolia*) tannins to reduce ETEC infection symptoms in post weaning diarrhea (PWD). Twenty Swiss Large White piglets from four litters weaned at 23 (± 1) days with an average BW of 8.6 (± 0.6) kg were included in this study. Piglets were randomly assigned by bodyweight (BW) to two groups, and housed by group in pen of 10. The Control group (C; n=10) had *ad libitum* access to a standard starter diet formulated according to the Swiss feeding recommendations for weaned pigs. The treatment group (T; n=10) was offered a starter diet containing 12.6% Sainfoin, corresponding to a Condensed Tannins (CT) concentration of 1%. The two diets contained the same ingredients (except Sainfoin) and were formulated to be isocaloric (13MJ/kg) and isonitrogenic (165g/kg). Four days after weaning, C and T pigs were infected with an ETEC F4 strain. Fecal score (n=9), BW (7) and ETEC shedding (6) were regularly measured throughout the study until 10 days after infection. Diarrhea was defined as a fecal score of 3 or above on a scale of 1 (no diarrhea) to 4 (watery diarrhea). Duration of diarrhea was the sum of days in diarrhea. Fecal score was analyzed with a two-way ordinal regression whereas individual BW, average daily gain (ADG) and duration of diarrhea with two way (repeated) measures ANOVA. Sainfoin tannins reduced fecal scores ($P=0.003$) and the duration of diarrhea ($P=0.002$). The ADG did not differ between C and T pigs ($P=0.633$). The CT from Sainfoin seem to be efficient to overcome PWD caused by ETEC infection. Further studies are required to refine Sainfoin CT dose for an optimal efficiency.

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Targeting gut inflammation in pigs with the use of olive fruit bioactives positively influences pig performance

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Prolonged inflammation derived from standard farming procedures drives animals to cytokine-induced anorexia accompanied to a negative energy balance, resulting in reduced animal performance. Previous work showed that the addition of olive fruit bioactives (OFB) in feed was able to reduce inflammation and prevent gut integrity side-effects on a model of LPS-challenged piglets. The present work was designed to evaluate the potential benefits of a new prototype based on standardized OFB and to test pig performance responses when added to transition diets. Anti-inflammatory activity was determined by gene expression analysis of IL-1 β and iNOS on LPS-stimulated Raw264.7 macrophages. Benefits over the intestinal barrier were determined on an IPEC-J2 cell model by transepithelial electrical resistance (TEER) measurement after 24h incubation with OFB. Finally, 192 21d-old weaned piglets ((LDxLW)x Pietrain) were blocked according to their initial body weight (BW) and distributed in pens (eight animals/pen) among three different dietary treatments: i) non-medicated diets in the pre-starter (weaning to 14d) and starter (15 to 42d) stages (C-), ii) pre-starter diet containing 300 ppm of amoxicillin and 2500ppm of ZnO followed by starter diet with 300ppm of amoxicillin (AM), iii) C- diets with 500ppm of OFB added (OFB). BW and feed disappearance were recorded weekly. Performance data were analysed as a RCBD using a mixed-effect model with repeated measures. TEER and gene expression data were analysed using a mixed model. Statistical analyses were performed with SAS v.9.4. Increase in the relative expression of inflammatory markers after macrophages stimulation was significantly ($P < 0.01$) reduced by OFB pre-incubation. Addition of OFB to IPEC-J2 cells resulted in larger relative TEER values ($P < 0.01$). Final BW improved in AM and OFB groups vs C- in heavy pigs ($P < 0.05$). In summary, the tested OFB prototype may improve performance of pigs fed with non-medicated diets through lowering inflammation and improving gut barrier function.

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Porcine gastric ulceration in weaners from high-risk Danish herds using finely grounded commercial diets fed ad libitumJ Peralvo Vidal¹, S Haugegaard², J Nielsen¹, N Rosager Weber² and A Øyan Pedersen¹¹University of Copenhagen; ²SEGES Pig Research Centre

Ulceration of the pars oesophagea is a problem primarily observed in growing-finishing pigs fed ad libitum and sows. It is often associated with an increased fluidity of the gastric content due to finely grounded and pelleted diets. Although weaners are normally fed pelleted diets ad libitum, little is known about the prevalence of ulceration in this age group. In this cross-sectional study, we assessed the occurrence of gastric ulceration in high-risk herds with weaners fed ad libitum on finely grounded commercial diets (av. particle size 75 % < 1 mm). We evaluated the solid phase percentage of the gastric content. The study consisted of 200 clinically healthy weaners from ten Danish farms. In each farm, 20 weaners with an average weight of 22 kg (Min. 12 kg and Max. 37 kg) were randomly selected. Post-mortem gastric ulceration assessment was based on the Danish score system, where a stomach scored index 0 is healthy; index 1 to 5 present minor to severe parakeratosis and minor scars; index 6 to 10 present consolidated scars, minor to severe ulcers, and oesophageal stenosis. Solid phase percentage of the gastric content was determined based on the sedimentation test. We observed that 35.5 % of weaners had ulceration in the pars oesophagea (index > 5). Logistic regression analysis showed a decrease in the odds of gastric ulcers (index >5) as the solid phase of the gastric content increased. Thus, for each percentage the solid matter increased the odds of gastric ulcers (index >5) decreased by 8 % (OR 0.92, CI95 %: 0.89-0.95). We have demonstrated that ulceration of the pars oesophagea is present in some Danish herds with weaners fed commercial diets ad libitum. However, it remains unanswered why 64.5% of weaners exposed to the same conditions in the herds do not develop gastric ulceration.

Evaluation of gastrointestinal functionality changes in a nutritional post weaning diarrhea model in early weaned pigletsE Perez Calvo¹, V Verlhac Trichet¹, J Schmeisser¹, A Klüenter² and P Celi²¹DSM Nutritional Products; ²DSM Nutritional Products

Weaning compromises gastrointestinal functionality, including changes on digestive and absorptive capacity, microbiology and immunology, resulting in clinical signs like post-weaning diarrhea (PWD). Both leguminous plant proteins and high level of dietary proteins can influence gastrointestinal functionality in weaned piglets (Pluske et al., 2002). The objective of the present study was to evaluate the changes on gastrointestinal functionality on early weaned piglets fed a high protein diet containing soybean meal to induce PWD. A total of 32 male pigs weaned at aged 21 d with initial BW of 6.1 ± 0.6 , were fed either a low protein control diet (LP; 167 g of CP/kg) or a high protein diet (HP, 210 g of CP/kg) for 19 days. Individual piglet weights and fecal samples were recorded on Study Days 0, 6, 12 and 19. Fecal scores were assessed visually daily using a scoring system from 0 to 3. Plasma samples were collected at days 12 and 19 for plasma urea nitrogen (PUN) and vitamins A and E analyses. At day 19, caecum content was collected for VFA determination. Differences between treatments were analyzed by one-way ANOVA. Results showed no significant differences in ADG and BW between the LP and HP fed piglets. Feeding HP diets progressively decreased DM content in feces ($P < 0.01$), increased diarrhea frequency (<0.05) and increased PUN ($P < 0.001$) on study day 19. No differences between diets were observed for vitamin A and E concentrations in plasma and for total SCFA at the caecum level, at day of 19, with the exception of a higher proportion of valeric acid in the LP fed piglets ($P < 0.05$). In conclusion, feeding high soy protein diet seems to be a valid model to generate PWD in early weaned piglets indicating its utility to be used to identify potential biomarkers for gastrointestinal functionality.

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Effects of dietary protease on growth performance and intestinal morphology of weaned pigs

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A study determined protease effects (75,000 PROT units/g; *Bacillus licheniformis*; Ronozyme[®] ProAct, DSM nutritional products, Switzerland) on growth performance and intestinal morphology in weaned pigs. A total of 75 weaned-pigs (7.06 ± 0.18 kg BW; 28 d old) were randomly assigned to 3 dietary treatments (5 pigs/pen; 5 pens/treatment) in a randomized CBD (block = BW). Treatments were 1) Positive Control (PC) corn/soybean diet to meet/exceed crude protein (CP) requirements (PC; CP = 24.17%), 2) Negative Control, reduced CP level (NC; CP = 23.51%), and 3) NC + 0.02% protease (PRO). Not spray-dried plasma, fishmeal, zinc oxide, or antibiotics was used to eliminate antibacterial or physiological effects. Treatments were offered during 6 weeks with blood collected (one pig/replicate) on d 1, 3, 7, and 14, after weaning. Ileum samples were collected (one pig/replicate) end of experimental period. Measurements included performance, frequency of diarrhea, packed cell volume (PCV) and ileal morphology. Data was processed (PROC GLM, SAS) assigning dietary effect and BW as a covariate. Pigs offered PRO and PC had higher ($P < 0.05$) ADG (323 and 322 vs. 220 g/d), G:F (0.456 and 0.431 vs. 0.304 g/g) and number of goblet cells (25 and 21 vs. 14) than those fed NC. Pigs fed PRO had higher ($P < 0.05$) villus height (318 vs. 282 μm) and villus height to crypt depth ratio (3.67 vs. 2.87 μm/μm) than those fed NC, but there was no difference on ileal morphology between PC and PRO. Interestingly PRO decreased ($P < 0.05$) frequency of diarrhea during first 2 weeks after weaning (16 vs. 36 and 41%) and PCV on d 14 after weaning (32.08 vs. 35.56 and 34.26%) compared with PC and NC. Adding protease in nursery diets with low protein level improved growth performance and decreased frequency of diarrhea of weaned pigs.

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Development of a pig model for inflammatory bowel diseases in humans

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Animal models of inflammatory bowel diseases (IBD) are important to provide insights into the pathogenesis of IBD and to evaluate modalities to prevent or ameliorate inflammation. Dextran sulfate sodium (DSS) was used to induce intestinal inflammation in weaned piglets in two experiments, each with 24 six-week-old piglets. The first study was a DSS dose-response experiment with either 0, 0.625 or 1.25 g DSS/kg body weight (BW) provided orally for five days. In the second study, the effect of red meat intake (control feed substituted with 15% red meat for two weeks) on the severity of DSS-induced inflammation was investigated. In both experiments, piglets were scored based on clinical measures (weight, bloody stools and overall performance) during the DSS-treatment period and blood samples were collected at day 0, 2, 4 and 5 for determination of inflammation markers. At slaughter, the intestines were evaluated for macroscopic appearance and tissue and digestive samples collected for expression of pro- and anti-inflammatory proteins. Experiment 1 showed that a dose of 1.25 g/kg BW decreased BW gain by more than 40% ($P < 0.05$) and increased the blood concentration of C-reactive protein (CRP) from 341 to 680 mg/l ($P < 0.01$), while haptoglobin, IgG, IgA and IgM were unchanged. Experiment 2 confirmed the DSS effect on BW gain ($P < 0.015$), while CRP and haptoglobin were increased with DSS treatment ($P < 0.001$) but not with meat intake. However, the clinical scores and IgM were higher in piglets fed the meat diet + DSS compared with piglets treated with DSS alone ($P < 0.01$) suggesting an increased sensitivity for DSS in meat fed piglets. In conclusion, we have developed an intestinal inflammation model in weaned piglets that can be used as a model for diet intervention studies in humans.

Soybean meal and β -mannanase affected immunoproteins in carotid artery and morphology and water channel proteins in small intestine of pigs

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One hundred Landrace \times Yorkshire barrows (BW Mean 9.49 kg, SD 1.18 kg) were used in a thirty-day 2 \times 2 RCBD trial to study the effects of soybean meal (SBM) and β -mannanase (Hemicell XT) on small intestine. Treatments were SBM (220 and 370 g/kg, making dietary soluble β -mannan to 2420 and 4070 ppm, respectively) and β -mannanase (0 and 200 mg/kg). Diets were isocaloric (3200 kcal/kg ME) and equal in SID Lys (12.5 g/kg). On Day 30, six pigs per treatment were sacrificed for blood from mesenteric vein, portal vein and carotid artery to measure serum IgM, IgG and complement C4 (C4) and for small intestine to measure morphology and relative mRNA of aquaporins (AQP1, AQP3, AQP8, AQP10, AQP11) that channel transmembrane water movement. Data were analyzed with GLM of SPSS. In carotid artery, β -mannanase increased C4 ($P < 0.01$) and SBM tended to increase IgM ($P = 0.08$). Treatment had no other effects on the measured immuno-proteins in the specified serum. In jejunum, SBM decreased villus height ($P = 0.02$) whereas β -mannanase increased it ($P < 0.01$). Treatment did not affect jejunal crypt depth. SBM tended to decrease jejunal villus/crypt (V/C) ($P = 0.07$) whereas β -mannanase increased it ($P = 0.03$). In ileum, SBM decreased villus height ($P = 0.02$) whereas β -mannanase increased it ($P = 0.01$). SBM and β -mannanase had no main effects on either crypt depth or V/C in the ileum but SBM \times β -mannanase affected them ($P = 0.01$). β -mannanase restored ileal crypt depth and V/C to the same levels as low SBM diets in pigs fed high SBM feed. In jejunum, SBM decreased AQP11 ($P = 0.01$) and β -mannanase increased AQP8 ($P < 0.01$) and AQP10 ($P = 0.02$). In ileum, SBM decreased AQP3, AQP8 and AQP11 ($P < 0.01$) whereas β -mannanase increased AQP3 ($P = 0.01$) and AQP11 ($P = 0.02$) and tended to increase AQP8 ($P = 0.06$). Dietary SBM and β -mannanase appeared to counteract on morphology and function of small intestine in the pigs.

Dietary soybean meal level and β -mannanase supplementation affected serum biochemicals and enzyme activities in nursery pigs

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One hundred Landrace \times Yorkshire barrows (BW Mean 9.49 kg, SD 1.18 kg) were used in a thirty-day 2 \times 2 RCBD trial to study the effects of soybean meal (SBM) and β -mannanase (Hemicell XT) on major serum biochemical constituents. Treatments were SBM (220 and 370 g/kg, making dietary soluble β -mannan to 2420 and 4070 ppm, respectively) and β -mannanase (0 and 200 mg/kg). Diets were isocaloric (3200 kcal/kg ME) and equal in SID Lys (12.5 g/kg). On Day 30, six pigs per treatment were sacrificed for blood from mesenteric vein, portal vein and carotid artery for the measurements. Data were analyzed with GLM procedure of SPSS. In mesenteric vein, SBM decreased total protein (TP) ($P = 0.04$), creatinine ($P < 0.05$), calcium ($P = 0.01$) and phosphorus ($P = 0.03$), and increased lactate dehydrogenase (LDH) ($P = 0.02$). β -mannanase increase albumin ($P < 0.01$), creatinine ($P < 0.01$) and calcium ($P < 0.02$), and decreased blood urea nitrogen (BUN) ($P < 0.01$), alanine aminotransferase (ALT) ($P < 0.01$) and glutamic oxaloacetic transaminase (GOT) ($P < 0.01$). SBM \times β -mannanase affected albumin ($P < 0.01$), creatinine ($P = 0.03$), calcium ($P < 0.01$), LDH and GOT ($P < 0.05$). β -mannanase improved TP, albumin, BUN, creatinine, LDH, GOT, calcium and phosphorus in high SBM diet close to the levels of low soybean meal diets. In portal vein, SBM increased GOT ($P = 0.01$) and β -mannanase decreased BUN ($P = 0.03$). SBM \times β -mannanase affected ALT ($P < 0.05$) and GOT ($P < 0.01$). β -mannanase restored ALT and GOT in high SBM diet to the levels of low soybean meal diets. In carotid artery, SBM increased TP ($P < 0.05$), albumin ($P < 0.01$), BUN ($P < 0.01$), LDH ($P < 0.05$) and GOT ($P < 0.01$), and decreased alkaline phosphatase (ALP) ($P < 0.01$). SBM \times β -mannanase affected LDH ($P = 0.03$) and GOT ($P < 0.01$). β -mannanase restored LDH and GOT in high SBM diet to levels of low soybean meal diets. SBM and β -mannanase appeared to counteract on nitrogen and mineral metabolism and, as indicated by serum enzyme activities, on body cavity tissue injuries.

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Gamma-aminobutyric acid and amino acid supplementation in weaner pigs subject to short-term production stressors do not influence intestinal permeability

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The aim of this study was to examine whether gamma-aminobutyric acid (GABA) supplementation in water and glutamine (Gln), glutamate (Glu) and tryptophan (Trp) supplementation in the diet reduced gastrointestinal tract permeability in weaner pigs exposed to production stressors. At weaning (d0), 36 male pigs were allocated to pens with their litter mates (3/pen) and allowed to acclimate for 14 days. At d14 of the study, all pigs were mixed with non-littermates, deprived of feed for 12 hours from 0600h to 1800h, and split into two treatment groups. At 0600h on d14, 18 of these pigs were provided with 50 mg/day GABA in water (from d14-18), and at 1800h with 0.3% L-Glu, 0.1% L-Gln and 0.34% L-Trp added to a commercial diet (from d14-22). The study finished on d28. On days 14 and 22, at 1700h, 12 pigs were orally dosed with 2.5ml/kg BW a solution comprising of 20% mannitol and 0.75ml/kg BW lactulose (66%) to measure sugar absorption. Twenty minutes after dosing, blood was collected from the pigs and analyzed for D-mannitol and lactulose. Although not statistically significant at d14 pigs the supplement treatment pens had lower plasma D-mannitol concentrations compared to un-supplemented pigs (14.4 vs 18.9nmol/L: $p=0.25$). No significant differences were observed at d22. These results indicate that GABA supplementation prior to exposure to acute stressors at weaning may reduce GIT permeability, but the large variation between pigs prevented statistical significance.

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Effect of short-chain fructooligosaccharides supplementation on performance and gut health of pigs

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Farmers face difficulties in redeeming their investment in large litters since this increase in size comes with a higher piglet mortality, which is partly attributed to these litters' low resilience. Dietary oligosaccharides (OS), given to the sow as a preventive measure, proved beneficial for the offspring's performance and resilience. However OS supplementation to the piglet - and not the sow - has been poorly explored. Therefore, this field trial studied the effect of short-chain fructooligosaccharides (scFOS)(no or 1g scFOS/kg BW) supplemented to piglets for 7 or for 21 days after birth. Parameters indicating overall performance (average daily gain, mortality) and gut health (microbiome, intestinal integrity, mucosal immune system, intestinal morphology) were measured from birth (d0, d7, weaning) till 2 weeks post-weaning and analysed using linear mixed modelling (fixed factors: treatment, age; random factors: sow, piglet).

Post-weaning survival of pigs that received scFOS was improved whereas average daily gain was not. No differences in the microbiome (Next Generation Sequencing via 16s rRNA Amplicon sequencing), short-chain fatty acids profile of feces and digesta (via gas chromatography) between the treatment groups were seen, albeit age affected these parameters, in accordance with the literature. Intestinal integrity, determined by measuring intestinal permeability (FD-4-translocation in Ussing chambers), immunohistochemistry against occludin, ZO-1, claudin-2 and real time qPCR for *MUC2*, and regenerative capacity (Western blot for PCNA and caspase-3), was similar between the treatment groups. Alike, intestinal architecture (villus length, crypt depth) was not affected by scFOS supplementation. The density of intra-epithelial lymphocytes and the expression profiles of anti-inflammatory (IL-10) and pro-inflammatory (IL-1 β , IL-6, TNF α and IFN γ) genes were used to assess mucosal immunity. Only IFN γ was lower in pigs that received scFOS.

In conclusion, the supplementation of scFOS to suckling piglets improved their survival, this effect not being associated with a superior gut health.

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Impact of intestinal disorders and respiratory diseases on performance and economics of growing pigs

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Intestinal disorders and/or respiratory diseases have a negative impact on animal welfare and profits for pork producers. It has been shown that these diseases reduce pig performance, however, little is known about the magnitude of the economic losses associated with intestinal disorders and/or respiratory diseases for growing pigs. The objective of this study was to quantify under field conditions the impact of intestinal disorders (ID), respiratory diseases (RD) and the combination (CO) on the performance and profits of growing pigs (25-115 kg BW). For this a dataset of 32 grower/finisher farms in the Netherlands was used including antibody titres measurements at week 0, week 5, week 10 and week 15 after pen entry. Farms were categorised ID with positive titre responses against *Lawsonia intracellularis*, *Salmonella* or PCV2 virus. Farms were categorised RD with positive titre responses against *Actinobacillus pleuropneumonia*, PCV2 virus, Influenza, *Mycoplasma hyopneumonia*, PRRS virus and *Ascaris suum*. Category CO was positive titres for both ID and RD. The performance of the categories ID, RD and CD was compared to farms with no health issues (NH). Data were analysed with analysis of variance using Genstat (version 18.0) with pig farm as the experimental unit and health category as fixed effect. Compared to NH the reduction in growth ($P < 0.01$) was 9.4% for ID, 9.0% for RD and 11.1% for CO. Compared to NH the mortality increased ($P = 0.04$) with 0.8% for both ID and RD and 1.4% for CO. Economic loss per delivered pig was calculated at €2.65 for ID, €2.56 for RD and €3.47 for CO. In conclusion, intestinal disorders and respiratory diseases cause significant reduction in performance and profits.

Mycotoxin binder increases growth performance and digestive health of finisher pigs offered wheat based diets grown under different agronomical conditions

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Cereal grains vary in nutrient composition due to different cultivars and agronomic conditions which in turn may affect animal performance. It was hypothesised that low quality wheat grain, grown under different agronomical conditions would negatively affect animal performance and immune responses in pigs compared to feeding high quality wheat grain. The addition of a mycotoxin binder would negate the effects of the low quality grain. Sixty-four pigs (38.7 kg (SD 3.48 kg)) were assigned to one of four dietary treatments: (T1) low quality wheat diet, (T2) low quality wheat diet plus a mycotoxin binder, (T3) high quality wheat diet, (T4) high quality wheat diet plus a mycotoxin binder. The inclusion of wheat was 500 g/kg. The mycotoxin binder used was a Hydrated Sodium-Calcium-Aluminum-Silicate, which also included calcium propionate and calcium formate. The low quality wheat grain had higher levels of zearalenone, aflatoxin and ochratoxin. The data was analysed using Proc Mix and the model included the effects of wheat, binder and their interaction. Pigs offered the low quality wheat diet had a lower average daily gain (ADG) ($P < 0.05$), average daily feed intake (ADFI) ($P < 0.001$) compared with pigs offered the high quality wheat diets. The inclusion of a mycotoxin binder improved ADG and ADFI ($P < 0.05$). Pigs offered the low quality wheat diets had increased ($P < 0.05$) expression of tumour necrosis factor alpha (*TNFA*) in the duodenum and colon and of claudin 2 (*CLDN2*) ($P < 0.001$) in the duodenum, compared to pigs offered the high quality wheat diets. The inclusion of a mycotoxin binder reduced the expression of *CLDN2* ($P < 0.05$). In conclusion, the low quality wheat reduced ADG, ADFI and modified the gene expression of genes involved in inflammation. The supplementation of a mycotoxin binder improved ADG, ADFI, and also improved digestive health through increases in tight junction gene expression.

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Maternal and direct supplementation with milk protein fraction and yeast beta glucan helps piglets overcome the post-weaning challenge

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The objective of this study was to investigate the use of a casein hydrolysate with a yeast β -glucan as a maternal supplement (MAT) and/or direct supplement post-weaning (PW), to improve piglet health and performance during the PW period. It was hypothesised that piglets that had been exposed to the supplement during gestation and weaning would have the healthiest faecal scores and improved gut architecture. Twenty gilts were assigned to either the supplemented diet (SD; n=10) or control diet (CD; n=10) from day 75 of gestation to weaning (MAT). At weaning, six piglets/sow were randomly assigned to one of 2 dietary groups based on their respective mother's diet. Four treatment groups were formed, CD-MAT/CD-PW (CD/CD), CD-MAT/SD-PW (CD/SD), SD-MAT/CD-PW (SD/CD) and SD-MAT/SD-PW (SD/SD). Piglets (3/pen) were housed on fully slatted floors (1.68 × 1.22 m) at an ambient temperature (30°C) with ad-libitum access to feed and water. The weights of piglets and feed offered-consumed were recorded at weaning and on day 10 PW. Faecal consistencies were scored twice daily using a scoring system, which assigns scores ranging from 1 to describe firm faeces to 5 which describes mucous-like faeces (severe diarrhoea) (normal scores between 2 and 3). Randomly selected piglets from each group were euthanised at weaning and 10 d PW for histological analysis of intestinal tissues. The faecal scores were analysed using repeated measures analysis using PROC MIX and the performance and histology data was analysed using PROC GLM. Maternal SD supplementation increased the duodenal villous height and crypt depth compared to CD group at weaning ($P < 0.05$). The SD supplementation (maternally and/or directly) was also associated with lower faecal scores ($P < 0.001$) and feed intake ($P < 0.001$) compared to CD group. In conclusion, piglets that were exposed to the supplement in-utero, lactation and post-weaning had the lowest faecal scores and healthiest villus architecture.

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The immune response of pigs divergent in feed efficiency differs in both basal unchallenged and lipopolysaccharide challenged colonic tissue

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Pigs that are more feed efficient are potentially more susceptible to disease due to having less available resources to fight an infectious challenge. The objective was to examine the gene expression profile of targets involved in the innate immune response and epithelial nutrient transport such as tight junction proteins, pattern recognition receptors, cytokines and volatile fatty acid transporters in: a) basal unchallenged colonic tissue; and (b) colonic tissue following an ex-vivo LPS challenge in pigs divergent for residual feed intake (RFI). Two experiments were conducted by examining a panel of genes (72 targets) using the nanostring multi-gene assay in both basal colonic and ex-vivo LPS challenged colonic tissue. For the analysis of the basal tissue, two populations of pigs (Group 1 + 2) were utilized while only one group (Group 2) was utilized for the LPS challenge experiment. In the basal unchallenged colon, low RFI (LRFI) pigs had increased expression of the LPS detoxification enzyme *AOAH* and increased expression of the transcription factor *API1/JUN* ($P < 0.05$), as well as an increased expression of the downstream cytokines (*IL1*, *IL8* and *TNF*; $P < 0.05$) compared to the high RFI (HRFI) pigs. Following the LPS challenge there was interaction between RFI group and LPS with the HRFI group having an increase in the gene expression of *IL8* ($P < 0.05$) while the LRFI pigs had reduced expression of *IL8* following the LPS challenge. The LRFI group had higher expression of *GPR43/FFAR2* and transcription regulators (*JAK2*, *NFAMI*) in the LPS challenged tissue suggesting changes in the capacity of LRFI pigs to respond to a challenge ($P < 0.05$). In conclusion the more efficient LRFI pigs have a heightened basal inflammatory state compared to the inefficient HRFI pigs. The differential response to the addition of LPS suggests changes in the regulation of the inflammatory response between HRFI and LRFI pigs.

Evaluation of intestinal morphology and inflammatory cell infiltration in a swine chronic heat stress model

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One challenge in swine health research is evaluation and quantification of immunologic and morphologic changes in the gastrointestinal tract in response to physiologic stress. The study objective was to evaluate morphologic and cellular infiltrative changes in porcine small intestine in response to heat stress. Commercial crossbred gilts (n = 100; initially 72.0 kg) were housed (5 pigs/pen) under either thermoneutral (TN; 18.9–16.7°C) or cycling heat (HS) conditions simulating seasonal chronic heat (30°C/26.7°C for 12h:12h on days 24–72) with acute heat waves (32–33°C/29–30°C for 12h:12h on days 21–24, 42–45, and 63–65) were used in a 70d experiment. On day 65, one pig/pen was euthanized, and a 4cm sample of mid-jejunum was collected, stored in 10% NBF, placed in paraffin blocks and subsequently stained with H&E and alcian blue. Slides were also immunohistochemically stained to detect HSP70 using a commercially available antibody. Stained slides were scanned with a Leica Aperio[®] whole slide imaging scanner and analyzed with ImageScope[®] software. Analysis included manual and automated counts of eosinophils, goblet cells, intraepithelial lymphocytes, and villus height and crypt depth. An ACVP-boarded pathologist also assigned scores based on qualitative assessment of inflammation. Data were analyzed with Proc GLM in SAS 9.4. Results indicate no differences ($P > 0.10$) between manually counted intraepithelial lymphocytes, eosinophils or goblet cells between HS and TN pigs. However, automated analysis indicated a greater number of eosinophils in TN compared to HS pigs ($P = 0.02$). TN pigs had greater ($P = 0.0145$) villus heights (468µm vs. 388.5µm) and tended ($P = 0.08$) to have deeper crypts (168µm vs. 150µm) compared to HS pigs. The pathologist's qualitative scores indicated no differences in inflammation between TN and HS slides. Based on these findings, chronic heat stress induced villus blunting but did not affect infiltration of inflammatory cell types in the porcine GI tract.

Effect of dietary *Lactobacillus plantarum* on innate immunity in young piglets challenged with ETEC K88

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In our previous study we found that *Lactobacillus plantarum* (LP), given to piglets in early life, improved performance and effectively prevented the diarrhea in young piglets induced by enterotoxigenic *Escherichia coli* (ETEC) K88 challenge. In this study, we investigated effects of LP on innate immunity of young piglets challenged with ETEC K88. Seventy-two male piglets (4-day-old, Duroc × Landrace × Large White) were assigned to 2 diets (antibiotic-free basal diet with or without LP, 5×10^{10} cfu/kg diet) and subsequently challenged or not with ETEC K88 (1×10^8 cfu per pig) on d 15 in a 2×2 factorial arrangement of treatments. Each treatment consisted of 6 replicates with 3 piglets per pen. Intestinal segments, mucosa were collected from one piglet in each pen, real-time PCR and western blotting analysis. Statistical analysis was performed using two-way ANOVA with the GLM procedure of SAS. Results showed that in young piglets challenged with ETEC K88, gene expression of jejunal (IL-1β, IL-8, pBD-2, MUC1, and MUC4), and ileal IL-8 and MUC4 were up-regulated ($P < 0.05$); The protein concentrations of IL-8, sIgA, pBD-2 and MUC4 in jejunal mucosa and ileal sIgA concentration increased ($P < 0.05$); TLR4 mRNA and protein expression, and phosphorylation of p38 and ERK1/2 MAPK increased ($P < 0.05$), TLR2 mRNA level in jejunum decreased ($P < 0.05$). There were interactions on these significant changes of innate immune defense, p38 and ERK1/2 MAPK pathways were inhibited by dietary LP ($P < 0.05$). In conclusion, supplementation of dietary LP improved performance and effectively prevented the diarrhea in young piglets induced by ETEC K88 challenge through protecting functional integrity of intestinal barrier via TLR2 signaling and inhibiting TLR4-induced activation of p38 and ERK1/2 MAPK pathways.

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L-glutamine restores microRNA-29a induced impairment of intestinal epithelial integrity in piglets with intrauterine growth restrictionY Zhu, T Li, W Wang, G Wu and J Wang
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Intrauterine growth restriction (IUGR) impairs the small-intestinal development of neonates. The role of microRNAs (miRNAs) and their nutritional regulation in IUGR caused intestinal dysfunction remains unknown. Using a pig model, this study was conducted to identify the responsible miRNA for the intestinal damage in IUGR neonates. Then maternal glutamine supplementation was performed to evaluate its effects on miRNA and protein expressions in the small intestine of neonatal pigs. Notably, IUGR is associated with profoundly increased miR-29 family and decreased expression of extracellular matrix (ECM) and tight junction (TJ) proteins in the jejunum. *In vitro* study using the porcine intestinal epithelial cell line (IPEC-1) showed that inhibition of miR-29a expression could improve the monolayer integrity by increasing cell proliferation and transepithelial resistance. MicroRNA-29a directly targeted integrin β 1, collagen I, collagen IV, fibronectin, and claudin-1, inhibited cell proliferation, impaired epithelial integrity, and negatively correlated with glutamine level in intestinal cells. Furthermore, maternal glutamine supplementation during late gestation enhanced glutamine concentrations in maternal and neonatal plasma, suppressed intestinal miR-29a levels, and increased ECM and TJ protein abundance, resulting in improvement of piglet small intestinal growth. In conclusion, IUGR-impaired intestinal barrier function is associated with downregulated ECM and TJ protein expression mediated by the upregulation of miR-29a. These findings suggest miR-29a as a novel candidate target for small-intestinal dysfunction caused by IUGR. The work also highlights the possibility of maternal glutamine supplementation in the prevention and treatment of IUGR both in livestock and human-being.

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iTRAQ-based membrane proteomic analysis reveals intestinal development is altered by intrauterine growth restriction in pigletsS Huang, N Li, T Li, W Wang, L Jiang, Z Li and J Wang
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Intrauterine growth restriction (IUGR) impairs fetal growth and development and reduces nutrient utilization efficiency during postnatal life of pigs. Membrane proteins are of paramount importance for mediating the transport and absorption of nutrients across the plasma membrane, which is closely correlated with metabolism. In the current study, we investigated the differentially expressed membrane proteins between the jejunum of IUGR and normal-birth-weight (NBW) piglets during their early life (Days 0, 7, 21 and 35) by isobaric tags for relative and absolute quantitation (iTRAQ). A total of 351 membrane proteins were annotated and enriched from all identification of the proteins. These proteins play important roles in biological processes including metabolism, stress response, signal transduction and biological regulation. Fourteen membrane proteins (four up-regulated, 10 down-regulated) were differentially expressed between IUGR and NBW piglets. These 14 proteins participate in the intermediary metabolism of nutrients (including lipid transport, protein transport, vitamin transmembrane transport) and cell differentiation. Interestingly, the IUGR piglets had a lower abundance of DNM1, PTPRK and STX4 than the NBW piglets, which are responsible for the transmission of synapses in the enteric neurons. Besides, the loading capacity of nutrients was reduced in the jejunum of IUGR piglets, derived from the lower abundances of ABCA4 and MRS2. In summary, this is the first comprehensive analysis of the jejunum membrane proteome in neonatal piglets and the results indicate altered signaling pathways of enteric nervous system and reduced efficiency of nutrient utilization in the intestine of IUGR piglets.

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Growth performance and gut health of weaned pigs fed berberine-containing diets

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Weaning process negatively affects growth performance and gut health of pigs but inclusion of antibiotics in diets for food animals to manage gut health is being discouraged. Thus, there is a need for alternatives to antibiotics. A study was conducted to determine the effects of including berberine in weaned pig diets on growth performance and electrophysiological properties of the small intestine mounted in Ussing chambers. A total of 216 three-week-old pigs (BW = 5.5 kg) were obtained in 2 batches of 108 pigs each. Pigs were housed at 6 pigs/pen and fed one of 3 diets (12 pens/diet) for 21 days. Diets were a basal diet without or with berberine (0.06%) or antibiotics (denagard, 0.71%; chlortetracycline, 0.20%). One pig/pen was used for gut health assessment. The ADG of pigs in Batch 1 increased ($P < 0.05$) by dietary antibiotics by 32% and tended to increase ($P < 0.06$) by dietary berberine by 16.6%, whereas ADG of pigs in Batch 2 was decreased ($P < 0.05$) by dietary berberine and not affected by dietary antibiotics. Dietary antibiotics increased ($P < 0.05$) jejunal transepithelial resistance (which reflects reduced gut permeability) in Batch 1 (by 35%) but not in Batch 2. Dietary berberine but not antibiotics increased ($P < 0.05$) jejunal short circuit current (which reflects improved nutrient absorptive capacity) in Batch 1 (but not in Batch 2) by at least 74%. Evidence of diarrhea was observed in pigs fed diets containing berberine or antibiotics in Batch 2 only. In conclusion, dietary berberine improved growth performance and short circuit current in intestine of pigs in Batch 1, but not in Batch 2 likely due to confounding effects of diarrhea. Thus, dietary berberine may improve performance of weaned pigs by increasing intestinal nutrient absorptive capacity.

Effects of dietary supplementation with antibiotics or yeast glycoprotein on intestinal mucosal morphology and Hsp70 in weanling piglets

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Antibiotics have traditionally been suggested the main means for the prevention and treatment of diseases induced by early-weaning stress. However, antibiotics in feed for swine and poultry production was recently banned in many countries. This study aimed to investigate the effects of dietary supplementation with antibiotics or yeast glycoprotein (YG) on intestinal mucosal morphology and immunity in weaned piglets. A total of 240 Duroc × Landrace Large × White barrows (weaned at 23 ± 2 d of age, with an average initial body weight (BW) 6.51 ± 0.05 kg) were randomly allocated to two groups with eight pens and 15 pigs in each pen. The two treatments included feeding the barrows either a diet supplemented with antibiotics (25% Quinocetone 200 mg/kg and 4% Enduracidin 800 g/t of the diet), or a diet supplemented with YG diet (800 mg/kg YG of the diet), respectively. After 15 d feeding, one pig with average body weight in each pen was selected for blood sample collection and intestinal samples. The results showed that there were no differences in the ADG, feed intake and diarrhea rate. Compared with the antibiotics group, serum diamine oxidase decreased in the YG group ($P < 0.01$), and piglets in the YG group had lower villous height ($P < 0.01$) in the jejunum, greater villous height ($P < 0.05$) and the villous height to crypt depth ratio ($P < 0.01$). Furthermore, piglets supplemented with YG upregulated ($0.05 < P < 0.1$) the gene expression of Hsp70 in the duodenum and ileum mucosa, downregulated the gene expression of Hsp70 ($0.05 < P < 0.1$) in the jejunum mucosa. We conclude that dietary supplementation with YG could improve intestinal mucosal morphology by Hsp70, when compared with that of antibiotics.

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Dietary supplementation with plant extracts or antibiotics have different effects on intestinal mucosal morphology and immune responses in weaned piglets

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This study aimed to investigate the effects of dietary supplementation with plant extracts or antibiotics on growth performance, intestinal mucosal morphology and immune responses in weaned piglets. A total of 240 Duroc × Landrace × Large Yorkshire barrows (average initial body weight 6.69 ± 0.03 kg, weaned at 25 ± 3 d of age) were randomly allocated into two treatment groups with eight pens per treatment. The barrows were fed with either a diet supplemented with antibiotics (25% Quinocetone 200 mg/kg and 4% Enduracidin 800 mg/kg of the basal diet), or a diet supplemented with plant extracts (1000 mg/kg of the basal diet), respectively. After two weeks of feeding, one piglet per pen in each group was selected for blood sample collection and dissection. There were no differences between the diets on average daily gain, average daily feed intake, mortality rate or serum concentrations of D-lactate, diamine oxidase. Plant extracts tended to increase villous width of duodenum ($P=0.072$), decreased villous height ($P=0.039$) and villous height to crypt depth ratio ($P=0.013$) of jejunum, as well as increased villous height to crypt depth ratio ($P=0.049$) of ileum. Plant extracts tended to upregulate the mRNA expression of ZO-1 in the duodenal mucosa ($P=0.066$), upregulated the mRNA expression of ZO-1 in the jejunal ($P=0.017$) and ileal mucosa ($P=0.030$). Plant extracts upregulated the mRNA expression of IL-1 β ($P=0.013$) and IL-17 ($P=0.039$) in the duodenal mucosa, downregulated the mRNA expression of IFN- γ ($P=0.038$) in the jejunal mucosa, as well as upregulated the mRNA expression of Toll-4 ($P=0.029$) and NF-kb ($P=0.036$) in the ileal mucosa. It is concluded that dietary supplementation with plant extracts could improve intestinal mucosal morphology, ameliorate the intestinal integrity and immune responses, when compared with that of antibiotics.

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Maternal uridine supplementation reduces diarrhea rate of piglets by influencing intestinal mucosal barrier and cytokines in suckling piglets

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Nucleotides play an important role in regulation of cellular energy and protein homeostasis to facilitate repair, recovery and repletion of tissue function. It was reported that uridine monophosphate represented 98% of all the monophosphate nucleotides present in pig colostrum, and 86-90% of all the nucleotides present in pig milk, regardless of the day of lactation. This study aimed to test the effects of maternal dietary supplementation with uridine (Ur) during late pregnancy and lactation on transport and immune function of small intestinal in suckling piglets. The experiment was started from d 85 of gestation to the end of weaning. Fifty-two pregnant sows with similar parity were housed individually in gestation crates, the sows were assigned randomly to Control group (corn-soybean-based diets) or Ur group (Ur 150g/t based diet) on d 85 of gestation. Reproductive performances were recorded, and intestinal samples of suckling piglets were obtained for Real-time PCR at age of 20 days. Results showed that, compared with the control group, supplementing Ur in sow' diet decreased the birth mortality of piglets by 51% ($P<0.05$), and decreased diarrhea rate of suckling piglets ($P<0.01$). RT-PCR results showed that maternal Ur supplementation increased claudin mRNA in duodenal and jejunal mucosa of suckling piglets ($P<0.05$); maternal dietary uridine supplementation increased mRNA expression of IL-6, IL-8 and IL-1b in duodenal, jejunal and ileal mucosa of suckling piglets ($P<0.05$), and increased IL-10 expression in both jejunal and ileal mucosa ($P<0.05$), as well as enhanced mRNA expression of IKB and TLR4 in ileal mucosa ($P<0.05$) of suckling piglets. It can be concluded that maternal Ur supplementation can reduces diarrhea rate of suckling piglets by influencing intestinal mucosal barrier and cytokines in suckling piglets.

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Maternal yeast-based nucleotides supplementation during late-pregnancy and lactation induced transport and immune function of small intestine in neonate piglets

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Nucleotides play an important role in regulation of cellular energy and protein homeostasis to facilitate repair, recovery, and repletion of tissue function. Yeast-based nucleotides (YN) is an enzymatic product of yeast and is rich in nucleotides. This study aimed to test the effects of dietary supplementation with YN of sow during late pregnancy and lactation on transport and immune function of small intestine in neonate piglets. Sixty-four pregnant sows with similar parity were housed individually and randomly assigned into corn-soybean-based diets supplemented with 0g/t (CON group) or 4000g/t YN (YN group) on d 85 of gestation. Intestinal samples of neonate piglets were obtained immediately after farrowing for HE staining and RT-PCR, the number of piglets born alive, birth mortality and diarrhea rate of suckling piglets were recorded. Results showed that, compared with the control group, maternal YN supplementation increased the number of piglets born alive by 11.84% (11.71 vs. 10.47, $P = 0.03$) and decreased the birth mortality by 59% (0.34 vs. 0.83, $P = 0.03$), also decreased total diarrhea rate ($P = 0.002$); maternal YN supplementation increased the villus height of ileum ($P = 0.009$) and villus width of jejunum ($P = 0.024$) in neonate piglets; maternal YN supplementation increased mRNA expression of SGLT1 in duodenum ($P = 0.007$), while decreased claudin in duodenum ($P = 0.003$) and jejunum ($P = 0.014$), decreased ZO-1 in jejunum ($P = 0.045$) and ileum ($P = 0.018$) in neonate piglets, and increased mRNA expression of IL-17 in jejunum and IL-10, TNF- α , TLR4 in ileum of neonate piglets ($P < 0.05$). IL-10 and TNF- α are essential for the tight junction barrier of small intestinal epithelium. It can be concluded that maternal YN supplementation can improve reproduction performances of sows by inducing intestinal nutrient transport, villi development and TLR signaling pathway on innate immunity of small intestine in neonate piglets.

Enteromorpha polysaccharides-zinc complex affected gut health and the immunity of piglets during weaning period

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Both polysaccharides and zinc have been shown to have immune function and antioxidant properties. As a novel organic zinc complex, enteromorpha polysaccharides zinc (EP-Zn) complex was developed based on the assumption that the synergic effect of EP and Zn might be able to strengthen their immune function and antioxidant capacity. This study aimed to investigate whether EP-Zn could be an alternative to antibiotics in weaned piglet feeds. A total of 224 weaned piglets (d 23) from 14 pens (16 piglets/pen) were randomly assigned into an antibiotics group (400 mg olaquinox/kg basal diet and 800 mg enduracidin/kg basal diet) or an EP-Zn group (600 mg EP-Zn/kg basal diet). One piglet per pen was chosen to collect plasma and intestinal tissue samples after 14 days feeding. Results showed that EP-Zn supplement significantly increased the plasma IgG ($P < 0.05$), IgM ($P < 0.001$), GSH-Px activity ($P < 0.01$), T-AOC capacity ($P < 0.01$) and MDA level ($P < 0.001$) without affecting the growth performance of weaned piglets when compared with the antibiotics group. Histopathologic evaluations also demonstrated that EP-Zn contributed to improving the intestinal development via increasing crypt depth ($P < 0.05$), and reducing the villus length/crypt depth (V/C) ratio ($P < 0.05$) of the ileum without affecting the villus length. Interestingly, EP-Zn significantly reduced the mRNA expression of IL-6 ($P < 0.001$), IL-8 ($P < 0.05$), IL-10 ($0.05 < P < 0.1$), IL-12 ($P < 0.05$), TNF- α ($P < 0.001$) in

jejunum, and IL-12 ($P < 0.05$) in ileum, but increased HSP70 level in the jejunum ($0.05 < P < 0.1$) or ileum ($P < 0.05$) when compared with those in the antibiotics group. In all, it is speculated that EP-Zn would be efficient in improving gut health and the immunity of weaned piglets.

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Pyrimidine nucleotides metabolism and function of gut in an early-weaned piglets model: effects of a uridine monophosphate or uridine diet

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Uridine monophosphate (UMP), as the richest nucleotide in milk of sows, is insufficient for earlier weaning period of piglets. The study probing into the function of UMP in sow's milk was conducted to evaluate the effects of oral administration with UMP and its gastro-intestinally metabolic product uridine (UR) on intestinal pyrimidine nucleotides metabolism, morphometry and intestinal health of early weaning piglets. Twenty-one Duroc × Landrace × Yorkshire piglets (weaned at 7 days old) were randomly allotted into 3 groups with 7 replicates and received milk replacer ad libitum. The piglets were given 0 mg UMP nor UR (control group), 476mg UMP (UMP group) and 348mg UR (UR group) per day for 10 days, respectively. All the piglets were sacrificed for blood and intestinal samples collection. Results showed that UMP and UR supplement increased ($P < 0.05$) the average daily gain (ADG) of piglets, and significantly decreased the diarrhea rate ($P < 0.01$) as well as diarrhea index ($P < 0.05$) when compared with those in the control group. Oral UR daily increased villus length/crypt depth (V/C) ($P < 0.05$) of jejunum compared to the control group. Compared with the control group, mRNA levels of pyrimidine nucleotide metabolic enzymes including UMPS, CMPK1, RRM2, UPRT, CTPS1 and CTPS2 increased in duodenal mucosa ($P < 0.05$) in the UR group; both UMP and UR decreased the relative mRNA levels of CAD and RRM2 at jejunal mucosa and CTPS1 at ileal mucosa respectively ($P < 0.05$). Compared to the control group, oral UR increased the relative expression levels of Claudin-3 and E-cadherin of duodenum mucosa ($P < 0.05$). These results indicated that oral UMP or UR could affect the small intestinal morphology, improved the intestinal development and health, and thus improved growth performance of weanling piglets.

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A comparative analysis for the growth performance and intestinal development of weanling piglets affected by UMP and UR supplements

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It is widely accepted that dietary supplementation with nucleotides could improve growth and development of intestine. However, little research has been conducted to show the effects of uridine 5'-monophosphate (5'-UMP) or uridine (UR) on the intestinal barrier of weaning piglets. This study aimed to evaluate the effects of UMP or UR on the intestinal development of weanling piglets. A total of 108 piglets (Large White × Landrace × Duroc) aged at 21 days were randomly assigned into 18 pens (6 piglets/pen), which were fed the three diets: 1) Piglets fed a basal diet formulated to meet nutrient specifications for weaned piglets (NRC 2012, Control), 2) Piglets were fed a basal diet containing 0.06% (wt/wt) UMP-Na₂ (UMP-Na₂ group), 3) Piglets were fed a basal diet containing 0.045% (wt/wt) UR (UR group). However, nonsignificant difference was observed in ADFI, ADG, F/G and diarrhea incidence

Abstract

($P > 0.10$). Plasma biochemical indexes showed that UMP- Na_2 significantly increased plasma Glu ($P < 0.05$), and UR had a trend ($0.05 < P < 0.10$) to reduce the plasma TC of piglets when compared with those in the other two groups. Moreover, UR supplementation exhibited an increased crypt depth in duodenum and ileum ($P < 0.05$), whereas the villus length and V/C ratio didn't reach the significant difference level ($P > 0.10$). Further study showed that the relative mRNA level of ZO-1 in the duodenum and jejunum ($P < 0.05$) from UR group, and occludin in the duodenum from UR and UMP- Na_2 groups were increased when compared with that in the other group. In conclusion, these results indicated that UR was more conducive to improving the intestinal development of weanling piglets than UMP.

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Activation of endoplasmic reticulum stress signaling in jejunum of early-weanling piglets

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The endoplasmic reticulum (ER) is the major organelle for the synthesis, folding and modifying the secretory and membrane proteins. Accumulation of unfolded or misfolded proteins in the ER lumen activates unfolded protein response to survival unfavorable conditions and restore homeostasis in both humans and animals. It is well known that weaning stress is associated with reduced growth performance due to impaired intestinal barrier function and nutrients absorption in piglets. However, a functional role of ER in the intestine of early-weaned piglets remains elusive. In our study, piglets were weaned at 21-days of age and fed with a soybean-corn basal diet formulated for weaner piglets according to NRC (2012). Piglets were slaughtered on 24-days of age for intestinal sample collection. Age-matched suckling piglets were used as controls. The data showed that the proteins abundances for BiP, ATF6 α , p-IRE1 α , and p-eIF2 α (proteins involved in unfolded protein response) and apoptosis-related proteins, such as CHOP, p-JNK, caspase12, cleaved caspase3, BAX and Bcl-2, were enhanced in jejunum as compared with that of controls. Quantitative real-time PCR analysis indicated that the mRNA level of inflammatory cytokines, including *IL-6*, *IL-8*, *IL-1 β* , were enhanced in jejunum tissue of weanling piglets. In addition, we found that the mRNA level of *FBXO32* and *MuRF-1*, genes implicated in protein degradation were greater in jejunum of weanling piglets. Taken together, we showed that the ER function is impaired in jejunum of weanling piglets, as evidence by increased proteins associated with unfolded protein response and enhanced inflammatory cytokines. Restoration of ER function might be a potential strategy to improve intestinal development of weanling piglets.

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Effects of dietary niacin on intestinal morphology and diarrhea of weaning piglet

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Introduction Vitamin deficiency is one of the important causes of intestinal dysfunction in piglets. Dietary vitamin is an important mean to improve intestinal function of piglets. The present study was conducted to determine the intestinal morphology and diarrhea of weaning piglets fed diets with different contents of niacin.

Material and methods Forty-eight piglets were weaned at 21 d of age and randomly assigned into 1 of 4 treatments, consisting of a basal diet supplemented with 22.5, 30, 45 or 75 mg/kg niacin, for a 14-d period with 12 pens (1 piglet per pen) per treatment. Six piglets were randomly slaughtered for tissue sampling on 7 and 14 d post-weaning, respectively.

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Results There are no significant differences in growth performance among the treatments, but dietary supplementation with 75 mg/kg niacin increased ($P < 0.05$) diarrhea rate compared with other groups from d0 to d14. Compared with 22.5 and 30 mg/kg niacin, dietary supplementation with 45 mg/kg niacin reduced ($P < 0.05$) duodenal villus height on d7. Compared with 22.5 and 30 mg/kg niacin, dietary supplementation with 45 or 75 mg/kg niacin reduced ($P < 0.05$) jejunal crypt depth on d7, while increased ($P < 0.05$) jejunal villus height and crypt depth on d14. In ileum, dietary supplementation with 45 mg/kg niacin reduced ($P < 0.05$) villus height and crypt depth compared with 22.5 and 30 mg/kg niacin from d0 to d7.

Conclusions Dietary supplementation with high niacin increases diarrhea rate and affects intestinal morphology of weaning piglets.

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Effects of folic acid in antibiotic-free diets on growth performance and diarrhea of weaned piglets

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Introduction The National Research Council (2012) recommendation of folic acid for weaning piglet is 0.3 mg/kg, which is lower than the commercial recommendation. There are evidences that oral administration of folic acid significantly shortened the duration of animal diarrhea. This study was conducted to determine the effect of dietary folic acid on growth performance and diarrhea of weaning piglets.

Material and methods A total of 28 piglets were weaned at 21 d of age and randomly allotted to 1 of 4 treatments, with 7 replicates per treatment and 1 piglet per replicate. The treatments consist of a basal diet (without antibiotics) and the basal diet supplemented with 3, 9, or 18 ppm folic acid. Feed intake and fecal score (0 = dry and hard feces, to 3 = liquid diarrhea) were determined every day. On d 14, the body weight of each piglet was measured.

Results Dietary supplementation with folic acid enhanced ($P < 0.05$) the average daily gain and average daily feed intake of weaned piglets compared with the control group. The diet with 9 ppm folic acid significantly decreased ($P < 0.05$) the fecal score compared with the basal diet and 18 ppm folic acid supplemented diet during the 14 d post-weaning.

In conclusion, the results of this experiment indicate that dietary supplementation with folic acid benefits growth performance and gastrointestinal health of weaning piglets when fed antibiotic-free diets.

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Effects of dietary protein on diarrhea and intestinal inflammation of weaned piglets

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Introduction Much attention had been paid to the post weaning diarrhea. However, the effect of dietary protein on diarrhea and intestinal inflammation has not been entirely understood. The present study was conducted to explore the effects of high protein diet on diarrhea and intestinal inflammation of weaned piglets.

Materials and methods A total of 16 piglets (Duroc × Landrace × Yorkshire; 35 d of age) with an average body weight of 9.47 ± 0.22 kg were randomly assigned into 1 of 2 treatments consisting of diets with 18% crude protein (LP) or 26% crude protein (HP). The piglets were slaughtered for intestinal tissue sampling when obvious and steady diarrhea symptoms occurred.

Abstract

Results There were no significant differences in average daily gain and feed conversion ratio between the 2 groups, but dietary HP tended to increase ($P < 0.10$) the average daily feed intake. The HP group had greater ($P < 0.01$) diarrhea rates than LH group. Moreover, dietary HP significantly elevated ($P < 0.05$) the number of goblet cells in ileal villi and colonic crypt of piglets. Compared with the LP treatment, the myeloperoxidase content was also significantly increased ($P < 0.01$) in ileum and colon of piglets from HP treatment. In addition, the relative abundance of LGALS3 in ileum and colon of HP piglets was also significantly elevated ($P < 0.05$) compared with that in LP piglets.

Conclusions High protein diets contribute to the diarrhea of piglets by inducing intestinal inflammation.

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Effects of dietary sulfur amino acids on serum biochemical variables, mucosal amino acid profiles, and intestinal inflammation in weaning piglets

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Introduction Sulfur amino acids (SAA) play critical roles in biological functions and protection against diseases. We hypothesized that SAA may affect metabolism and intestinal inflammation of weaning piglets.

Material and methods Forty piglets (Duroc × Landrace × Yorkshire) were weaned at 21 d of age and randomly assigned to 1 of 5 diets that contained 0.53, 0.63, 0.74, 0.85, or 0.96% SAA, which corresponded to 70, 85, 100, 115, or 130% of the SAA:Lys ratio recommended by National Research Council (NRC, 2012). At the 35 d of age, all the piglets were fasted overnight and slaughtered for blood and tissue sampling. Biochemical variables were measured using an analyzer (Beckman CX4 Chemistry Analyzer; Beckman Coulter, USA). Jejunal mucosa amino acid contents were determined by Hitachi L-8900 automatic amino-acid analyzer. Mucosal gene expression was conducted by RT-PCR for tumor necrosis factor alpha (TNF- α), transforming growth factor- β (TGF- β), IL-1 β , cysteine desulfurase (NSF1), and cystathionine beta-synthase (CBS).

Results Dietary supplementation with SAA significantly enhanced ($P < 0.05$) serum creatinine and low density lipoprotein. Serum glucose was reduced ($P < 0.01$) and serum total cholesterol and triglycerides tended to be decrease ($P < 0.1$) in response to dietary SAA supplementation. No significant difference was observed in jejunal mucosal amino acid profiles, but dietary supplementation with SAA lowered ($P < 0.05$) jejunal content of Ala and tended to decrease ($P < 0.1$) the content of His. Dietary SAA reduced ($P < 0.05$) the mRNA expression of genes involved in SAA metabolism (NSF1 and CBS), as well as genes related to inflammation (TNF- α , TGF- β , and IL-1 β).

In conclusion, dietary SAA affects lipid and intestinal SAA metabolism, and alleviates intestinal inflammation of weaning piglets.

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Effects of dietary vitamin B6 on the growth performance, intestinal morphology and inflammation response in weaned piglets

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Vitamin B6 (VB6) plays an irreplaceable role in animal's health. However, there were few studies focused on the effects of VB6 on intestinal health of weaned piglets. This study was conducted to investigate the effects of dietary VB6 on growth performance, intestinal morphology and inflammatory cytokines expression in weaned piglets. Twenty-four piglets (Landrace × Large White × Duroc) weaned at 21 d of age (7.03 ± 0.15 kg body weight) were randomly assigned to 1 of 4

treatments that contained 0 (V0), 2 (V2), 4(V4), or 7 (V7) mg/kg VB6 for 14 d. The growth performance, intestinal morphology, and mRNA expression of inflammatory cytokines (IL-1 β , IL-10, TNF- α , and TGF- β) were determined. The results showed that the average daily feed intake, the gain to feed ratio and diarrhea ratio were not significantly affected by the dietary treatments, while the average daily gain of group V0 and V7 was greater ($P < 0.05$) than other groups. No significant differences ($P > 0.05$) in villus height, crypt depth, and the ratio of villus height to crypt depth in jejunum were observed. However, ileal crypt depth for group V2 was reduced ($P < 0.01$) compared with V0 and the ratio of villus height to crypt depth for group V4 was lower ($P < 0.05$) than V2. In jejunum, the mRNA expression of IL-10 and TGF- β for group V2 and V4 was significantly decreased ($P < 0.05$) compared with group V0. In ileum, the mRNA expression of IL-1 β in group V7 was significantly increased ($P < 0.05$) compared with V0. The mRNA expression of TNF- α and TGF- β in group V4 was significantly increased ($P < 0.05$) compared with V2. In conclusion, dietary supplementation with VB6 affects ileal morphology and intestinal inflammation in weaned piglets.

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Swine-derived *Lactobacillus reuteri* LR1 modulates the activation of M2 macrophages in the intestine of weaning piglets

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Probiotics are reported as antibiotic alternatives to improve intestinal health of weaning piglets. We previously found a strain of swine-derived *Lactobacillus reuteri* (*L. reuteri*), LR1, to significantly improve intestinal barrier integrity over in-feed antibiotics in piglets. This work further investigated the effect of LR1 on innate immunity, specifically on the activation of classical (M1) and alternative (M2) macrophages within the intestine of piglets. A total of 144 male piglets (25 d old) were assigned to 3 groups (8 replicate pens of 6 piglets), provided with antibiotic-free basal diet (the control) or basal diet added with antibiotics (Olaquinox and aureomycin at 100 and 75 mg/kg diet) or LR1 (5×10^{10} CFU/kg diet). On both d 14 and 42, 1 piglet from each pen was sacrificed for intestine sampling. On d 14, antibiotics and LR1 similarly up-regulated the mRNA abundance of macrophage markers CD68 and F4/80 (all $P < 0.01$) in ileum when compared with the control, while having no difference in affecting the abundance of M1 or M2 macrophage markers. Likewise, mRNA abundance of CD68 and F4/80 were elevated by both antibiotics and LR1 in the colon (all $P < 0.001$). Moreover, the mRNA and protein abundance of M2 macrophage markers, Arg-1, IL-10 and Lamtor1, were increased by LR1 in colon when compared with either control or antibiotics (all $P < 0.05$). On d 42, no changes in mRNA or protein abundance of the macrophage markers were observed in ileum among groups. However, compared with either the control or antibiotics, LR1 significantly increased mRNA and protein abundance of CD68 and F4/80, as well as those of Arg-1 and IL-10 in colon (all $P < 0.05$). Collectively, *L. reuteri* LR1 preferentially activate M2 macrophages in the intestine, which might favor wound repair and barrier development in the intestine of weaning piglets.

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Responses in gut permeability, nutrient digestibility and growth performances in weanling pigs fed the diet supplemented with aureomycin

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Aureomycin is widely used as a growth promoter in commercial weanling swine diets even though its modes of actions are largely unknown. A total of 104 crossbred (barrows) were weaned at d 19, with an average initial BW of 6.81 kg; and were randomly assigned to two groups according to a randomized complete block design. Both groups of pigs were fed a corn and SB-based diet for 3 weeks; and the antibiotic-treatment diet was supplemented with 550 mg

Abstract

aureomycin per kg diet. Titanium dioxide was added at 0.3 % as a digestibility marker. Pigs had free access to water and feed for 22 days. Pigs were weighted and received an oral bolus D-mannitol (0.3 g/kg BW) 4 h prior to collect the plasma every week. The results showed that pigs fed with the dietary antibiotic increased (0.350 ± 0.016 vs. 0.295 ± 0.015 kg/d, $P < 0.05$) the average daily gain by 19% during d 0-22, and increased (0.17 ± 0.01 vs. 0.13 ± 0.01 kg/d, $P < 0.05$) the average daily feed intake by 31% during d 0-7. However, the apparent total tract digestibility of dry matter in the pigs fed with antibiotic was decreased (77.84 ± 0.65 vs. 82.29 ± 0.80 %, $P < 0.05$) by 6%. Compared with the day-1 weaning baseline, in vivo gut permeability was higher ($P < 0.05$) during the weeks 1 and 2 weaning; and then became lower ($P < 0.05$) at the end of week-3 weaning in the antibiotic-fed pigs compared with the control. Results of this study indicated that feed antibiotic use improved the growth performances with decreased in vivo gut permeability as an adaptive response; however, reduced total tract dry matter digestibility was not a contributing factor to the feed antibiotic-associated growth promotion in the weanling pig.

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The effect of spirit-based distillers' grains on the intestinal microbes in piglets

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Due to the huge annual output of distiller's grains in China, the development and utilization of Spirit-based distillers' grains (SDGS) is not only an effective way to solve the shortage of feed resources, but also an important means to realize the transformation of waste resources into treasure, the development of recycling economy, and the reduction of environmental pollution. SDGS is a new feed ingredient, abundant at amino acids nutrients and contains a large amount of vitamins, enzymes, organic acids, etc. With the lack of research on affection of SDGS on the metabolism, growth and colonization of microbes in the intestine, this study evaluated the changes in intestinal microbes of piglets in response to SDGS. A total of 24 weaning pigs were randomly allocated into a 4×2 factorial design and fed with a basal diet or a 4% adjunction of SDGS in dietary. The piglets were weaned at 21 days and slaughtering after 30 days feeding. Samples of intestinal contents collected 30 days later and microbial communities were analyzed. The results showed that the alpha diversity of a 4% adjunction of SDGS in dietary higher than those of CON ($p = 0.039$) in jejunum. The beta diversity analysis also showed that, a 4% adjunction of SDGS in dietary improve the difference in species complexity, as beta diversity of jejunum and Ileum from 0.431 to 0.649. The colonization of *Mycoplasma* (reduce 82%) and *Clostridium* (reduce 51%) in jejunum was significantly inhibited with a 4% adjunction of SDGS in dietary. The same results showed in Ileum. Meanwhile it greatly promoted the colonization of *Prevotella* (increase 76%) and *Ruminococcus* (increase 82%), and inhibited the colonization of *Streptococcus* (reduce 78%) in jejunum. Moreover, a 4% adjunction of SDGS in dietary promoted the colonization of *Lactobacillus* (increase 63%) and *Peptostreptococcus* (increase 59%, $P = 0.005$) in Ileum. In conclusion, our results suggest that, a 4% adjunction of SDGS in dietary greatly influence the intestinal microbiota composition of weaning pigs. It improve the microbiota diversity and the species complexity in jejunum. Also, SDGS have an actively effect on the digestive ability and healthy of jejunum and ileum.

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Dietary fatty acid patterns affect intestinal health of weaning piglets

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Lipids are important energy resources and fatty acids play a vital role as building blocks for lipid synthesis in animals. The fatty acids composition of sows milk is likely matched to optimise intestinal growth of piglets. Oil emulsification is one strategy that improves oil stability, solubility and flavor and may improve the value of dietary vegetable oils and we hypothesize that providing a higher diversity of fatty acids similar to sow milk combined with oil emulsification could improve intestinal health. Eighteen weaned piglets at 21 days (5.47 ± 0.32 Kg) were randomly allocated to one of three dietary groups and were fed individually for 2 weeks with water freely available via nipple drinkers. The dietary treatments were weaner diets containing either 2% soybean oil, compound oil (containing 0.64% soybean, 1.2% palm and 0.16% coconut oils) or emulsified compound oil. The unsaturated fatty acid percentage of soybean oil was 84.9%, whereas the compound oil was 60.2%. All other nutrients were the same and designed to meet the demands of piglets. After 2 weeks of feeding the pigs were slaughtered and Intestinal segments and chime were collected for analysis. The ratio of villus length and recess depth greatest in the duodenum of pigs fed soybean oil whereas this ratio was higher in the jejunum of pigs consuming the compound oil. Bacterial diversity in the jejunum as indicated by OTUs ($P=0.041$), chao1 ($P=0.041$) and ACE ($P=0.05$) was greatest in pigs consuming the soybean diet. At the level of genus, fatty acid diversity improved the colonization of *Clostridium* in the jejunum and ileum as the richness was highest (8.56% and 3.81%) in pigs consuming the compound oil. Emulsification of oil improved growth of *Lactobacillus* since richness in the jejunum and ileum are 62% and 33% higher than the other two diets at the level of both genus and family. Prevotellaceae mainly existed in the cecum and colon and was improved by oil emulsification. In conclusion, these data showed that higher unsaturated fatty acid contents could improve the health status of duodenum while fatty acid diversity could improve gut health status by improving intestinal morphology and intestinal microbial composition diversity.

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Effects of dietary carboxymethylpachyman supplementation on oxidative stress induced by diquat in weaned piglets

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The carboxymethylpachyman(CMP) is a polysaccharide abstracted from medicinal fungi *poria cocos*, which has antioxidant, anti-inflammatory and immunoregulatory activity. In this study, we studied the effects of carboxymethylpachyman on the oxidative stress induced by diquat in weaned piglets. A total of 40 weaned piglets (landrace \times large \times white) with similar weight (6.7 ± 0.50 kg) were randomly divided into four groups ($n = 10$) according to a 2×2 factorial design: control (basal diet), negative control (9 mg diquat/kg BW injected to piglets fed with basal diet), and basal diet treatments containing CMP group in which piglets were fed 0.002% CMP, and CMP diquat –induced group in which piglets were fed 0.002% CMP and injected with 9 mg diquat/kg BW. Growth performance were monitored and blood samples were collected to determine antioxidative and immune parameters. The results show that the supplementation of CMP can significantly improve growth performance, including body weight, average daily weight gain, and feed intake ($P < 0.05$), significantly reduce the diarrhea rate ($P < 0.01$). Meanwhile, the concentration of IgG in the serum of CMP group, CMP diquat-induced group and negative control group was significantly higher than that in the control group ($P < 0.05$). The UREA concentration in serum can be increased by the injection of diquat, but the concentration of UREA in CMP diquat-induced group was significantly lower than that in the negative group ($P < 0.05$). Furthermore, the addition of CMP can significantly improve the anti-oxidant factors activity of catalase (CAT), glutathione peroxidase (GSH-PX), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) ($P < 0.05$), and significantly elevated malonaldehyde (MDA) concentration in serum ($P < 0.05$). The results indicated that dietary supplementation with CMP confers beneficial effects on diquat-induced oxidative stress in piglets.

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Effect of oral supplementation of *Lactobacillus plantarum* JCM1149 on growth performance, diarrhea incidence, and intestinal health in growing pigsY Yin, C Hurtado¹, Y Martínez² and G Liu²¹University of Córdoba, Faculty of Veterinary Medicine and Zootechnic, Department of Livestock Sciences; ²Institute of Subtropical Agriculture, Chinese Academy of Sciences

The growth promoters of antibiotics in farm animals are still used in many countries. Some *Lactobacillus* spp. are used as probiotics in pig diets, which is important to maintain acceptable levels of performance and ensure the safety of food free of antibiotic residues for consumers. Oral use with *Lactobacillus plantarum* may stimulate beneficial intestinal bacteria and nutritional metabolic process and therefore improving the gut health and growth performance in growing pigs. A total of 120 (Landrace x Duroc x Pietrain) pigs, at five weeks of age were randomly allotted to three treatments during 77 days. At the end of the experiment, immune and visceral organ weights, hematology parameters, blood biochemical and gut morphometry were measured. The treatments were: the basal diet, oral supplementation of 5×10^6 of *Lactobacillus plantarum* JCM1149 (T1) and 0.2 g/kg of dietary ciprofloxacin (T2) ($n = 40$ per group). Data were subjected to analysis of variance (ANOVA) and Duncan multiple range test was done. *L. plantarum* improved ($P < 0.05$) average daily gain, body weight and feed/gain ratio ($P < 0.05$), and reduced ($P < 0.05$) the diarrhea incidence compared with the non-additive and antibiotic groups. In addition, this probiotic increased the small intestine length and the relative weight of the cecum and spleen. Results indicated that the *L. plantarum* decreased ($P < 0.05$) the serum contents of cholesterol, triglycerides, glucose, β -hydroxybutyrate and blood urea nitrogen with relation to the control, without changing the hemogram of these pigs. In the cecum, T1 caused an increase in muscular and mucosal thickness, and lactic acid bacteria, without varying the **crypts depth**. These findings indicate a beneficial effect of oral use with *Lactobacillus plantarum* JCM1149 on body weight, diarrhea syndrome, intestinal health, immune and intestinal organs, harmful lipids and serum glucose, which may offer an effective alternative to antibiotics for growing pigs.

The effect of benzoic acid and essential oils on nutrient digestibility and colonic microbiota in piglets

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Previous trials showed consistent improvement in growth performance in piglets supplemented with a combination of benzoic acid and essential oils (thymol, eugenol, and piperine), while the underlying mechanisms were not fully investigated. Therefore, the current trial was conducted to explore this additive's effect on nutrient digestibility and colonic microbiota. The hypothesis was that benzoic acid and essential oils could improve nutrient digestibility and modulate the composition and function of colonic microbiota. Twenty-four barrows and gilts were assigned to two levels of benzoic acid and essential oils: 0 and 2 g/kg feed, 12 piglets per treatment. Titanium dioxide was used as an indigestible marker to determine the total tract digestibility of dry matter, gross energy, and crude protein, and apparent ileal digestibility of amino acids. Colonic microbiota was categorized by the 16s rRNA sequencing technique. The results showed that the supplementation of benzoic acid and essential oils decreased the abundance of phylum of *Bacteroidetes* ($p = 0.05$). In the phylum of *Bacteroidetes*, the abundance of genera of *Prevotellaceae* *Prevotella* ($p = 0.06$) and *Paraprevotellaceae* *Prevotella* ($p = 0.04$) decreased, and the genus of *unclassified* S24-7 increased ($p = 0.01$). Principal coordinate analysis based on Bray-Curtis distance could separate the microbial communities between the control and treatment groups ($p = 0.02$). The microbial function analysis showed that the dietary supplementation positively affected the metabolism of carbohydrate, amino acids, and lipoic acid, and degradation of limonene, pinene, and caprolactam ($p < 0.05$), and suppressed the NOD-like receptor signaling pathway as a component of immune system ($p < 0.05$). In conclusion, the results in the current trial proved the modulatory effect of benzoic acid and essential oils on colonic microbiota composition and functions, while the effect on energy and nutrient digestibility was not detected.

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The protective effect of resveratrol against oxidative stress injury induced by deoxynivalenol in intestinal porcine epithelial cells

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Deoxynivalenol (DON) is a common mycotoxin that affects human health and animal production owing to its high toxicogenic potentials. Resveratrol has various activities including antioxidant, anti-inflammatory and chemopreventive effects. Whether resveratrol can protect intestinal porcine epithelial cells against DON induced oxidative stress remained unclear. Here, the established intestinal porcine epithelial cell line (IPEC-J2) was used to test the hypothesis that resveratrol might prevent the IPEC-J2 cells from oxidative stress injury induced by DON. Cells were pretreated with 15 μ M resveratrol for 24 h followed by incubation with 0.50 μ g/mL DON for 12 h. Cell viability, cell proliferation, cell apoptosis, gene expression, and oxidative stress associated indicators were determined. Data were subjected to two-way ANOVA analysis using SPSS (18.0) software. The results demonstrated that pretreatment of cells with resveratrol prior to DON exposure improved ($P < 0.05$) cell viability, cell proliferation and mitochondrial membrane potential, and attenuated ($P < 0.05$) DON induced cell cycle arrest. Moreover, pretreatment of resveratrol decreased ($P < 0.05$) intracellular reactive oxygen species (ROS) production and activation of caspase-3, and increased ($P < 0.05$) the ratio of Bcl-2/Bax, thereby preventing cell apoptosis induced by DON exposure. Furthermore, resveratrol dramatically induced ($P < 0.05$) the nuclear translocation of nuclear factor-erythroid 2-related factor-2 (Nrf2) in IPEC-J2 cells. Resveratrol also upregulated ($P < 0.05$) the mRNA and protein expression of Nrf2 target genes Cu/Zn-superoxide dismutase (SOD1), the catalytic (GCLC) and modulatory (GCLM) subunits of γ -glutamylcysteine ligase in IPEC-J2 cells exposed to DON. In addition, small interfering RNA (siRNA) knockdown of Nrf2 expression abrogated ($P < 0.05$) the protective effects of resveratrol on IPEC-J2 cells against DON induced cytotoxicity, oxidative stress, and mitochondria-dependent apoptosis. These results suggest that resveratrol protects IPEC-J2 cells against DON induced oxidative stress injury at least partly via Nrf2 pathway.

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Use of essential oils as an alternative to antibiotic growth promoters in piglets

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Bacterial resistance to antibiotics (AGPs) is increasing and thus their use as growth promoters limited. Essential oils (EO) stand as a natural alternative due to their beneficial effects in performance and gut health. The objective of this study was to assess the effect of EO-FIT[®] Swine (NOREL), which contains carvacrol, thymol, cinnamaldehyde and clove, as an alternative to AGPs in piglet diets. A group of 240 piglets weaned at 28d were assigned to 3 treatments with 8 replicates each. T1 – Negative Control NC (basal diet), T2 – Positive control PC (NC + colistin at 120mg/kg + zinc oxide at 3000ppm), T3 – NC + EO at 0.5kg/ton. Animals were weighed at 28d, 42d and 70d of age. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were determined globally and for each period. Results were analyzed by one-way ANOVA using GLM procedure of SSPS v. 24.0. At the end of the trial (70d), animals from the positive control (PC) were 13.33% (23.8^akg) significantly heavier ($P < 0.0001$) than those from the negative control (NC) (21.0^bkg) and EO treatment (5.71%, 22.2^bkg). Globally (from 28d to 70d) no differences were observed regarding ADFI. PC (T2) achieved better figures in ADG ($P < 0.0001$) than the EO group (T3) and the NC (T1). Pigs treated with EO had a significantly better FCR ($P = 0.0006$) than the NC and similar to the PC (1.58^a, 1.73^b and 1.50^a respectively). That is a consequence of a significantly higher ADG ($P = 0.0006$) during the starter period (43d to 70d) for pigs treated with EO (416.9^ag/d) compared to the NC (375.3^bg/d), being statistically similar than the PC (442.2^ag/d). These results confirm the potential of essential oils as natural growth promoters, although further investigation is required to increase their effects.

Reducing body weight loss during lactation in sows: a meta-analysis on the use of a non-starch polysaccharide-hydrolyzing enzyme supplement

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A meta-analysis was performed on 8 trials, which included a total of 949 parity 1 to 8 lactating sows, to evaluate the effects of feeding xylanase (Rovabio[®] Excel, Adisseo, France) supplement throughout lactation on sow performance factors: body weight loss, feed intake, backfat depth and piglet growth. Even a short period of enzyme supplementation during lactation led to a reduction in body weight loss of approximately 3 kg per sow ($P = 0.003$). This reduction represented 1-2% of the body weight of sows. This effect is explained by an increase in feed energy intake and enhanced feed digestibility. Sows fed enzyme-supplemented diets exhibited greater dry matter, organic matter and gross energy digestibilities (3.4, 3.9, and 4.2% increases, respectively; $P < 0.001$) than sows fed control diets. During lactation, sows lost from 19 to 25 kg of BW (i.e., approximately 10% of BW), with a difference between parity groups ($P < 0.001$). Body reserve mobilization was decreased in sows fed enzyme-supplemented diets (-2.9 kg, $P = 0.003$), with a more pronounced effect in primiparous than multiparous sows when body weight loss is expressed relative to total body weight (-2.27 vs -0.59%, respectively; $P = 0.058$). Enzyme supplementation also increased litter weight gain up to weaning, with a greater effect in litters from multiparous sows than those from primiparous sows (5.4 vs 0.6 kg, respectively; $P = 0.009$). Results could be explained in part by the relationship between their net energy (NE) intake and either variations in body weight or litter weight gain ($R^2 = 0.51$ and 0.49 , respectively; $P < 0.001$). Finally, the meta-analysis suggests that there are differences in the partitioning of the NE intake between growth and milk production and in relation to the sow's parity. Extra energy released by enzyme is used for one of these functions.

Effects of increased amino acids supply as protein or free amino acids on performance and carcass of heat stress pigs

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Exposure to high ambient temperature (AT), i.e. > 30 °C reduces voluntary feed intake (VFI) and growth of pigs. Increased dietary supply of amino acids (AA) by adding free AA may be helpful to compensate the negative effect of heat stress (HS). Two 21-day experiments were conducted with growing (Landrace/Hampshire \times Duroc) pigs. In Exp. 1, 30 pigs (initial BW 31.1 ± 1.2 kg) were used to determine the effect of HS on pig performance. Dietary treatments included: thermos-neutral pigs fed a 22% CP control diet (TN-C); HS pigs fed the control diet (HS-C); HS pigs fed a 14% CP, AA supplemented diet (HS-AA). Compared with TN-C, HS pigs had lower ($P < 0.05$) ADG and Lys utilization efficiency. In Exp. 2, 25 pigs (initial BW 33.6 ± 0.65 kg) were used to evaluate the effect of extra dietary AA supply either as protein-bound or free AA on performance and carcass traits of HS pigs. Diets included: wheat-soybean meal-free Lys, Thr and Met control (CON); CON + 30% more CP (HSxCP); CON + 25% more AA by adding free AA (HSxAA). Daily feed and NE intake ($P > 0.10$) were not affected, but Lys, Thr, and Met intake were higher ($P < 0.05$) for HSxCP or HSxAA compared with CON. ADG was not affected ($P > 0.10$) but G:F tended to be higher ($P < 0.10$) for HSxCP than CON pigs. HSxAA pigs had higher ADG ($P < 0.05$) than CON pigs. Loin muscle weight was higher ($P < 0.05$) for HSxAA than HSxCP pigs. Kidney weight and serum urea in HSxCP pigs were higher ($P < 0.05$) compared with CON and HSxAA pigs. These results showed that HS reduces the VFI, and increased AA supply using free AA rather than protein-bound AA better ameliorate impaired performance of HS pigs.

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Estimation of body composition of piglets at weaning using live weight and deuterium space

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Piglet body fat at weaning is important for the post-weaning growth performance. This study aimed to develop equations that predict body composition of four wk old piglets using deuterium oxide (D₂O). A total of 42 piglets were weighed and enriched with D₂O at d 28 (weaning) and blood was sampled to assess D₂O space. Piglets were euthanized and the entire gastrointestinal tract was emptied for digesta. Next, the carcass including organs was grinded to obtain a homogenous mass, which was analyzed according to standard procedures for content of dry matter, ash, and protein (N × 6.25), whereas body fat content was calculated by difference. Piglet live weight (LW; mean and range) was 8,527 g [5,747;11,797], and D₂O space 5,818 g [3,948;7,846], while the mean carcass pools [and ranges] were 290 g ash [198;392], 1,350 g protein [927;1,858], 5,550 g water [3,724;7,366], and 1,337 g fat [631;2,206]. Linear regression models to predict body pools from LW and D₂O space were as follows: **ash-pool**, $g = 44 (\pm 38; P = 0.26)$, $g + 0.020 (\pm 0.028; P = 0.47)$, $g/g \times LW$, $g + 0.013 (\pm 0.044; P = 0.76)$, $g/g \times D_2O \text{ space}$, g , ($R^2 = 0.60$), **protein-pool**, $g = -5 (\pm 47; P = 0.91)$, $g + 0.148 (\pm 0.034; P < 0.001)$, $g/g \times LW$, $g + 0.017 (\pm 0.054; P = 0.76)$ $g/g \times D_2O \text{ space}$, g , ($R^2 = 0.97$), **fat-pool**, $g = -300 (\pm 118; P < 0.05)$ $g + 0.666 (\pm 0.086; P < 0.001)$, $g/g \times LW$, $g - 0.694 (\pm 0.136; P < 0.001)$, $g/g \times D_2O \text{ space}$, g , ($R^2 = 0.92$), and **water-pool**, $g = 262 (\pm 96; P < 0.01)$, $g + 0.167 (\pm 0.070; P < 0.05)$, $g/g \times LW$, $g + 0.664 (\pm 0.111; P < 0.001)$, $g/g \times D_2O \text{ space}$, g , ($R^2 = 0.99$). The D₂O space improved prediction of body water and body fat but not prediction of body protein and body ash. This is a powerful technique to evaluate the effect of sow nutrition and energy intake of suckling piglets on body composition at weaning.

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Alternatives to medicinal zinc for weaner pigs

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A very large part of zinc absorbed through the feed is excreted in the manure. The supply of zinc to the farmland should be reduced for environmental reasons and the risk of causing MRSA in pigs. A majority of Danish piglets are fed 2,500 ppm medicinal zinc to reduce diarrhoea after weaning, but this will stop in 2022 due to an EU ban. The need for alternatives are therefore urgent. A trial to test three alternative products (seaweed, probiotics and yeast/probiotics) was conducted, including six groups, 60 replicates (pens) and a total of 4,500 piglets.

The weaner pigs were fed either 2,500; 1,500 or 0 ppm of medicinal zinc for 14 days after weaning or given three alternative products throughout the period from seven to 30 kg. These alternative products were based on literature supposed to improve gut health and reduce diarrhoea frequency and thereby improve productivity compared to the 0 ppm zinc group.

The allocation of 2,500 ppm or 1,500 ppm of medicinal zinc for 14 days resulted in 20 gram higher daily gain (7-30 kg) ($p < 0.001$), 0.03 improved FCR ($p < 0.001$) and reduced diarrhoea frequency measured by number of antibiotic treatments ($p < 0.01$) with app. 60 % compared to no medicinal zinc or the allocation of the alternative products. The allocation of these three products in the period from seven to 30 kg did not show better productivity or reduced treatment rate compared to the group without medicinal zinc.

There was no production or health difference between the allocation of 2,500 or 1,500 ppm medicinal zinc in piglet feed for 14 days after weaning. Using 1,500 instead of 2,500 ppm medicinal zinc, the zinc consumption in the piglet production can be reduced by 40 % without compromising productivity and health.

The influence of dietary calcium concentration on the efficacy of supplementary phytase in finisher pigs

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High levels of phytase have been shown to elicit improvements in pig performance, beyond that observed due to improved P nutrition. While the mode of action for this response remains unclear, it has been suggested that improved *myo*-inositol bioavailability is important. This study set out to determine the influence of phytase on finisher pig performance and plasma *myo*-inositol when added to high and low calcium diets. A total of 568 pigs (BW 34.54 kg) were used in this grower-slaughter trial (*ca.* 70 days). Dietary treatments included: a positive control (PC), a negative control (NC; calcium and dig-P reduced by 1.5 and 1.3 g/kg respectively) and the NC + 500 or 1000 FYT/kg. Each of the four treatments were applied to a normal (N) and a low (L) Ca diet to create a 4x2 factorial design. The N-PC diet was formulated to meet the nutritional requirements of the pig (Ca at 7.5 g/kg and dig-P at 0.25 g/kg) and calcium was reduced by 2 g/kg to create the L-PC diet. Each treatment was replicated 8 times with each replicate pen containing 8-10 mixed sex pigs. At the end of the study, plasma samples were obtained from two pigs/pen and analysed for *myo*-inositol. Data were analysed using the GLM function of SPSS with the pen serving as the experimental unit. Phytase linearly improved pig ADG and FCR ($P < 0.05$), independently of calcium concentration. Plasma *myo*-inositol was influenced by a Diet x calcium interaction ($P < 0.01$): phytase increased *myo*-inositol in a quadratic fashion when added to the L diet, but linearly when added to the N diet. Correlation analysis revealed a direct relationship between pig ADG and circulating MYO ($r = 0.203$; $P < 0.05$). In conclusion, phytase improved finisher pig performance, independently of calcium concentration. Moreover, phytase increased plasma *myo*-inositol concentration, however, this response was dependent on dietary calcium concentration.

Allometric development of gastrointestinal tract of pigs from 30 to 150 kg

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Allometric development of visceral organ weight has been reported as relatively early-developing in pigs. However, allometric development of length and volume of the gastrointestinal tract (GIT) remains ill-defined. This study slaughtered 48 pigs from 30 to 150 kg to describe the allometric development of stomach, small intestine (SI), cecum, and large intestine (LI). Crossbred pigs were fed a 5-phase common diet supplemented with a constant level of 20% DDGS that met or exceeded NRC (2012) requirement estimates. At the beginning of the study (30 kg) and 50, 75, 100, 125, and 150 kg, 8 pigs (4 females and 4 males) were slaughtered for measurements of weight, volume, and/or length of GIT segments. All data were analyzed for relationship to bodyweight by GLM procedures in SAS. The absolute weight and length of GIT segments increased, but the weight relative to bodyweight decreased as bodyweight increased (linear and/or quadratic, $P < 0.05$). Relative to total intestinal length, the length of SI (0.814 to 0.799) and LI (0.186 to 0.200) decreased and increased, respectively, with increasing bodyweight (linear, $P < 0.05$). The absolute volume (mL) of GIT segments (stomach: 621, 831, 1864, 1949, 2854, and 2921; SI: 1385, 2203, 3339, 3877, 3892, and 4615; cecum: 606, 1427, 1842, 2069, and 2520; LI: 1290, 2184, 3927, 5434, 5723, and 8037) increased with increasing bodyweight (linear and/or quadratic, $P < 0.05$). The volume relative to total volume decreased for cecum and SI (linear and/or quadratic, $P < 0.05$), increased for LI (linear, $P < 0.0001$), but was not altered for stomach with increasing bodyweight. Development of GIT volume was hypoallometric for SI, isometric for cecum, and hyperallometric for stomach and LI; whereas GIT weight and/or length all developed hypoallometrically except for LI weight, which developed isometrically. The results indicated that GIT segments had relatively greater development of volume than weight and/or length in growing-finishing pigs.

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Benzoic acid replaces high level of CuSO₄ as growth promoter in piglet feed

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Copper sulfate, CuSO₄ is used as growth promoter in most piglet feed. As Cu is concentrated in the soil and is toxic it is desirable to find alternative growth promoting feed additives. The ability of benzoic acid to act as a growth promoter with same efficiency as copper sulfate was tested in this trial.

The hypothesis is that the level of Cu in piglet feed can be reduced from 150 ppm to 25 ppm to prevent diarrhea, without reducing productivity in piglet production and increasing use of antibiotics.

The trial was carried out at the Pig Research Center trial station Groenhoej, Denmark. A total of 720 piglets were randomly allocated to 4 treatments with 56 replicates. The piglets entered the trial after weaning, at approx. 7 kg live weight (4 weeks of age) and left the trial when reaching 30 kg. The treatments were, feed containing 1) 150 ppm Cu, 2) 25 ppm Cu without benzoic acid, 3) 25 ppm Cu with 0.5 % benzoic acid and 4) 25 ppm Cu with 1.0 % benzoic acid. The feed was pelleted and offered *ad libitum*.

Daily feed intake, daily gain, and number of treatments with antibiotics were registered and the feed conversion rate (FCR) calculated.

Proc mixed and proc glimmixed from SAS were used to statistically analysis. Bonferroni correction was used in comparison of groups.

The results revealed that the productivity and concentration/level of antibiotics were similar for group 1, 3 and 4. Piglets in group 2 had lower daily gain ($P < 0.001$), poorer FCR ($P < 0.001$), and higher use of antibiotics ($P < 0.001$) than the other groups.

In conclusion, high level of Cu (150 ppm) in piglet feed can be replaced by benzoic acid, at concentrations down to 0.5 %, without reducing productivity or increasing use of antibiotics.

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Effects of combining essential oils, increased zinc oxide and copper sulfate, on nursery pig performance

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Changes in antibiotic use regulations, concerns over antibiotic resistance, and consumer preferences are pressuring the swine industry to find growth promoting alternatives. Phytogetic compounds and increased dietary zinc and copper are promising alternatives. This experiment was conducted to compare growth performance of nursery pigs fed diets containing carbadox, essential oils blend (Victus LIV), elevated levels of zinc and copper, or their combinations.

Two hundred-eighty nursery pigs (Line 200 X 400; DNA, Columbus, NE) initially weighing 5.17 kg were used in a 35-d study. There were 5 pigs / pen and 8 replications / treatment. Pigs were weaned at 21 d of age and blocked by initial bodyweight (BW). Within BW blocks, diets were randomly assigned. Data were analyzed as a random complete block using GLIMMIX in SAS. There were 7 dietary treatments which were: 1) Negative Control (NC); 2) Positive Control (PC, 55 ppm carbadox); 3) Supplemental copper sulfate (125 ppm) and zinc oxide (3000 ppm d 0-7, 2000 ppm d 7-35); 4) Low essential oils blend (145 ppm, Victus LIV) (LEOB); 5) High essential oils blend (435 ppm, Victus LIV) (HEOB); 6) LEOB + Cu Zn; 7) HEOB + Cu Zn.

Overall, feeding carbadox, essential oils at 145 ppm, or increased levels of Cu and Zn improved ADG ($P < 0.05$) of nursery pigs compared to NC. The use of high levels of Cu + Zn or LEOB, alone or in combination, allows for competitive ADG and G:F with carbadox. In summary, pigs fed the combination of Zn + Cu and/or essential oils at 145 ppm had similar growth performance to those fed carbadox ($P = 0.97$).

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In-feed antibiotics or protected sodium heptanoate on performance in post-weaned pigletsC Sol¹, M Puyalto¹, M Gracia² and J Mallo¹¹Norel SA; ²Imasde Agroalimentaria

This study evaluated the effect of sodium heptanoate protected with sodium salt of coconut fatty acid distillate (HEP) on performance parameters in post-weaned piglets. A total of 240 piglets, with 28d of age (7.3 ± 0.30 kg) were distributed according to their initial body weight (BW) into 24 pens with 10 piglets per pen. There were three treatments: CON-, control diet with no additives nor antibiotics; CON+, control diet plus 120mg/kg of colistin and 3000ppm of ZnO; HEP, control diet plus HEP at 3kg/t of feed in pre-starter diet (28d to 42d of age) and 1 kg/tn of feed in starter diet (43d to 70d of age). Performance results were analyzed by one-way ANOVA using GLM procedure of SSPS v. 19.0, with initial BW as covariate.

At 42d, CON+ piglets were significantly heavier than CON- and HEP (11.4 vs 10.5 and 10.6 Kg, CON+, CON- and HEP, $P=0.002$); had higher ADG (291, 224 and 236 g, CON+, CON- and HEP, $P=0.002$); however, FCR was not different between CON+ and HEP and was statistically lower than CON- (1.00, 1.11 and 1.33, CON+, HEP and CON-, $P=0.023$). At 70d, piglets of CON+ were significantly heavier than CON- and HEP (23.8 vs 21.0 and 22.2 Kg, for CON+, CON- and HEP, $P>0.001$); FCR tended to be lower in CON+ than CON-, but not different from HEP (1.67, 1.87 and 1.79, for CON+, CON- and HEP, $P=0.079$). There were no significant differences in mortality, it was very low for CON+ (1.3%), low for HEP (2.5%) and higher for CON- (5%).

It can be concluded that the good results obtained by medicated diets (CON+) can also be achieved by sodium heptanoate as showed by the results in FCR and in lower mortality. These results reinforce sodium heptanoate as a good alternative to antibiotics in pig diets.

Sodium salts of medium chain fatty acids on performance in pigletsC Sol¹, M Puyalto¹, M Gracia² and J Mallo¹¹Norel SA; ²Imasde Agroalimentaria

The study evaluated the effect of sodium salt of coconut fatty acid distillate (DIC) as a source of medium chain fatty acids on performance parameters in piglets in starter feeds (42 to 60d of age). A total of 320 piglets, with 42d of age (9.2 ± 0.40 kg) were distributed according to their initial body weight (BW) into 8 pens with 20 piglets per pen. There were two treatments: CON, control diet with no additives nor antibiotics; DIC, control diet plus DIC at 1kg/t of feed. Performance results were analyzed by one-way ANOVA using GLM procedure of SSPS v. 19.0, with initial BW as covariate. Microbial quantification of *lactobacillus*, *enterobacteriaceae*, *bacteroides*, *bifidobacteria* and total bacteria was performed by qPCR. Furthermore, the Gut Index that relates three bacterial groups, such as *lactobacillus* and *bifidobacteria* considered as beneficial bacteria and *enterobacteriaceae* associated to dysbiosis processes, was also calculated. The performance results showed that at 60d, piglets in DIC were significantly heavier than CON (16.6 vs 16.9 Kg, for CON and DIC, $P=0.02$) and there were no differences in feed intake nor in feed conversion ratio. The most important numerical differences observed in microbiology were for *lactobacillus* (7.69 vs 7.84 log CFU, for CON and DIC, $P>0.10$), however no significant differences between treatments were observed in any bacterial population or in the Gut Index. It can be concluded that sodium salt of coconut fatty acid distillate could be used as a performance booster for piglets. Further studies are needed to demonstrate the beneficial properties of medium chain fatty acids in early stages of piglets not only in performance but also in gut health.

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Validation of nutrient matrix values for a novel bacterial 6-phytase in weaned pigletsD Torrallardona¹, L Hall² and P Ader³¹IRTA; ²BASF Australia Ltd.; ³BASF SE

The hydrolysis of phytic acid by phytases increases the availability of energy and other nutrients, in addition to that of phosphorous. To account for this, it is common practice that phytases are assigned matrix nutritional values for feed formulation. A trial was conducted to validate matrix nutritional values of a new phytase (6-Phy). One hundred and forty four piglets (26d-old; 8.3 kg BW) in 48 pens were offered six treatments: a positive control (PC; 14.0/13.8 MJ ME/kg; 12.8/12.0 g SIDLys/kg; 3.87/2.91 g digP/kg between 0-21/21-42d post-weaning, respectively); two negative controls (NC1 and NC2) with reductions (per kg feed) of 0.22 and 0.29 MJ ME/kg; 0.11 and 0.15 g SIDLys/kg; 1.4 and 1.9 g Ca/kg; and 1.12 and 1.52 g digP/kg, respectively; NC1 with 500 FTU/kg of 6-Phy (NC1 + 500); and NC2 with either 1000 or 1500 FTU/kg of 6-Phy (NC2 + 1000 and NC2 + 1500). Pigs fed NC2 and grew less than those fed PC (399 vs. 473 g/d; $P < 0.05$), and the gain:feed ratio of pigs fed NC1 and NC2 was poorer than that of pigs fed PC (0.66 and 0.64 vs. 0.69 g gain/g feed; $P < 0.05$). Relative to NC1, NC1 + 500 numerically improved weight gain and feed efficiency, but these were not different from PC or NC1 ($P > 0.05$). Compared to NC2, supplementation of NC2 with either 1000 or 1500 FTU improved weight gain (454 and 477 vs. 399 g/d; $P < 0.05$) and gain:feed (0.67 and 0.68 vs. 0.64 g gain/g feed; $P < 0.05$), but they did not differ from PC. No differences were observed between the two doses of 6-Phy used to supplement NC2. It is concluded that nutrient specifications in feed can be reduced according to the proposed 6-Phy matrix nutritional values without affecting performance.

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Supplementation of a wheat-barley-rye based diet with xylanase/glucanase improves the apparent total tract digestibility of nutrients in lactating sowsD Torrallardona¹, L Hall² and P Ader³¹IRTA; ²BASF Australia Ltd.; ³BASF SE

A trial was conducted to evaluate the efficacy of a xylanase/glucanase preparation on the performance, and on the total tract digestibility of nutrients in lactating sows. A total of 32 sows [Landrace x Large White] were involved in the trial from 2-3 weeks before farrowing until weaning at day 28 post-farrowing. At the start of the trial, pairs of sows with similar age, parity number and initial body weight were randomly assigned to two experimental treatments, consisting of a wheat-barley-rye-soybean-rape seed basal diet with or without supplementation with 100 ml/ton of Natugrain TS L (BASF SE, Ludwigshafen, Germany), equivalent to 560 TXU/kg feed of xylanase activity and 250 TGU/kg feed of β -glucanase activity. The body weight and feed intake of the sows and the performance of their litters were monitored weekly during the whole trial. During the third week of lactation, fresh faeces were sampled individually for each sow, analysed for dry matter, ash, crude protein, crude fat, crude fibre, ADF, NDF, lignin and energy and the apparent total tract digestibility (ATTD) of nutrients was calculated using TiO_2 as indigestible marker. No statistically significant differences between treatments were observed for performance parameters of the sows or their litters ($P > 0.05$). On the other hand, xylanase/glucanase supplementation significantly improved ($P < 0.05$) the ATTD of dry matter (79.7 vs. 80.7%), organic matter (82.7 vs. 83.7%), fat (42.3 vs. 47.0%), energy (79.9 vs. 81.2%), NDF (66.4 vs. 69.0%), ADF (46.7 vs. 50.1%), hemicelluloses (78.9 vs. 81.1%) and cellulose (53.2 vs. 57.1%). It is concluded that the xylanase/glucanase preparation tested significantly improved nutrient digestibility in lactating sows, despite no effects on performance were observed.

Nutrient and energy sparing with a novel bacterial 6-phytase in weaned pigletsD Torrallardona¹, L Hall² and P Ader³¹IRTA; ²BASF Australia Ltd.; ³BASF SE

Phytases improve the utilization of energy and other nutrients besides phosphorous. To validate the matrix nutritional values of a new phytase (6-Phy), 196 piglets (21d-old; 4.8 kg BW) in 49 pens were offered seven treatments: a positive control (PC; 14.0/13.8 MJ ME/kg; 12.8/12.0 g SIDLys/kg; 3.82/2.88 g digP/kg between 0-21/21-42d post-weaning, respectively); three negative controls (NC1, NC2 and NC3) with reductions (per kg feed) of 0.16, 0.22 and 0.29 MJ ME/kg; 0.08, 0.11 and 0.15 g SIDLys/kg; 1, 1.4 and 1.9 g Ca/kg; and 0.8, 1.12 and 1.52 g digP/kg, respectively; and the three NC diets with 6-Phy (300, 500 or 1000 FTU/kg, respectively). Performance did not differ among pigs fed the PC, NC1 or NC1 + 300 diets ($P > 0.05$). However, pigs fed NC3 grew less than those fed PC (303 vs. 356 g/d; $P < 0.05$), and the gain:feed ratio of pigs fed NC2 and NC3 was poorer than that of pigs fed PC (0.69 and 0.67 vs. 0.73 g gain/g feed; $P < 0.05$). Supplementation of NC2 with 500 FTU improved weight gain relative to NC2 and PC (404 vs. 316 and 356 g/d; $P < 0.05$) and gain:feed relative to NC2 (0.74 vs. 0.69 g gain/g feed; $P < 0.05$). Finally, supplementation of NC3 with 1000 FTU improved weight gain (390 vs. 303 g/d; $P < 0.05$) and gain:feed (0.73 vs. 0.67 g gain/g feed; $P < 0.05$), relative to NC3. At the end of the trial, *Os metacarpale III* of pigs fed NC1, NC2 and NC3 had less ash than PC pigs (0.97, 0.87 and 0.76 vs. 1.21 g/bone; $P < 0.05$). Supplementation of NC diets with 6-Phy increased bone ash (1.24, 1.28 and 1.17 vs. 0.97, 0.87 and 0.76 g/bone; $P < 0.05$) and it was not different from PC. In conclusion, with the proposed 6-Phy matrix nutritional values, nutrient specifications can be reduced while maintaining performance and bone mineralization.

Effect of encapsulating an orally administered iron supplement over the prevention of anaemia in suckling pigletsC Valenzuela¹, J Figueroa², S Guzmán² and I González¹¹Universidad de Chile; ²Pontificia Universidad Católica de Chile

This work aimed to determine the effect of encapsulating an oral iron supplement on the prevention of iron anaemia in piglets. Heme and non-heme iron sources, such as bovine erythrocytes (BE) or ferrous sulphate (FS), were encapsulated with maltodextrin by spray-dried. The final supplement comprised 2 grams of water-soluble mixture, at 1:1 ratio, of both microparticles. We prepared an unencapsulated supplement based on the same iron sources. Then, 72 neonatal piglets were assigned to 3 groups: 1) control group, which received one dose of parenteral iron; 2) non-encapsulated group, that received 4 oral doses of unencapsulated iron supplement, and 3) encapsulated group, that received 4 oral doses of encapsulated iron supplement. Haemoglobin (Hb) and serum ferritin (SF) were determined at days 1 and 21. The Cut-off were: Hb: < 9 g/dL and SF: < 12 μ g/L. The behavior of the pigs after oral supplementation was observed. ANOVA and Tukey test were used for the analysis ($p < 0.05$). The encapsulated and unencapsulated iron supplements contained 65.2 and 65.0 mg/iron per dose, respectively. One-dose of parenteral treatment is not enough to ensure an adequate nutritional state of iron for piglets (Hb: 9.3 g/dL and SF: 8.4 μ g/L). Even though these piglets are not anaemic, they have shown SF levels below the cut-off point, that indicate a state of iron depletion. Unlike the parenteral treatment, the oral supplements effectively prevented development of anaemia in piglets. However, we found that encapsulating iron with maltodextrin (Hb: 11.6 g/dL and SF: 19.9 μ g/L) did not have any effect vs. the unencapsulated supplement (Hb: 11.3 g/dL and SF: 17.4 μ g/L). Nonetheless, we observed for the oral supplements a decrease of behavioral manifestations indicating serious discomfort on piglets assigned to the unencapsulated group (sharp and sideways head movements, tongue protrusion, rubbing their snouts on wood shavings).

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The effect of early intervention with galactooligosaccharides on the intestinal development of small intestinal in suckling piglets

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Galactooligosaccharides (GOS) were often focused on its prebiotics effects on hindgut. However, its beneficial effects on small intestinal (SI) were ignored. Therefore, this study hypothesized that early intervention with GOS could improve intestinal development of SI. Six litters (10 piglets/ litter) neonatal piglets were equally assigned to control (CON) group and GOS (GOS) group in each litter. Piglets in the GOS group were orally administrated with 10 mL GOS solution (reach 1 g/kg body-weight) per day from the age of 1 to 7 days; the piglets in the CON group were treated with the same dose of physiological saline. At the age of 8 and 21 days, six piglets from each group were euthanized. Data were evaluated by independent samples t-test. The results showed that GOS intervention increased the SI weight ($P < 0.05$) and trended to increase the SI length ($P = 0.077$), SI weight/ SI length ($P = 0.076$), SI weight/ body weight ($P = 0.096$) on day 8. Jejunum and ileum had a higher villus height/ crypt depth in GOS group than CON group on day 21 ($P < 0.05$). In addition, the positive rate of proliferating cell nuclear antigen was decreased in ileum ($P < 0.05$) and jejunum ($P = 0.084$) on day 21. The lactase activity in jejunum was increased on day 8, and the sucrase activity in jejunum and ileum were improved on day 21 ($P < 0.05$). For intestinal growth factors, on day 8, the concentration of GLP-2 (glucagon-like peptide 2) in jejunum and GLP-1 in ileum were increased, the concentration of GLP-1 in jejunum and epidermal growth factor (EGF) in ileum had a tendency to increase; on day 21, the concentration of GLP-2 and EGF in jejunum trended to increase. In conclusion, the early intervention with GOS promotes intestinal development.

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The reliability of tabular values to estimate the composition and digestibility of pig feeds

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Tabular values of feed ingredients are commonly used in pig feed formulation. It regroups mean values of chemical composition and digestibility coefficients. However, composition and digestibility within the same feed may vary to a large extent. To evaluate the reliability of the Dutch (CVB2016) and French (INRA 2017) tabular values of the ingredients, 20 compound feeds were formulated and the correlation (R^2) and the mean absolute difference between the tabular and the determined chemical composition and digestibility coefficients was calculated (Excel 2016). The 20 compound feeds had a variable nutrient and ingredient composition and were fed to fattening pigs for a digestibility trial. Compounds feeds, feed ingredients and faeces were analyzed for chemical composition and were used to calculate the digestibility coefficients of organic matter (OM).

The R^2 between the analyzed parameter on the compound feeds and the calculated values using the Dutch and French tables for the ingredients amounted to respectively 0.83 and 0.68 for moisture, 0.90 and 0.88 for crude protein, 0.95 and 0.95 for crude fat, 0.95 and 0.96 for starch, 0.78 and 0.52 for sugars, 0.79 and 0.76 for crude ash, 0.93 and 0.95 for nsp. The correlation between the *in vivo* digestion coefficients of the compounds and the digestibilities derived from the Dutch and French tables was 0.93 and 0.92 for OM, 0.91 and 0.86 for crude protein and 0.41 and 0.19 for crude fat.

The Dutch and French tables are quite reliable to calculate the chemical composition of compound feeds, with somewhat lower accuracy for moisture, ash and sugar. These tables are also reliable to calculate the digestibility of OM and CP, but less for crude fat digestibility. For most parameters the Dutch tables appear slightly better than the French ones.

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Evaluation of methods for estimation of the net energy content of pig feedsL Paternostre¹, J De Boever¹ and S Millet²¹*Flanders Research Institute for Agricultural, Fisheries and food (ILVO);* ²*Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University*

The net energy (NE) content of the feed strongly determines pig performances, so that an accurate and rapid estimation is very important. We designed an experiment to evaluate the use of feed tables, chemical analyses and *in vitro* organic matter digestibility (OMd) for the prediction of the *in vivo* NE. 20 compound feeds with variable nutrient and/or ingredient composition were formulated and each feed was fed to six pens of three pigs in three weight intervals at an initial mean life weight of 52, 72 and 94 kg, respectively. The faeces were spot-sampled during five days and nutrient digestibility was determined using acid insoluble ash as marker. The compound feeds as well as their ingredients were analysed for chemical composition and *in vitro* Omd according to Boisen and Fernandez (1997). The *in vivo* NE content of the feeds was calculated according to the Dutch feed evaluation system (CVB,2016) and amounted to 10.84 ± 0.92 MJ/kg DM (mean \pm sd), varying from 8.31 to 12.01 MJ/kg DM. The sd of the NE due to variation between pens averaged 0.08 MJ/kg DM and was mainly caused by the differences in weight interval. The calculated NE content increased with 0.0021 MJ/kg DM per kg bodyweight.

Besides, the NE content of the 20 feeds was calculated from the NE of the composing ingredients, using either directly the tabular NE-values or the analyzed proximate composition in combination with the tabular digestion coefficients, which resulted in a prediction error of 0.34 and 0.19 MJ/kg DM, respectively. Further, multiple regression equations were developed using chemical parameters and *in vitro* Omd. The best equation based on chemical parameters (crude fat, starch, ADL and crude ash) resulted in an error of 0.13 MJ/kg DM, whereas the incorporation of *in vitro* Omd could slightly lower this error (0.11 MJ/kg DM).

Effects of dietary serine supplementation on growth performance and amino acid contents in growing-finishing pigsX Zhou¹, Y Zhang², X Wu¹ and Y Yin¹¹*institute of subtropical agriculture, the chinese academy of sciences;* ²*Hunan Co-Innovation Center of Animal Production Safety, CICAPS, College of Animal Science and Technology, Hunan Agricultural University*

An experiment was conducted to investigate the effects of dietary serine supplementation on growth performance, serum biochemical parameters and amino acid contents in serum and *longissimus dorsi* muscle in growing-finishing pigs. A total of 14 male pigs with an initial body weight (BW) of 13.10 ± 0.42 kg were randomly allotted to two groups (7 replicates each group) fed with basic diet met the National Research Council (NRC, 2012) requirement (CON) and basic diet supplemented with 0.2% serine (SER), respectively. At the end of the feeding experiment, pigs with BW of 91.00 ± 4.57 kg were electrically stunned, exsanguinated and eviscerated. Then, samples of blood and *longissimus dorsi* muscle were collected and data obtained in CON and SER groups were compared by one-way analysis of variance using the General Linear Model procedure of SAS. The results showed that there was no significant difference in growth performance, and serum levels of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and glucose ($P > 0.05$) between pigs in CON and SER group. Serine, glycine and cysteine concentrations in serum and *longissimus dorsi* muscle were significantly higher ($P < 0.05$) while homocysteine was significantly lower ($P < 0.05$) in SER group when compared with CON group. No significant difference was observed in other amino acid concentrations between pigs in CON and SER group. These results indicated that dietary serine supplementation during growing-finishing period may have beneficial effects on homocysteine metabolism and amino acid composition in *longissimus dorsi* muscle.

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Dietary methionine deficiency promotes muscle fibre type transformation in weanling piglets

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Dietary methionine deficiency (MD) has been proved to regulate intramuscular fat content in weanling piglets. However, whether MD has any effects on muscle fibre type composition is still unknown. This study was conducted to investigate the effects of MD diet on myofibre transformation in weanling piglets. Piglets weaned at the age of 21 day with an initial body weight of 6.5 kg were fed diets with either an adequate (methionine and cysteine, 0.75%) or a methionine deficient (methionine and cysteine, 0.48%) diet for 21 days ($n = 7$ per group). At the end of the feeding experiment, samples of *longissimus dorsi* muscle and soleus muscle were collected and data obtained in the control and MD groups were compared by one-way analysis of variance using the General Linear Model procedure of SAS. The results showed that pigs fed the MD diet had higher mRNA expression of myosin heavy chain I (MyHC I) ($P < 0.05$) while they had lower mRNA expression of MyHC IIb ($P < 0.05$) in muscles, when compared with pigs fed the control diet. Moreover, pigs fed the MD diet also had higher protein expression of troponin I and myoglobin (markers of type I muscle fibre), and cytochrome c and cytochrome c oxidase 1 (COX1) (mitochondrial proteins) ($P < 0.05$). In addition, pigs fed the MD diet had increased mitochondrial genomic DNA copy numbers ($P < 0.05$). These results suggested that MD drove the formation of slow-twitch muscle fibres. Furthermore, peroxisome-proliferator-activated receptor- γ co-activator-1 (PGC-1 α) and myocyte-specific enhancer factor (MEF2c) protein levels were also higher ($P < 0.05$) in the piglets fed the MD diet. Collectively, these findings indicated that MD diet promoted muscle fibre transformation to type I fibre in weanling piglets and PGC-1 α - MEF2c pathway may play a role in this process.

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Effects of dietary serine supplementation on meat quality in growing-finishing

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An experiment was conducted to investigate the effects of dietary serine supplementation on backfat thickness, meat color, water holding capacity, pH value, intramuscular fat content and mRNA expression of myosin heavy chain in growing-finishing pigs. A total of 14 male pigs with initial body weight (BW) of 13.10 ± 0.42 kg were randomly allotted to two groups (7 replicates each group) fed with basic diet met the National Research Council (NRC, 2012) requirement (CON) and basic diet supplemented with 0.2% serine (SER), respectively. At the end of the feeding experiment, pigs with BW of 91.00 ± 4.57 kg were electrically stunned, exsanguinated and eviscerated. Then, samples of *longissimus dorsi* muscle was collected and data obtained in CON and SER groups were compared by one-way analysis of variance using the General Linear Model procedure of SAS. The results showed that there was no significant difference in backfat thickness, meat color (L, a and b value) and water holding capacity ($P > 0.05$) between pigs in CON and SER group. When compared with pigs in CON group, pigs in SER group had higher intramuscular fat content and mRNA expression of myosin heavy chain I ($P < 0.05$) in *longissimus dorsi* muscle. In addition, pigs in SER group had higher pH value at 45 min and 24 h postmortem ($P < 0.05$) when compared with pigs in CON group. These results indicated that dietary serine supplementation during growing-finishing period may be beneficial for producing favorable meat with higher intramuscular fat level.

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Impact of adding insoluble dietary fiber (IDF) on ileal and fecal digestibility of fiber components in growing pigs

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This experiment tested the hypothesis that adding IDF would decrease the utilization of various fiber components in the gastrointestinal tract, and to determine if the response to IDF was different under constant nutrient or constant ingredient conditions. Twenty-one crossbred gilts (33 ± 0.4 kg BW) fitted with T-cannulae at the terminal ileum were randomly allocated to one of seven treatments over three sample collection periods. Treatments consisted of a 0% corn DDGS basal diet plus diets containing 15, 30 or 45% of DDGS as a source of IDF formulated using one of two different approaches: constant nutrient (CN) where nutrients were held equal to the basal diet, or where DDGS were added at the expense of corn and all other ingredients remained constant (CI). Chromic oxide was added at 0.5%. The MIXED procedure of SAS was used to test linear estimates with pig and collection as random effects. Each 1% of additional IDF decreased the apparent ileal digestibility (AID) of IDF (-1.97%, CN and -2.77%, CI; $P < 0.01$), the AID of soluble dietary fiber (SDF; -3.26%, CN and -2.25%, CI; $P < 0.01$), and the AID of total dietary fiber (TDF; -2.10%, CN and -2.74%, CI; $P < 0.01$). Likewise, each 1% of additional IDF decreased the apparent total tract digestibility (ATTD) of IDF (-1.57%, CN and -2.25%, CI; $P < 0.01$), and ATTD of TDF (-1.24%, CN and -1.86%, CI; $P < 0.01$). However, each 1% of additional IDF increased the ATTD of SDF (1.52 and 1.13% for CN and CI respectively; $P < 0.01$). There were no differences between the SN and CI on any of the linear estimates ($P > 0.05$). The addition of IDF decreased the use IDF and TDF along the intestinal tract. Adding IDF increased fermentation of SDF in the large intestine. Adjusting diets for ingredient or nutrient composition resulted in similar responses to IDF.

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Evaluating the effect of adaptation length on apparent ileal and total tract digestible energy in weaning pigs

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This study investigates the effect of adaptation length to corn and wheat middlings (WM) based diets on apparent ileal and total tract digestible energy (DE) of corn and WM in pigs using the difference method. Twenty-one ileal cannulated growing pigs (BW = 34.1 kg) were allotted in a 3 x 3 factorial arrangement of treatments using the RCBD with 3 diets (reference, corn-, or WM-based) and 3 adaptation periods (4, 8, and 12 days). Each pig was fed 4% of the BW of the lightest pig within each block. Ileal and fecal samples were collected on days 4, 8, and 12. Corn- and WM-based diets were produced by replacing 30% of the reference diet (corn-SBM-based) with corn or WM, respectively. Proc mixed model of SAS was used to analyze the data and a repeated statement was included to account for correlated observations made on the same animal. There was an interaction ($P < 0.05$) between adaptation length and diet for ileal DE, DM, and energy (EN) digestibility. Addition of WM to the reference diet decreased ($P < 0.05$) ileal and total tract DE, DM and EN digestibility. Increasing adaptation length resulted in both linear (increase) and quadratic ($P < .0001$) effect on DE, DM and EN digestibility. Total tract DE was highest ($P < .0001$) in the reference diet (3,935 kcal/kg) and lowest ($P < .0001$) for the WM-based diet (3,569 kcal/kg). The DE derived for corn and WM was highest ($P < .0001$) on d 12. Corn's total tract DE was higher ($P < 0.05$) than that of WM by 804 kcal/kg. Regardless of feed ingredient, a linear increase ($P < 0.001$) in ileal DE was observed with increasing adaptation length. Results from this study showed that longer adaptation periods may be needed for feed and ingredients' DE evaluation.

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Phytate degradation pattern in piglets fed diets differing in P, Ca and phytase level

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Intestinal phytate degradation products (inositol phosphates and isomers) were analysed in the digesta of control and phytase-fed piglets. After weaning, 160 barrows (Hampshire × Duroc × Yorkshire × Landrace) were assigned in a randomized complete block design to four treatments, 10 replicates each. Corn-soyabean meal based diets adequate (PC) or reduced (NC) in P and Ca were fed without or with one of the two phytase doses (modified *E. coli* 6-phytase; 500 or 2000 FTU per kg feed; NC500 and NC2000). Duodenal and ileal digesta, sampled on d49 after weaning, were analysed for inositol phosphates (InsP₂₋₆) and myo-inositol (MI). Results show that dietary P/Ca level had no influence on duodenal and ileal InsP₂₋₆ and MI levels (PC compared to NC; $P > 0.05$). InsP₆ disappearance to the end of the ileum was low in PC and NC piglets (35.7 and 25.4%, respectively) but increased ($P < 0.05$) in NC500 and NC 2000 piglets (90 and 97.9%, respectively). No relevant further degradation occurred in PC and NC piglets as revealed by the low ileal InsP₂₋₆ disappearance (31.5 and 25%, respectively) compared to NC500 (73%) and NC2000 treatments (94%). Ileal InsP₃₋₄ levels increased in NC500 ($P < 0.05$) but not in NC2000 piglets. Ins(12356/12345)P₅ and Ins(1234/1236)P₄ were the major InsP₄₋₅ intermediates in phytase fed piglets, confirming the typical degradation pattern for *E. coli* phytases. In the ileal digesta, 5 and 7 fold more MI (NC500 and NC2000, respectively) was detected in phytase fed compared to NC piglets ($P < 0.05$), confirming that phytate degradation down to MI was enhanced by adding phytase, especially at the high dosing level. The reduction of intestinal InsP₃₋₄ and increased MI levels can partly explain the improved ADG as also noted in this trial in NC2000 piglets (Lu et al., unpublished).

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Effect of serine protease in piglet diets containing either soy bean meal or canola meal

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This study was conducted to determine the effect of a serine protease on animal performance in three wheat based piglet diets containing either soy bean meal (SBM) or two different sources of canola meal, solvent extracted canola meal (CMS) or cold pressed expeller canola meal (CME). A total of 168 Large White x Landrace male piglets fed in single pens were submitted to one of 6 treatments from 5 to 21 days of age. T1, Standard SBM diet containing 23.5% SBM. T2, CMS diet containing 25.3% CMS. T3, CME diet containing 26.5% CME. T4, same as in T1 with the inclusion of 500g/ tonne protease. T5, same as T2 with the inclusion of 500g/ tonne protease. T6, as in T3 with the inclusion of 500g/ tonne protease. Data was analysed using one way anova and information was grouped using Fisher method. Animals in treatments T1 and T2 responded with similar intakes (10.37 kg and 10.36 kg respectively). T3 animals had lower intake (8.44kg) than either T1 or T2 ($P = 0.014$). T2 grew less (6.78kg) than T1 (7.96kg) over the trial period with feed conversion ratio (FCR) of 1.57 and 1.31 respectively ($P = 0.031$). T3 fed animals performed with similar efficiency with a FCR of 1.52 however overall growth at 5.63kg was significantly lower than T1 and T2 ($P < 0.001$). The addition of protease had no effect on piglet performance when consuming the SBM diet or the CME diet, however piglets consuming T5 CMS diet showed greater growth ($P < 0.001$) and FCR ($P = 0.031$) than in the CMS diet T2. There was no effect of treatment on morbidity or mortality in any treatment group in this study. The addition of protease to feed containing solvent extracted canola meal significantly improved growth and efficiency of piglets in this study.

Both dietary copper(I)oxide and copper sulphate stimulate growth performance in pigs but differentially affect copper absorption and metal transporter genes

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Introduction Copper sulphate (CuSO₄) is commonly used as Cu source in pigs diets, both at nutritional and pharmacological levels, but copper(II)oxide (CuO) was found to be ineffective as Cu source. We observed previously that high dietary Cu downregulated the expression of duodenal DMT1, which may impact absorption of other divalent metals. We hypothesized that copper(I)oxide (Cu₂O) would be an effective Cu-source because monovalent Cu may be readily absorbed by CTR1, but not by DMT1, thus minimizing competition with divalent metals for uptake by DMT1. We aimed to determine efficacy of Cu₂O and its interaction with other metals, in comparison to CuSO₄.

Methods We conducted a 2x3 factorial experiment with Cu-source (CuSO₄ and Cu₂O) and supplementation (15, 80 or 160 mg Cu/kg diet) as factors in 600 weaned pigs during 5 weeks. On day 35, blood and gut tissues of eight pigs/treatment were harvested to determine enzyme activities, contents of Cu, Zn, and Fe, and expression of genes related to their homeostasis in the pig by RT-qPCR.

Results Both CuSO₄ and Cu₂O enhanced ($P < 0.001$) feed intake, feed efficiency and body gain, with a final BW of 18.2 and 21.2 kg for 15 and 160 mg Cu/kg, respectively. Cu₂O tended ($P = 0.085$) to enhance body gain more than CuSO₄. Liver Cu-content and duodenal MT1A expression increased more with incremental dietary CuSO₄ than Cu₂O (interaction $P < 0.001$ and $P = 0.008$, respectively). Increase of both Cu-sources upregulated DMT1 and downregulated ZIP4 expression. In conclusion, contrary to earlier results with CuO, the growth promoting effect of Cu₂O was at least equal to CuSO₄, whereas the absorption of Cu was less at 160 mg/kg monovalent Cu from Cu₂O than divalent Cu from CuSO₄. The increase in DMT1 expression and reduction in ZIP4 suggests that these transporters may be less specific in pigs than earlier described.

Low phosphorous diets in gestation and lactation stimulate calcium absorption in sows and reduce calcium and phosphorous absorption in offspring

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Introduction Insight in calcium (Ca) and phosphorous (P) metabolism of sows is required to optimise reproductive performance and improve P utilisation in sows and their offspring. This study was conducted to determine i) the early life programming of P metabolism in weaned pigs via maternal low dietary P exposure during gestation and lactation, and ii) consequences for Ca-P interactions in sows and nursery pigs.

Methods A 2 × 2 factorial arrangement was used with 2x14 sows on low (LP) and high P (HP) diets (50% and 100% of recommendations) in gestation and lactation and LP and HP nursery diets (60% and 110% of recommendations) during 5 weeks post weaning. Faeces, urine and blood of sows was collected in early and late gestation and lactation. Piglets were sacrificed at birth, 21 days of age and 35 days post-weaning, and serum, jejunal mucosal scrapings and kidney cortex harvested to determine expression of Ca and P transporter genes with qPCR. Faeces were collected in d 32-35 post weaning.

Results The reduction in dietary P content enhanced apparent Ca-digestibility ($P < 0.001$) in sows, but not in nursery pigs. This effect was associated with a drastic increase in serum 1,25-OH₂-D₃ and an increase in expression of calcium binding protein CaBP9K, Ca channel TRPV6 and phosphate transporter NPT2c, as observed in nursery pigs. Jejunal PIT1 expression was increased ($P = 0.069$) in nursery pigs originating from LP

sows, suggesting an effect on the capacity of intestinal P uptake. Digestibility of Ca ($P = 0.003$) and P ($P = 0.02$) in weaned pigs was lower in offspring of LP sows irrespective of the P-content of the nursery diets. Conclusion: Results indicate differential Ca-P interactions in the GI-tract between sows and growing pigs, and a long term programming effect of the P-reduction in sow diets in the offspring.

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Effects of two direct fed microbials on digestibility of amino acids and energy in diets fed to growing pigs

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The hypothesis that two direct fed microbials (DFM) improve apparent ileal digestibility (AID) of AA, CP, and GE, apparent total tract digestibility (ATTD) of CP and GE, and hindgut digestibility (HGD) of CP and GE was tested. The two DFMs included DFM1 based on *Bacillus amyloliquefaciens* (DSM 25840) and DFM2 based on *Bacillus subtilis* (DSM 25841). Three diets based on corn, soybean meal, distillers dried grains with solubles, and no DFM (control) or DFM 1 or DFM 2 were formulated. Twenty-four growing barrows (22.69 ± 1.48 kg) with a T-cannula in the distal ileum were individually housed and allotted to a 24×3 incomplete Latin square design with 24 pigs and three 21-d periods. There were eight pigs per diet in each period and 24 total observations per diet. Fecal samples were collected from d 13 to 18 and ileal digesta were collected on d 20 and 21 of each period. Data were analyzed by ANOVA. There were no differences in ATTD of CP, but the AID of CP was reduced ($P < 0.05$) in the DFM2 diet compared with the control and DFM1 diets, whereas the HGD of CP was greater ($P < 0.005$) for the DFM2 diet compared with the other diets. The AID of total indispensable, total dispensable, and total AA was greater ($P < 0.05$) in the DFM1 diet compared with the control diet. There were no differences among diets in ATTD of GE, but the AID of GE was greater in the DFM1 diet than in the control and DFM 2 diets ($P < 0.001$), whereas HGD of GE was less ($P < 0.05$) in the DFM1 diet compared with the other diets. In conclusion, supplementation of *Bacillus* sp. in diets fed to growing pigs enhanced the AID of AA and GE.

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Impact of *Brachyspira hyodysenteriae* on intestinal amino acid digestibility and endogenous amino acid losses in pigs

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Brachyspira hyodysenteriae (Bhyo) is an economically significant infection in grow-finish pigs worldwide. Our objectives were to determine the impact of Bhyo infection on ileal basal endogenous AA losses (BEL), standardized ileal digestibility (SID) of AA, and hindgut disappearance of nutrients in growing pigs. Thirty-two Bhyo negative gilts (38.6 ± 0.70 kg BW) were fitted with a T-cannula in the distal ileum. Over two replicates, pigs were fed a complete diet (seven control, 10 Bhyo) or nitrogen-free diet (NFD; four control, 11 Bhyo). The 21 Bhyo pigs (62.6 ± 1.39 kg BW) were inoculated with Bhyo on day post inoculation (dpi) 0, and 11 control pigs were sham inoculated. Feces were collected from nine to 11 dpi and ileal digesta collected from 12 to 13 dpi. Feed, feces, and digesta were analyzed for DM, N, and GE. Feed and digesta were analyzed for AA. Within the complete diet and NFD treatments, data were analyzed to determine pathogen effects. All control pigs remained Bhyo negative, and five challenged pigs in each replicate were confirmed Bhyo positive within nine dpi. Infection with Bhyo reduced BEL of Pro ($P < 0.05$). When SID values were calculated, Bhyo significantly reduced ($P < 0.05$) SID of N, Arg, Lys, Ala, Gly, Pro, and Ser compared to the controls. In the hindgut of Bhyo pigs, there was generally an

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appearance of nutrients rather than disappearance. In pigs fed a complete diet, hindgut appearance of N and GE increased ($P < 0.05$) by 58 and nine-fold, respectively. Similarly, in NFD fed pigs, hindgut appearance of N and GE was increased by 172 and 162%, respectively. Altogether, Bhyo infection reduced SID of N, Arg, Lys and some nonessential AA, BEL of several AA were unaffected, while hindgut loss of GE and N increased. This suggests an increased need for AA and energy during Bhyo infection.

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The effect of physicochemical properties of feed grade zinc oxide sources in dissolution kinetics

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Zinc oxide (ZnO) is widely used in pig production to improve intestinal health. However, the mechanism of action of ZnO is still not completely elucidated. Studies reported improvement of intestinal barrier function and impact on the microbiota, but there is still controversy about the effect of Zn^{2+} ions and/or ZnO itself. Different sources of ZnO showed different results on animal performance. This study hypothesized the different sources of feed grade ZnO have various physicochemical properties that lead to distinct dissolution kinetics. Over 40 samples of ZnO have been collected from the feed industry worldwide. Samples were analyzed for density, tapped density, particle size, shape, specific surface area. Dissolution kinetics was assessed in vitro from a pH range of 2 to 4 in water at 40°C with an automatic burette and zinc concentration measured at times 3, 15, 30 and 60 minutes with ICP-OES. A principal component analysis (PCA) was performed to define the most relevant physicochemical characteristics analyzed affecting dissolution. Density, agglomerate size and specific surface area (PC1 40.02%; PC2 28.55%; and PC3 16.36%, respectively), were the components with highest relevance among properties analyzed. The results implied that there is not only one variable influencing the kinetics of dissolution. It suggests a dissolution mechanism in 2 steps, in which there is the dissolution of ZnO into Zn^{2+} ions from the surface of ZnO elementary particles, followed by diffusion to the bulk solution through the aggregates of elementary particles where the liquid is in a quiescent state. The speed of dissolution can determine the fate of zinc oxide products in the gastrointestinal tract and therefore may explain the different results in animal performance.

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Protein and amino acid digestibility of *Camelina sativa* co-products for growing pigs

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Camelina sativa is an oilseed cultivated in Europe and its oil is used for biofuel production. Solvent-extracted camelina meal (CM) and expeller-extracted camelina expellers (CE) are the co-products that remain after oil extraction. The standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in these two camelina co-products was determined using growing pigs. Thirty-three Pietrain x (Landrace x Large white) barrows of 82.0 ± 2.57 kg body weight were allotted to three treatments (11 pigs/treatment). The experimental diets were a cornstarch-based diet with 30% CM or 35% CE as the sole source of CP and AA; and a N-free diet that was used to determine basal endogenous losses of CP and AA. All diets contained titanium dioxide as an indigestible marker. Pigs were fed at 3 times the maintenance requirement for metabolizable energy. The SID of CP and AA was determined using the direct method. After 7 days of feeding, pigs were slaughtered and the terminal ileum contents were obtained for CP and AA analyses. Solvent-extracted camelina meal contained more protein (419 vs. 381 g/kg dry matter) and most AA, compared with CE. The SID of CP in CE was less than in CM (56.4 vs. 69.3 %, $P = 0.01$). The SID of AA in the CE also tended to be lower ($P < 0.10$) compared with those in CM. Among indispensable AA, methionine had the highest SID both in CM and CE (88.4 and 81.0%, respectively) and lysine and threonine had the lowest SID in

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CM and CE (69.5 and 56.7% for lysine and 70.0 and 51.9% for threonine, respectively). Results from this experiment indicate that the SID of AA was somewhat less in CE than in CM, and that camelina co-products may be included as a protein source in diets fed to pigs.

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Next-generation non-starch polysaccharide-degrading, multi-carbohydrase complex rich in xylanase and arabinofuranosidase to enhance pig feed digestibility

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The objective of this study was to evaluate the effect of a multi-carbohydrase complex (MCC) rich in xylanase (Xyl) and arabinofuranosidase (Abf) on overall feed digestibility of pigs. Energy utilization and digestibility of dry matter (DM), organic matter (OM), protein, starch, fat, and insoluble and soluble fibers were measured using the markers method at ileal and fecal level. The experiment was carried out on 48 pigs (35 kg). Pigs were distributed over 6 treatments to evaluate the effect of the dietary arabinoxylan content and nutrient density with and without MCC (Rovabio Advance). The graded content of arabinoxylan (AX) was obtained using different raw materials (wheat and wheat by-product). Diet-energy density was modified by the increase of dietary fiber. Measurements indicated that nutrient density and AX content had a significant negative effect on most digestibility parameters. Digestible energy (DE) was significantly increased (86 kcal kg⁻¹) by MCC for the diet with highest AX content. The addition of MCC also resulted in significant improvement in the digestibility of all evaluated nutrients, with average improvements of 3.5, 3.3, 1.2, 1.9 and 1.6% units for ileal OM, ileal protein, fecal OM, fecal CP and energy utilization, respectively. The interaction between MCC and diet composition was significant for the digestibility of energy. Nutrient digestibility and diet DE were negatively correlated with AX content ($P < 0.001$). However, the addition of MCC resulted in a reduction of this negative effect ($P < 0.001$). This study confirms that the presence of AX in wheat-based diets and cereal byproducts reduces nutrient digestibility in pigs. Furthermore, the dietary addition of MCC, which is rich in Xyn and Abf, reduced deleterious effect of fiber and improved overall nutrient digestibility in pig diets. Additional measurement will be provided for the congress in term of amino acids and other nutrients digestibility.

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Comparison of lactose digestive capacity in the small intestine between gilt and sow progeny around birth and weaning

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Differences between gilt (GP) and sow progeny (SP) in terms of gut structure and function and digestive capacity may contribute to reduced growth in GP. We hypothesised that colostrum and milk from gilts would contain lower levels of lactose than that of sows, and GP would have lower specific lactase activity in the small intestine than SP with a lower weight to length ratio (W:L). Colostrum and milk samples were collected from 10 gilts and 10 sows (parities two and three) at farrowing (0h), 24h, and d21 of lactation and analysed for lactose concentration. Additionally, 36 GP and 37 SP were euthanised either at birth (0h), 24h after birth (24h), weaning (PRW), or 24h after weaning (POW). The length and weight of the jejunum and ileum were recorded. A mucosal scraping was taken from the proximal jejunum and the distal ileum and frozen at -80°C, thawed, homogenised and assayed for specific lactase activity. Data were analysed as linear mixed models in SPSS with dam parity, section,

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timepoint and interactions as fixed factors. Specific lactase activity ($\mu\text{mol}/\text{min}/\text{g}$ protein) was higher ($P=0.020$) and W:L of the small intestine tended to be lower ($P=0.082$) in GP compared to SP in the birth cohort (0h and 24h). In the weaning cohort (PRW and POW) there was no difference between GP and SP in specific lactase activity ($P=0.80$), but GP had a lower ($P=0.021$) small intestine W:L. Gilts and sows had similar ($P\geq 0.10$) concentrations of lactose at 0h and 24h; however, lactose tended to be higher ($P=0.056$) at d21 in sows' milk compared to gilts. In conclusion, intake and digestion of lactose in GP may be compromised due to lower lactose levels in gilt milk and reduced gut growth before and after birth compared to SP, despite higher specific lactase activity around birth in GP.

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The effect of soluble dietary fibre on the metabolite profile of pig intestinal tissue

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The intake of soluble dietary fibre (SDF) is known to confer positive health effects including; increased satiety, lowering of dietary cholesterol and reduced insulin response. These health effects may be attributed to the production of short chain fatty acids (SCFA) when the SDF is fermented by bacteria in the large intestine.

Although it has been shown that there is an increase in SCFA levels in the large intestine of pigs fed an arabinoxylan-rich diet, it is not known whether this increase is observed in the surrounding tissue. Tissues were obtained from a cohort of 40 pigs on 4 diets, low meat with and without arabinoxylan (LM, LMAX) and high meat with and without arabinoxylan (HM, HMAX). The large intestine was divided into four sections; caecum, proximal colon, mid colon and distal colon and the extracted SCFAs were analysed using ^1H NMR spectroscopy.

The NMR analysis revealed that pigs fed diets containing arabinoxylan had increased levels of propionate and acetate in their large intestinal tissue compared with pigs on diets without arabinoxylan. Increases in these SCFAs were also observed for the corresponding digesta samples.

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Dietary phytate and phytase levels influence mineral utilisation in weaned pigs

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Phytases are efficient feed enzymes in liberating low bioavailable phosphorus (P) from the anti-nutritional compound, phytate. The hypothesis of the present study was to verify if mineral utilisation in pigs could be affected by different dietary levels of phytate and if graded amounts of phytase could be beneficial.

Seventy-two 28-d old weaned pigs were randomly assigned in 6 groups of 12 animals each. They were fed *ad libitum* throughout a 28-d period a corn/soybean meal based diet according to NRC (2012) recommended nutrient levels excepted for digestible P (0.29%) and total calcium (0.63%) and containing chromium oxide as indigestible marker. The experiment was conducted in a 2x3 factorial design with two dietary phytate levels (0.19 and 0.31%), and three RONOZYME HiPhos phytase concentrations (0.0, 1,000 and 2,000 FTU/kg feed). Feces were collected at the end of the study, frozen at -20°C , freeze-dried, ground, mineralized and analysed for P, calcium (Ca) and chromium content. Apparent total tract digestibility (ATTD) and fecal excretion of P and Ca were calculated. Multi-factorial ANOVA with phytate and phytase dietary levels, and their interaction data, and Student-Newman-Keuls test were used to evaluate the significance among means.

ATTD of P and Ca were reduced ($P < 0.01$) with increasing phytate level and improved ($P < 0.01$) dose-dependently with phytase. Fecal P and Ca excretion were increased ($P < 0.01$) with increasing dietary phytate. Fecal P excretion was dose-dependently reduced ($P < 0.01$) with phytase, whereas fecal Ca excretion was reduced ($P < 0.01$) irrespective of the phytase dosage. High levels of phytate had a negative effect on mineral utilization, probably due to its anti-nutritional effects. Phytase improved mineral utilization and could reduce the deleterious effect of phytate, especially when the dietary level of this latest was high. High concentrations of phytase were needed to partly reduce the decreased mineral utilization in presence of high dietary phytate.

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Effects of dietary phytate and phytase levels on growth performance in weaned pigs

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Phytases are efficient feed enzymes in liberating low bioavailable phosphorus (P) from the anti-nutritional compound, phytate. P is well known to stimulate growth performance in pigs. The hypothesis of the present study was to verify if growth performance in pigs could be influenced by different dietary levels of phytate and especially when combined to graded amounts of phytase. Seventy-two 28-d old weaned pigs were randomly assigned in 6 groups of 12 animals each. They were fed *ad libitum* throughout a 28-d period a corn/soybean meal based diet according to NRC (2012) recommended nutrient levels excepted for digestible P (0.29%) and total calcium (0.63%). The experiment was conducted in a 2x3 factorial design with two dietary phytate levels (0.19 and 0.31%), and three RONOZYME HiPhos phytase concentrations (0.0, 1,000 and 2,000 FTU/kg feed). Average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were assessed for the pre-starter period (d0-14), the starter period (d14-28) and overall (d0-28). Multi-factorial ANOVA with phytate and phytase dietary levels, and their interaction data, and Student-Newman-Keuls test were used to evaluate the significance among means. ADWG was improved ($P < 0.01$) with increased levels of phytate and phytase, during the starter and overall periods. The high phytate level increased ($P < 0.01$) the ADFI during the starter and overall periods. There was a trend for an improved ($P < 0.10$) FCR with increased phytase supplementation during the pre-starter and overall periods. Even there was no dose effect, dietary phytase improved performance with an additional boost effect in diets containing a high phytate level. Growth performance in weaned pigs was positively influenced by dietary high levels of phytate and phytase.

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Phytate esters degradation with graded amounts of phytase at the proximal duodenum in T cannulated pigs

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Feed phytases are hydrolysing the anti-nutritional compound, phytate, in acidic conditions. In pigs, the proximal duodenum, before the income of the endogenous enzyme secretions, could give a good picture of the level of phytate degradation happening in the stomach. Graded amounts of phytase could influence the level and profile of phytate hydrolysis. Five proximal duodenal cannulated pigs (~40 kg initial BW) were fed 5 mash diets in a 5 × 5 Latin square arrangement. All diets were based on corn/SBM/RSM/rice bran and had a 0.34% phytate content. Five diets without addition of mineral P, but fitting the NRC (2012) recommendations for the other nutrients, were supplemented with RONOZYME HiPhos phytase at 0.0, 500, 1,000, 2,000 or 3,000 FTU/kg feed. Pigs were fed *ad libitum* after 16 hours fasting and housed in metabolism pens. Duodenal digesta were collected in plastic bags, 1, 2, 4 and 6 hours

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postprandial. Samples were frozen at -20°C , freeze-dried, ground and analysed for phytate esters (InsP6 to InsP3) and *myo*-inositol content, expressed in $\mu\text{mol/g DM}$. Multi-factorial ANOVA with phytase dietary levels and sampling times, and their interaction data, and Student-Newman-Keuls test were used to evaluate the significance among means. InsP6 and InsP5 duodenal concentrations were reduced ($P < 0.01$) in the phytase groups and with the increasing sampling time ($P < 0.01$). InsP4 and InsP3 concentrations were the lowest ($P < 0.01$), without phytase, mainly due to a lack of InsP6 and InsP5 degradation. On the opposite, InsP4 and InsP3 concentrations were reduced ($P < 0.01$) with increasing phytase inclusion. *Myo*-inositol concentration was higher in the 3000 FTU/kg group. These observations indicated, that total phytate degradation was driven by the phytase concentration especially for a diet with a high dietary phytate content. The phytate esters degradation level and profile at the proximal duodenum was influenced by the dietary phytase inclusion and the sampling time.

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Dietary xylose effects on digestibility, fermentation, and metabolism in pigs

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If xylanase releases free xylose in the small intestine, it is important to understand if, or to what extent, the pig can utilize xylose as an energy source. The experimental objectives were to determine dietary xylose concentration and adaptation time effects on diet digestibility, fermentation characteristics, and xylose metabolism. It was hypothesized that increased xylose would impact fermentation and increase urine xylose and metabolite concentrations. Forty-eight pigs were housed in metabolism crates and randomly assigned to 1 of 4 treatments with increasing D-xylose levels ($n = 12/\text{treatment}$) in a 21-d experiment with 3 collection periods, in 2 replications. The control diet was xylose-free (0%), to which either 2, 4, or 8% D-xylose was added. Adaptation time effects were assessed during 3 fecal and urine collection periods: d 5-7, 12-14, and 19-21. Nutrient digestibility, energy balance, and urine xylose and metabolite concentrations from each collection were analyzed. On d22, pigs from the 0 and 8% treatments were euthanized; cecal and colon digesta were collected. Data were analyzed using SAS with treatment and collection as fixed effects and replication as a random effect. The interaction of treatment collection was analyzed using repeated measures. Dietary xylose did not affect ATTD of DM, GE, or CP; digesta VFA concentrations and molar proportions, and cecal pH were not different ($P > 0.10$). Xylose-containing diets decreased colon pH (6.52 vs. 6.17, $P < 0.05$). As dietary xylose concentration increased, urine GE was increasingly attributed toward xylose, threitol, and other metabolites and less toward N-containing compounds ($P < 0.05$). Urinary threitol represented 11–15% of consumed xylose and increased as xylose consumption increased ($P < 0.05$). In conclusion, xylose was absorbed and partially retained but did not impact digestibility or most fermentation characteristics. Threitol was a major urinary metabolite and increasing dietary xylose decreased its utilization efficiency but pigs may possess adaptation mechanisms to more efficiently utilize xylose.

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Protein digestion in the gastrointestinal tract of pigs involves a one-by-one type of hydrolysis mechanism

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The present study aimed to evaluate the mechanism of protein digestion in the gastrointestinal tract (GIT) of pigs. It was hypothesized that intact proteins are gradually hydrolysed first into intermediate molecular weight (MW) peptides in the proximal part of the GIT and subsequently hydrolysed further into low MW peptides in the small intestine. Forty growing pigs were randomly allocated to one of the five experimental diets containing soybean meal (SBM), wheat gluten (WG), rapeseed meal (RSM), dried porcine plasma protein (DPP), and black soldier fly larvae (BSF) as

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the only source of protein. Diets were fed restrictedly and provided in small portions prior to dissection of the pigs to collect digesta samples from the stomach and four small intestinal segments of equal length. Freeze-dried digesta samples were solubilised in a 2 % SDS solution with 100 mM dithiothreitol and analysed for MW distribution using a size-exclusion chromatography. The chromatograms obtained were separated into MW ranges of >10 kDa, 10-5 kDa, 5-3 kDa, 3-1 kDa and <1 kDa. The amount of 5-10 kDa peptides increased during protein digestion in the stomach for each treatment. In each segment of the small intestine, proteins and peptides mainly had a MW > 5 kDa or < 1 kDa. The MW distribution of proteins and peptides was rather similar in each part of the small intestine and for each of the dietary treatments. A substantial quantity of peptides with a MW < 1 kDa was present in the distal segment of the small intestine of pigs fed the SBM, RSM and BSF diets. In conclusion, protein digestion in the GIT of pigs follows a “one-by-one” type of hydrolysis mechanism, in which digestible dietary proteins are hydrolysed into absorbable small peptides and free amino acids in one sequence once after initial denaturation.

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Protein sources differ in *in vitro* and *in vivo* digestion kinetics in the small intestine of pigs

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Kinetics of protein digestion provides information on the timing of release and absorption of amino acids along the gastrointestinal tract of pigs. The aim of the present study was to determine both *in vitro* and *in vivo* protein digestion kinetics up to the end of the small intestine of pigs for five protein sources (soybean meal (SBM), wheat gluten (WG), rapeseed meal (RSM), dried porcine plasma protein (DPP), and black soldier fly larvae (BSF)). For the *in vitro* study, protein sources were incubated with pepsin at pH 3.5 for 0-90 min and subsequently with porcine pancreatin at pH 6.8 for 0-210 min at 39°C. *In vitro* protein digestion kinetics was described as the kinetics of nitrogen solubilisation. In the *in vivo* study, 40 pigs were randomly allocated to one of the five experimental diets containing the respective protein sources as the only source of protein. TiO₂ was used as marker. Pigs were fed frequently in small portions prior to dissection to collect digesta quantitatively from four small intestinal segments of equal length. Apparent crude protein digestibility and retention time of digesta in each segment were determined to calculate protein digestion kinetics using a first order reaction rate equation. *In vitro* protein digestion rates ranged from 0.026 min⁻¹ for RSM to 0.122 min⁻¹ for WG during the incubation with pepsin, and from 0.027 min⁻¹ for RSM to 0.330 min⁻¹ for DPP during the incubation with pancreatin. *In vivo* protein digestion rates ranged from 0.011 min⁻¹ for RSM to 0.035 min⁻¹ for DPP. *In vitro* protein digestion kinetics during the incubation with pancreatin correlated well to *in vivo* digestion kinetics ($r=0.97$; $P<0.05$). In conclusion, protein sources differ in *in vivo* digestion kinetics in the small intestine of pigs. Differences in protein digestion kinetics can be predicted *in vitro*.

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Digestibility of amino acids and energy in soybean products fed to growing pigs without or with multi-enzyme composite

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Indigestible fiber-protein-phytate complexes reduce feeding value of soy products. We investigated effects of multi-enzyme supplement (MES) on standardized ileal digestibility (SID) of AA and DE content in expeller soybean meal (ESBM) and roasted full fat soybean seed (FFSB) fed to growing pigs. The ESBM was a commercial product and FFSB was roasted at 118-120°C for 75 min in a local farm. The CP was 42.8 and 33.4% DM in ESBM and FFSB, respectively and corresponding values for crude fat were 11.8 and 17.4% DM. Semi-purified diets with 50% of either ESBM or FFSB as sole source of AA were prepared without or with MES supplying phytase, protease, xylanase, β-glucanase at 1,500, 5,625, 270 and 70 U/kg of feed, respectively. Diets had TiO₂ indigestible marker and ratio of

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cornstarch to sucrose and corn oil was identical to our published N-free diet to calculate DE by difference method. Eight ileal-cannulated barrows (BW = 22.1 ± 0.61 kg) were fed 4 diets in a replicated 4 × 4 Latin square design to give 8 replicates/diet. Period lasted for 9 d; 5-d for acclimation, 2-d for fecal and 2-d for ileal digesta samples. Fixed effects of soy product, MES and two-way interactions were evaluated. There was no ($P > 0.05$) interaction or MES effect on SID of AA; ESBM had higher ($P < 0.05$) SID of CP, His, Leu and Lys. There was an interaction ($P = 0.039$) between soy products and MES on DE such that MES increased DE content in FFSB than in ESBM. The DE values for ESBM without and with MES were 4,010 and 4,539 kcal/kg DM, respectively and corresponding values for FFSB were 4,371 and 5,080 kcal/kg DM. In conclusion, MES had no effect on SID of AA, however, improvement in DE content was dependent on soy product type reflective of differences in energy yielding substrates

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Effects of protease on growth performance, fecal gas emission of growing pigs fed low or high density diets

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Volatility in feed ingredient prices prompts animal nutritionists to modulate nutrient density of diets in order to control feed cost. Protease enzyme is an interesting tool to reduce costs and secure animal performance by improving raw material digestibility. This trial aimed to demonstrate usefulness of protease enzyme in grower pigs fed either high or low-density diets. A six weeks pen study was conducted using 140 pigs [(Landrace × Yorkshire) × Duroc] with an initial body weight (BW) of 24 kg equally distributed in 28 pens with seven replications fed one of the following four treatments: HD: High density diet with 3400 kcal ME/kg and 19.5 % CP; HDP: HD + 125 g/t Jefo Protease (Jefo, Canada); LD: Low density diet with 3300 kcal ME/kg, and 17.6 % CP; and LDP: LD + 125 g/t Jefo Protease. Data were subjected to statistical analyses (GLM procedure, SAS 1996) as a randomized complete block design using a 2 × 2 factorial arrangement. Differences among treatment means were determined using Duncan's multiple range test with level of significance at $P \leq 0.05$. Overall, higher density diets ($P < 0.05$) and protease supplementation ($P = 0.05$) significantly improved gain/feed (GF) in pigs. Protease significantly improved GF in HD diets (0.488 vs 0.461) and numerically in LD (0.454 vs 0.440) with GF in LDP not statistically different than in HD treatment. Protease numerically improved pigs final BW in LD (54.0 vs 53.2 kg) and HD (55.8 vs 54.4) diets. NH₃ gas emission tended to be lower ($P = 0.06$) in low and high-density feeds supplemented with protease (4.6 vs 4.9 ppm and 4.5 vs 4.8 ppm for low and high-density feeds, respectively). In conclusion, protease supplementation has potential to improve gain/feed and allow growing pigs fed on low-density diet to achieve growth performance level comparable to pigs fed on a higher nutrient density diet without protease.

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Join dynamics of feeding behavior and plasmatic metabolites in voluntary fed pigs

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Variations in feeding behavior between animals result from individual variations in their metabolism as affected by diet composition. This study aimed to link the dynamics of voluntary feed intake and those of blood metabolites in 36 pigs (BW 35 kg) fed *ad libitum* with diets differing by fiber content (13 or 18% NDF for LF and HF by addition of wheat bran, soybean hulls and sugar beet pulp) and aleurone supplementation (0, 0.2, or 0.4% for A0, A2 or A4). Feeding behavior was individually recorded during one week. The kinetics of plasmatic metabolites was followed 1 hour after a voluntary test meal. Dietary fiber increased the number and size of meals (8.2 meals of 178 g vs 9.4 meals of 150 g, $P < 0.01$) without change in daily feed intake. Aleurone supplementation (A4 vs A0) decreased the number of meals (-1.5, $P < 0.01$) and daily feed intake (-270 g/d; $P < 0.01$). Dietary fiber affected insulin and urea kinetics ($P < 0.05$) while aleurone modified those of insulin, alpha-amino nitrogen and free fatty acid ($P < 0.05$), and

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this of lactate ($P=0.06$). Multifactorial Factor Analysis discriminated four clusters (5, 6, 5 and 6 pigs) according to feeding behavior, preprandial free fatty acid and urea concentrations, and size of the test meal. Postprandial lactate, urea, free fatty acid and alpha-amino nitrogen kinetics differed between clusters. Dietary treatment only partially agreed with cluster classification due to large individual variability of the voluntary test meal and associated metabolic responses. To conclude, metabolic responses were more affected by animal characteristics than diet composition. Individual variability should be considered when diet composition is used to modulate feeding behavior.

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Meat or fish but not blood meal can replace half of protein contribution of 22% soybean meal in weaner diet

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Replacing soybean meal (SM) in weaner pig diets with quality animal proteins may improve growth performance. Two experiments were conducted to investigate whether blood meal (BM; 88% protein), meat meal (MM; 60% protein) or fish meal (FM; 64% protein) can replace half of the protein contribution of SM (47% protein) in a control weaner diet with SM as the main protein source. The control SM diet contained 22% SM which constituted 49.5% total dietary protein. The BM, MM and FM diets replaced 10% dietary SM (equivalent to 45% its protein contribution) using 5.7%, 9.0% and 8.3% respectively. Essential amino acids, energy, calcium and phosphorus were balanced, and analysed crude protein was 21% in all diets. Sixty and 40 individually housed 26-day-old male weaners (av. 6.1 kg) were allocated into the four diets for 21 days in Experiment 1 (*ad libitum* fed) and 2 (restricted to 2.5 times of maintenance energy requirement) respectively. Growth performance and constipation (Bristol's stool score = 1) frequency were analysed by ANOVA and *Chi*-square analysis respectively. In Experiment 1 BM reduced average daily feed intake (ADFI) and average daily gain (ADG) by 20% (both $P < 0.05$) compared with the other diets that had similar ADI and ADG. The FM diet tended to have the greatest gain to feed ratio (G:F) ($P = 0.067$), whereas G:F was similar among the other diets. Constipation was only observed in BM (38%) and FM (14%) group ($P < 0.05$). In Experiment 2 the feed restriction eliminated palatability-caused difference in ADFI ($P = 0.34$). The FM diet achieved the greatest ADG and G:F (both $P < 0.05$), while the other diets were similar among each other. In summary, replacing 10% dietary SM with 5.9% BM in a weaner diet caused constipation and reduced ADFI; however, the replacement with 9.0% MM and 8.3% FM achieved similar and improved growth performance respectively.

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The influence of liquid feeding on growth performance, nutrient digestibility and intestinal microbiota of fattening pigs

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An experiment was carried out to evaluate the effect of liquid feed added enzymes and soaking in fattening pigs. One hundred sixty male and female Pietrain*(Landrace*LargeWhite) pigs, weighing 25kg liveweight were distributed into 8 blocks according to sex and liveweight, and housed in 40 pens (4/pen) until reaching the slaughter weight. Intra-block pigs were randomly allocated to 5 treatments corresponding to a diet without (T1, T3) or with carbohydrases inclusion (T2, T4, T5), distributed as dry pellets (T1, T2) or as liquid feed prepared just before distribution (T3, T4) or after 8h soaking (5). Faeces samples were collected at around 50kg bodyweight for major nutrient digestibility analysis. At the end, one pig per pen was slaughtered to collect samples of ileum digesta and to determine gut microbiota profile. Despite a numerical improvement on final bodyweight, differences on growth performance or feed conversion did not reach the statistical significance ($P > 0.10$). Feed intake during growing phase was higher for pigs fed with liquid ($P < 0.001$) than on dry form and a similar tendency was observed overall ($P < 0.10$). Furthermore,

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digestibility of dry and organic matter, crude protein ($P < 0.01$), energy ($P < 0.05$), arabinose and xylose, and phosphorus ($P < 0.001$) was improved with soaking. No effect of enzyme supplementation was observed on any parameter analysed. The microbiota profile of the ileum showed some significant differences: *Lactobacillus* spp were higher on pigs fed with liquid diets whereas *Clostridium* spp were more abundant on dry fed pigs. In conclusion, liquid and soaking diets increase nutrient utilisation and gut health, and can be beneficial to improve growth performance of fattening pigs.

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The influence of soaking and phytase inclusion on growth performance, nutrient digestibility and intestinal microbiota of liquid fed pigs

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An experiment was conducted to determine the effect of soaking and enzyme addition on pigs raised with liquid feeding. One hundred forty-four male and female Pietrain*(Landrace*Large White) pigs of 30kg liveweight were distributed into 12 blocks according to sex and liveweight, and housed in 36 pens (4/pen) until reaching the slaughter weight. Intra-block pigs were randomly allocated to 3 treatments corresponding to a diet without (T1) or with phytase inclusion, distributed as liquid feed immediately after preparation (T2) or after 8h soaking (T3). Faeces samples were collected at around 60kg bodyweight for major nutrient digestibility analysis. At the end, one pig per pen was slaughtered to collect ileum and cecum digesta samples and determine gut microbiota profile. Soaking improved feed intake during the finishing phase and overall ($P < 0.001$). A tendency to improve weight gain with soaking was also detected ($P < 0.10$) during the growing phase but differences were not significant at the end of the trial, and no effect on feed efficiency was detected. Major nutrient digestibility was not affected by dietary treatments. However, ash, calcium and phosphorus digestibility was significantly improved with both phytase inclusion and soaking ($P < 0.01$). Microbiota profile showed clear differences between ileum and cecum samplings but only minor differences were observed due to dietary treatments. In conclusion, adding phytase or soaking diets of liquid fed pigs increased mineral utilisation and may be beneficial to improve gut health, and growth performance of fattening pigs.

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Mechanisms and kinetics of starch digestion in growing pigs fed processed and unprocessed cereal based diets

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Starch is the main source of energy in commonly used pig diets and digestion kinetics is a.o. influenced by structural properties of starch, the feed matrix, and processing conditions. Variation in digestion kinetics affects feeding patterns, energy partitioning and protein utilization in pigs. We hypothesised that *in vitro* starch digestion kinetics would correctly predict *in vivo* starch disappearance throughout the small intestine of growing pigs. We assigned 90 pigs (23 kg BW) to one of 9 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. After an adjustment period, pigs were hourly fed for 6h to reach a steady state, after which digesta retention times and starch digestion coefficients (DC_s) were measured in 4 parts of the small intestine (SI1-4) using inert markers. Granule degradation was monitored by scanning electron microscopy and soluble degradation products were studied by chromatography. From proximal to distal SI, DC_s ranged between 0.42-0.97 for barley diets, 0.42-0.94 for corn diets and 0.44-0.71 for high amylose corn diets. From proximal to distal SI, 47-74% of the undigested starch consisted of oligomers in pigs fed barley diets,

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while this fraction was only 30-39% for corn and 20-26% for high amylose corn. Microscopy analysis revealed that in pigs fed ground corn and high amylose corn diets, part of the starch granules was still intact in SI4. The maximum DC_s of these diets was generally reached in SI3. After adjusting the incubation time of *in vitro* analysis (120 min) to *in vivo* measured SI retention times (210-310 min), *in vivo* digestion kinetics could be accurately predicted for most diets. However, this analysis underestimated high amylose corn DC_s in the proximal SI, while it overestimated ground corn DC_s in the distal SI.

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Rate of amino acid disappearance in the small intestine depends on the amount of digested protein arriving from the stomach

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Several studies have suggested that the rate of amino acid absorption in the small intestine (SI) is influenced by either the extent of gastric digestion or gastric emptying rate. Actinidin, a cysteine protease in kiwifruit (KF), increases both parameters in beef-muscle protein. This effect was used to determine how both parameters individually or combined (rate of digested protein entering the SI) affect rate of amino acid disappearance in the SI. Ninety entire male pigs (28 ± 2.9 kg (± SD) bodyweight) received a beef meat-based diet for three days with actinidin (green KF pulp or gold KF pulp supplemented with actinidin) or without actinidin (gold KF pulp alone). On day 3, pigs were euthanized at 0.5, 1, 3, 5 and 7 h postprandially. Protein present in stomach chyme was used to determine the rate of digested protein entering the SI, while proximal, medial and distal SI digesta were used to determine the apparent amino acid digestibility (AAAD) at set-time points. Factorial (diet x time) and correlation analyses were performed in SAS. The rate of digested protein entering the SI and the AAAD at the proximal and medial SI were higher (P < 0.05) at set-times with actinidin, but not in general for the distal SI AAAD (P > 0.05). For the proximal SI, the AAAD was more closely correlated with digested protein entering the SI (r = 0.73, P < 0.001; n = 57) than it was with gastric emptying (r = 0.64, P < 0.001; n = 57) or gastric protein digestion (r = 0.57, P < 0.001; n = 57). Similar trends were observed for the medial SI. In conclusion, the rate of digested protein entering the SI is an accurate predictor of the rate of AAAD in the SI. Proteins entering the SI highly digested are mainly digested and absorbed in the first half of the SI, while proteins entering the SI poorly digested are digested and absorbed throughout the whole SI.

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Can intestinal phosphorous absorption and excretion in urine be used to quantify phytate-phosphorus release by phytase in phosphorus (P)-adequate diets?

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Microbial phytase is widely used to enhance digestibility of phytate-P. By tradition, P-deficient diets are used to quantify phytate-P release by phytase, but P-adequate diets may be more physiologically relevant. We tested the hypothesis that P digestion and urinary P excretion would increase quantitatively as phytase level increased in a P-adequate diet, and that quantification of urine P output would estimate P release. Three replicates of 24 barrows each (BW = 22.95 ± 1.87 kg) were randomly assigned to one of eight treatments, housed in individual pens for 21d, then moved to metabolism crates for five-d urine and fecal collections. A basal corn-soybean meal diet (T1) was formulated at 0.36% STTD-P and total Ca:STTD-P at 2.03. Phytase (*modified E. coli*) was added at 200, 400, 600, and 800 FTU/kg (T2-T5, respectively). For T6, monocalcium phosphate was used to increase STTD-P to 0.52%. For T7, monocalcium phosphate was reduced to lower STTD-P (0.21%, slightly below requirement) and 200 FTU/kg

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phytase was added to T7 to create T8. Pig was the experimental unit; replicate was a fixed effect. For T1-T5, orthogonal polynomial contrasts tested linear and quadratic effects of phytase within P-adequate diets. Phytase increased percent ATTD (quadratic $P < 0.0001$) and quantity of absorbed P (linear $P < 0.0001$; quadratic $P = 0.069$). Urinary P increased linearly with phytase inclusion ($P < 0.0001$). In conclusion, increasing phytase resulted in a linear increase in urinary P, indicating that it may be a candidate to quantify P release when diets are not deficient in P. By relating increases in urinary P to phytase level, P release may be estimated.

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Basal ileal endogenous losses of amino acids in pigs determined by feeding nitrogen-free diet, low-casein diet, and regression analysis

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Basal ileal endogenous losses (BEL) of amino acids (AA) have been determined by feeding nitrogen-free diet (NFD) or diet containing low concentration of casein, or by regression analysis. Thus, this study was conducted to test the hypothesis that three methods, feeding NFD, low-casein diet, or regression analysis, give similar estimates of BEL of AA in pigs. Eighteen barrows (initial body weight = 50.5 ± 4.46 kg) surgically fitted with T-cannulas at the distal ileum were used. Pigs were assigned to triplicate 6×3 incomplete Latin square design with six diets and three periods. Nitrogen-free diet was prepared based on cornstarch and dextrose. Three diets were prepared to contain 60, 100, or 140 g/kg casein. Two additional diets were prepared for another objective of experiment. The BEL of AA as mg/kg dry matter intake (DMI) in pigs fed NFD and diet containing 60 g/kg casein (CAS60) were calculated based on the assumption that AA contents in ileal digesta samples were originated from endogenous losses. Regression analysis between apparent ileal digestible and dietary AA concentration (mg/kg DMI) in diets containing casein was conducted to estimate the BEL of AA as y-intercept. Pigs fed CAS60 had greater ($P < 0.05$) BEL of Ile, Glu, and Ser than those fed NFD; however, the BEL of other AA in pigs fed NFD was not different from those in pigs fed CAS60. The BEL of AA in pigs determined by feeding NFD or CAS60 was not different from the values determined by regression analysis. The BEL of Lys in pigs determined by NFD, CAS60, and regression analysis was 306, 494, and 464 mg/kg DMI, respectively. In conclusion, there was no difference among the BEL of AA, except for Ile, Glu, and Ser, in pigs determined by feeding NFD, low-casein diet, and determined by regression analysis.

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Grain digestibility affects the extent of large intestinal fermentation and growth of pigs

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Fermentation in the large intestine (LI) reduces available energy and pig growth rate due to heat of fermentation, methane release and microbes voided in faeces. Sorghum is less digestible than wheat, but steam-flaking increases digestibility of both grains. The hypothesis was that reduced grain digestibility in the small intestine (SI) would increase LI fermentation with reduced faecal-pH and increased SCFA-concentration, concomitant with reduced average-daily-gain (ADG) and increased feed-conversion-ratio (feed:gain; FCR). Four nutritionally balanced diets: milled-sorghum (MS), steam-flaked-sorghum (SFS), milled-wheat (MW) and steam-flaked-wheat (SFW) were randomly assigned to eight male Large White pigs (16.83 kg (mean); 0.640 (sd)) per diet. After one week acclimatisation, pigs were fed *ad libitum* for three weeks. Average-daily-feed-intake (ADFI), ADG and FCR were determined for periods 7- 14, 14-21, 21-28 and 7- 28d. Faecal-pH and SCFA-concentrations were measured on 7, 14, 21 and 28d. A linear-mixed model was fitted to

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ADFI, ADG, FCR, faecal-pH and SCFA-concentration with grain-type, process-type, and grain-type: process-type interaction as the main effects for each time period. A linear model was fitted between FCR and: 1) pH, 2) SCFA-concentration. Sorghum showed 5% and 7% higher FCR than wheat (1.511, 1.490 cf 1.429, 1.39) during 7-14d ($P < 0.05$) and 14-21d ($P = 0.001$) respectively. Sorghum showed 5% lower ADG than wheat (0.877 cf 0.928 kg) at 14-21d ($P = 0.022$). On 14, 21 and 28d, faecal-pH for sorghum was lower than wheat ($P < 0.001$) by 0.4 units. SCFA-concentrations were higher for sorghum than wheat at 7d and 28d ($P < 0.1$). SCFA-concentration was positively correlated to FCR during 14-21d ($R^2 = 0.80$, $P = 0.105$) and 21-28d ($R^2 = 0.83$, $P < 0.1$). Faecal-pH was correlated negatively with FCR during 7-14d ($R^2 = 0.84$, $P < 0.1$), 14-21d ($R^2 = 0.96$, $P = 0.02$), and 21-28d ($R^2 = 0.84$, $P < 0.1$), and positively with ADG during 14-21d ($R^2 = 0.91$, $P < 0.05$). In conclusion, assumed low grain digestibility in the SI increased LI fermentation, which subsequently reduced ADG in pigs.

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Fibre-type affects intake of a highly digestible diet – Potential manifestation of feed hydration capacity and colonic fermentation controlling digesta-passage-rate

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Since fibre is not digested in the small intestine (SI) and adds to the digesta bulk, it stimulates digesta-passage-rate, and therefore feed intake (FI) up to the point where gastric distension slows gastric-emptying-rate. Fermentable fibre in the large intestine (LI) stimulates the colonic brake (CB), which reduces digesta-passage-rate and FI.

The hypothesis was that fibre-type and amount alters FI of a highly digestible starch-based diet. Poorly fermentable oat hulls (OH) and more fermentable wheat bran (WB) were added at 2.5, 5, 10, 15 & 20% and 5, 10, 15, 25 & 35%, respectively to a base diet containing starch, dextrose, fishmeal and soy-proteins as the main ingredients. Five pigs [male, Large White, BW: 19.7(0.88)kg: mean(sd)] were assigned randomly to each diet. *Ad libitum* average-daily-feed-intake (ADFI) was measured for periods 0-7, 7-14, 7-21 and 0-21d. Base diet intake (BDI) during 7-21d was calculated from ADFI. A linear-mixed model was fitted to ADFI data. Hydration capacity (HC, ml/g), a measure of swellability, of the feeds was measured and analysed using One-Way ANOVA.

ADFI of OH diets was higher ($P = 0.053$) than WB-diets for 0-7, 7-21 and 0-21d. BDI was related inversely with HC of WB and OH diets. WB dose dependently increased ($P < 0.001$) the diet HC. OH (10%) showed higher ($P < 0.001$) HC than other OH diets. The lower BDI with 10% OH could be explained by its higher HC than other OH diets. WB at 5-10% caused a decline of ~4% in BDI, and from 10% to 35% WB resulted in a steady decline of up to 30%.

Overall, fibre-type affected ADFI. BDI highlights a potential interaction of three mechanisms affecting intake: initial increase in intake and digesta-passage-rate due to increased fibre bulk; slow gastric-emptying-rate from higher HC reducing FI, and decrease in digesta-passage-rate and FI due to CB.

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Mycotoxin binder influences digestibility and nutrient transporters gene expression of pigs offered wheat based diets grown under different agronomical conditions

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Abstract

Cereal grains vary in nutrient composition due to different cultivars and agronomic conditions which in turn may affect nutrient digestibility in animals. It was hypothesised that low quality wheat grain, grown under different agronomical conditions would negatively affect nutrient digestibility and intestinal nutrient transporters in pigs compared to feeding high quality wheat grain. The addition of a mycotoxin binder would negate the effects of the low quality grain. Sixty-four pigs (38.7 kg (SD 3.48 kg)) were assigned to one of four dietary treatments: (T1) low quality wheat diet, (T2) low quality wheat diet plus a mycotoxin binder, (T3) high quality wheat diet, (T4) high quality wheat diet plus a mycotoxin binder. The inclusion of wheat was 500 g/kg. The mycotoxin binder used was a Hydrated Sodium-Calcium-Aluminum-Silicate, which also included calcium propionate and calcium formate. The low quality wheat grain had a higher levels of zearalenone, aflatoxin and ochratoxin. The data was analysed using Proc Mix and the model included the effects of wheat, binder and their interaction. Pigs offered the low quality wheat diet had a reduced coefficient of apparent total tract digestibility (CATTD) ($P < 0.05$) of nitrogen (N) and gross energy (GE) compared to the high quality wheat diets. The inclusion of a binder increased the CATTD of N and GE. There was a wheat \times binder interaction on the expression of peptide transporter 1 gene (*SLC15A1/PEPT1*) and sodium-glucose linked transporter 1 gene (*SLC5A1/SGLT1*) ($P < 0.05$) in the duodenum. Pigs offered the low quality wheat with a mycotoxin binder had lower expression of *SLC15A1/PEPT1* and *SLC5A1/SGLT1* expression compared to the low quality wheat diet only. However, there was no response to mycotoxin binder supplementation with the high quality wheat diet. In conclusion, the low quality wheat reduced nutrient digestibility and modified the gene expression of genes involved in intestinal nutrient transport.

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Effects of nutrient solubility and feeding level on digesta passage rate through the proximal gastrointestinal tract of growing pigs

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To better predict the kinetics of metabolic nutrient availability in pigs, more knowledge is needed on nutrient digestion kinetics, and the underlying process of digesta passage through the gastrointestinal tract (GIT). It is known that, stomach emptying of liquids is faster than of solids, and nutrient solubility varies between feed ingredients. An increase in dietary nutrient solubility (S) or feeding level (F) was expected to decrease stomach emptying via the triggering of feedback mechanisms in the proximal GIT. We hypothesized that soluble nutrients, that pass with liquids, quickly reach the small intestine after ingestion thereby triggering the feedback mechanism. Therefore, the effect of S and F on the mean retention time (MRT; i.e. inverse passage rate) of digesta was studied in 40 male growing pigs. Four dietary treatments were evaluated: low S, at low and high F (LF-LS vs. HF-LS); medium S at high F (HF-MS); high S at high F (HF-HS). The MRT of solids (TiO₂) and liquids (Cr-EDTA) in the stomach, proximal-, and distal small intestine was determined, using the slaughter method in pigs fed to steady-state. Fixed effects of S and F were analysed using a general linear model. When S increased (HF-LS vs. HF-MS vs. HF-HS), stomach MRT of solids and liquids decreased from MS to HS (8.0 and 3.4 vs. 5.5 and 1.7 h; $P < 0.01$). When F increased (LF-LS vs. HF-LS), stomach MRT of solids and liquids increased (5.1 and 1.5 vs. 7.0 and 2.8 h; $P < 0.05$), and the MRT of solids decreased (4.4 vs. 3.6 h; $P = 0.01$) in the distal small intestine. Concluding, dietary nutrient solubility affected the stomach MRT in a non-linear fashion. Increasing feeding level increases the MRT of solids and liquids in the stomach, but decreases MRT of solids in the distal small intestine.

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The effects of diet viscosity on the passage rate and physicochemical properties of digesta in the digestive tract of pigs

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To better predict the kinetics of metabolic nutrient availability in pigs, more knowledge on nutrient digestion kinetics is needed. The kinetics of nutrient digestion is affected by digesta passage through the gastrointestinal tract (GIT). Diet viscosity can alter digesta passage kinetics by physically increasing the resistance of digesta to flow, and by affecting nutrient degradation and absorption. The effects of diet viscosity, induced by oat beta-glucans (BG; PromOat), on digesta mean retention time (MRT; i.e. inverse passage rate) was studied in a dose-response trial. Twenty male growing pigs were fed one of five nutrient balanced diets with incremental BG content (BG0%, BG2.5%, BG5%, BG7.5%, BG10%). The MRT of solids (TiO₂) and liquids (Cr-EDTA) in six consecutive GIT segments (stomach; small-, and large intestine (each two parts); and caecum) was determined, using the slaughter method in pigs fed to steady-state. Effects of BG on digesta MRT and physicochemical properties were analysed using linear regression. The stomach MRT of liquids, but not of solids, increased with increasing BG intake (BG0% to BG10%: 1.3 to 3.3 h; P=0.008), while digesta viscosity, dry matter content, and water-binding capacity in the stomach decreased. Moreover, the separation of solids and liquids in the stomach, and their difference in stomach MRT, decreased when BG intake increased. In the distal large intestine, the MRT of liquids tended to be increased (BG0% to BG10%: 6 to 9.2 h; P=0.08), and digesta water-binding capacity increased when BG intake increased. Beta-glucan intake did not affect the MRT in other GIT segments. In conclusion, diet viscosity increases the stomach MRT of liquids, and reduces the difference in stomach MRT between solids and liquids. Also, diet viscosity tends to increase the MRT of liquids in the distal large intestine. In addition, diet viscosity affects physicochemical properties of digesta in these GIT segments.

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Neonatal iron deficiency anemia in pigs: state of art, new oral procedures of IDA prevention using heme and Sucrosomial[®] iron

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Neonatal iron deficiency anemia (IDA) commonly occurs in pigs regardless of the breed and the system of piglet rearing. The major etiological factors include low hepatic iron stores in newborn piglets (resulting from the deployment of iron transferred from the mother among increasing number of fetuses), their intensive growth, inadequate levels of iron content in sow's milk and the immaturity of molecular mechanisms of iron absorption. To counteract development of IDA, an early administration of exogenous iron to piglets is an obligatory treatment. Whereas dietary iron supplements show limited preventive/therapeutic efficacy, the intramuscular injection of large amounts of iron dextran (FeDex) has been proved to be beneficial in correcting IDA. However, our studies clearly show that this procedure poses a risk of toxicity induced by redox active iron unduly stored in the liver. Furthermore, increased hepatic iron deposits entail overexpression of hepcidin, which inhibit both iron absorption from the gut and its redistribution from the liver to the circulation. We therefore established several optimized protocols of piglets supplementation with iron aiming at meeting iron demand for erythropoiesis, attenuating upregulation of hepcidin expression, and minimizing iron toxicity. Importantly, we used heme as a source of highly bioavailable dietary iron to rescue piglets from severe IDA and we determined pathway of its absorption across the duodenum. Finally, we demonstrated for the first time a high therapeutical potential of orally given Sucrosomial[®] Iron (SI) to combat IDA in pig neonates. We suggest that oral application of SI to newborn piglets may be an effective and reliable option for IDA treatment and hypothesize that high bioavailability of SI does not rely on the canonical iron absorption pathways in duodenum.

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Processing of soybean meal enhanced ileal digestibility of protein and amino acids in weanling pigsM Ton Nu¹, H Schulze¹ and R Zijlstra²¹Agilia; ²University of Alberta

Processing of soybean meal (SBM) aims to reduce anti-nutritional factors (ANF), unfold and denature of SBM protein, and alter fibrous structure. Consequently, better access to digestive enzymes is provided that may increase ileal protein and amino acid (AA) digestibility in young pigs. The objective of the study was to investigate effects of thermo-mechanical, enzyme-facilitated processing of SBM on ileal digestibility of protein and AA in weanling pigs. Eight barrows (Large White × Duroc) were weaned at 21 days and surgically fitted with a T-cannula at the terminal ileum (BW 9.43 ± 0.40 kg). Pigs were randomly assigned to a Youden square with 3 diets over 4 periods. The study included a N-free diet and two experimental diets using 40% SBM, either processed or non-processed, as sole source of AA. Cr₂O₃ was used as an indigestible marker. Each period included sequentially a 5-d adaptation, 2-d collection of feces and 2-d collection of ileal digesta. Data were analyzed with pig as experimental unit, processing as fixed effect and period and pig as random effects. Processing reduced content of ANF like lectin, trypsin inhibitor and β-conglycinin in SBM by up to 90%. Processing of SBM increased (P < 0.001) standardized ileal digestibility (SID) of protein from 88.4% to 90.3% and increased (P < 0.001) the SID of all indispensable AA on average by 2.9%. Processing did not decrease chemically-available lysine (Lys) content or ratio of Lys to protein in SBM but increased (P < 0.001) SID of Lys and chemically-available Lys by 3% compared to non-processed SBM. In conclusion, processing of SBM enhance digestibility of Lys and protein without damaging Lys, thereby making processed SBM a more suitable protein source for weaned pigs. Additionally, understanding the impact of processing enables an accurate means to predict the nutritive value of processed SBM.

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Effect of controlled cereal fermentation and carbohydrase supplementation on growth and digestibility in grow-finishing pigsA Torres-Pitarch^{1,2}, G E Gardiner³, J V O'Doherty² and P G Lawlor¹¹Teagasc, Animal and Grassland Research and Innovation Centre; ²School of Agriculture and Food Science, University College of Dublin; ³Department of Science, Waterford Institute of Technology

Feeding a liquid diet where the cereal fraction is fermented can improve growth. Xylanase + β-glucanase (XB) are carbohydrases used to improve nutrient digestibility but may also influence the fermentation of cereals. It was therefore hypothesized that feeding cereal-fermented (cFerm) diets ± XB would improve growth by increasing nutrient digestibility. A total of 252 pigs (31.0kg ± 0.65 SEM; same sex pens of 7 pigs/pen) were allocated to 1 of 4 dietary treatments in a 2x2 factorial arrangement: (1) non-fermented liquid feed (nFLF); (2) cFerm liquid feed (cFLF); (3) nFLF + XB and (4) cFLF + XB. The cereal fraction of the diet (35% wheat, 38% barley, 11% pollard) was fermented using a *Pediococcus acidilactici*/*Lactobacillus plantarum* starter culture. Pigs were fed the liquid diets (28.6% DM) for 55 days and average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Feed and faecal samples were collected two days before slaughter for apparent total tract digestibility (ATTD). At slaughter, carcass weight, muscle depth and fat depth were recorded and digesta from the terminal ileum was collected for apparent ileal digestibility (AiD). The data were analysed using the MIXED procedure of SAS. Pigs fed cFLF and cFLF + XB had higher final live weight than pigs fed nFLF (P < 0.01). No cFerm × XB interaction was found for any other variable of interest. Pigs fed cFerm diets had increased ADG (P < 0.01); higher dry matter (DM), organic matter (OM) and crude protein (CP) ATTD (P < 0.001); heavier carcasses (P < 0.01) and lower lean meat

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percentage ($P < 0.05$). Pigs fed XB diets had an improved FCR ($P < 0.05$); higher AiD of DM, OM and CP ($P < 0.05$) and higher ATTD of DM, OM and CP ($P < 0.001$) than non-supplemented pigs ($P < 0.05$). In conclusion, cForm improved pig growth due to increased ATTD and XB supplementation improved FCR due to increased AiD and ATTD of nutrients.

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Concentrations of digesta metabolites in growing pigs are influenced by dietary energy level, but not by dietary amino acid level

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The formulation of swine feeds affects the composition of intestinal digesta, but the degree of influences is not defined. In this study, total 160 pigs (initial weight: 31.77 ± 3.57 kg) were allotted to 40 pens and four treatments with 4 pigs per pen and 10 replicate pens per treatment. Pigs were fed experimental diets for 90 days using a 3-phase feeding program. Diets were formulated using a 2×2 factorial design with 2 levels of dietary energy (high or low) and 2 levels of dietary amino acids (high or low). In these diets, concentrations of amino acids were adjusted mainly by the inclusion rates of soybean meal, and energy concentrations were adjusted by the inclusion rates of choice white grease and distillers dried grains with solubles. On d 90, one pig per pen was harvested and samples of ileal and cecal digesta were collected. Samples were immediately stored in liquid nitrogen and later analyzed using liquid chromatography-mass spectrometry (LC-MS)-based targeted and untargeted metabolomic analysis. The LC-MS data were modeled by multivariate analysis, and concentrations of metabolites in digesta were compared using Proc GLM of SAS. The distribution of samples in the multivariate models indicated that dietary energy concentration, but not amino acid concentration, resulted in compositional differences in both ileal and cecal digesta. Increased dietary energy, but not increased amino acid concentration, increased ($P < 0.05$) concentrations of essential amino acids (lysine, threonine, methionine, and valine), and fatty acids in the cecal digesta. Reduced dietary energy was associated with increased ($P < 0.05$) bile salts in the ileal digesta. Overall, the results were consistent with the growth performance data of the animals, indicating that dietary energy level influences metabolic status of growing pigs to a greater extent than dietary amino acid level.

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Comparison of dietary protein bioavailability using a new dual stable isotope ratio method with direct ileal digestibility assessment in pigs

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Objectives Ileal digestibility of proteins is a crucial factor in selecting high quality proteins for human and pig diets. However, measuring ileal digestibility is difficult because the ileum is only accessible via fistulation or naso-ileal intubation. Therefore less invasive methods to measure bioavailability of proteins are needed. We aim to develop a dual stable isotope method to evaluate bioavailability of proteins. Here, we evaluated differences between this new isotope method and direct assessment of ileal protein digestibility, with the hypothesis that both methods should not differ.

Methods Eight pigs were fitted with jugular catheters and ileal T-cannulas. At the test day, feed, containing intrinsically labelled ¹⁵N-milk protein (2.3 atom%, 147g/kg DM) and ¹³C-spirulina (98 atom%, 0.019g/kg DM), was provided every half hour, during 4h. Ileal digesta and ten jugular blood samples were collected over nine hours.

Abstract

Digesta samples were analyzed for isotopic enrichment, nitrogen, and titanium contents. For the dual isotope method, serum total amino acid enrichment was measured. The serum $^{15}\text{N}/^{13}\text{C}$, relative to that in the diet was considered as measure of relative difference in protein digestibility between the two protein sources.

Results Using the standard ileal digestibility assay, apparent and true nitrogen ileal digestibility of the milk protein equaled $86.6 \pm 3.57\%$ and $97.0 \pm 0.65\%$ respectively. The average true AA digestibility of spirulina was $73.9 \pm 2.71\%$. The calculated protein digestibility of spirulina was $51.9 \pm 13.72\%$ with the dual stable isotope method, assuming 97% digestibility of milk protein.

Conclusions With the current approach, detectable enrichment of ^{13}C and ^{15}N in serum total free amino acids were obtained and, together with the enrichment levels in the meal, allowed the calculation of the digestibility spirulina protein, relative to milk protein. The spirulina protein digestibility was noticeably lower when using the dual stable isotope ratio method, suggesting that its protocol and analysis needs further optimization before application.

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Growth performance of pigs fed diets differing in resistant starch

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Microbes present in the intestinal tract ferment starch that escapes enzymatic digestion, so called resistant starch (RS). Predictive net energy (NE) equations used for pig feed formulation assume that RS has a 30% lower NE value than digested starch, and consequently, negatively affects growth performance. However, others have shown that the difference in NE efficiency is much smaller (Gerrits *et al.*, 2012; Da Silva *et al.*, 2014). This study aims to investigate the effect of feeding diets high (H-RS) or low in RS (L-RS) on growth performance and slaughter characteristics in pigs. We hypothesized that a more gradual energy supply by feeding H-RS vs. L-RS reduces fat deposition, while growth rates remain unaffected. Twenty pigs (40 ± 2.82 kg) were fed either a diet containing 50% waxy maize starch (L-RS) or high-amylose maize starch (H-RS), with TiO_2 as indigestible marker. After 14 days of adaptation to the diets, pigs were fed hourly for 6h to reach steady state, dissected, and rectal digesta samples were collected to measure starch fermentation (Gerrits *et al.*, 2012). Simultaneously, 48 groups of six pigs (80 ± 0.48 kg) were fed one of the two diets and slaughtered at 115 kg. Feed intake, growth, and slaughter characteristics were measured. Total tract starch fermentation was greater for H-RS than L-RS (33% vs. 10%; $P < 0.001$), resulting in 15% less enzymatic digested starch for H-RS ($P < 0.001$). Feed efficiency was 1%-unit greater for H-RS than L-RS ($P = 0.027$), however, coincided with a 1%-unit reduction in carcass percentage ($P < 0.001$) resulting in a similar carcass growth efficiency between diets. Diets did not affect carcass growth rate, back-fat-thickness, and lean meat percentage differently. In conclusion, a substantial increase in RS did not affect growth performance, indicating that when carcass growth rates and efficiencies are considered as response parameters, NE values of digested starch and fermentable RS are similar.

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Ileal starch fermentation in pigs fed diets differing in resistant starch

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Resistant starch (RS) escapes enzymatic digestion and becomes a substrate for microbial fermentation. Starch fermentation may already start in the small intestine, but this is often ignored. The energetic value of fermented starch is lower than that of digested starch; thus ignoring small intestinal starch fermentation may result in an overestimation of the energetic value of the total starch content. The aim of this study was to quantify ileal starch

fermentation in pigs fed diets high (H-RS) or low (L-RS) in RS. We expected a greater level of starch fermentation in the small intestine with high dietary RS inclusion. Twenty pigs (40 ± 2.82 kg) were fed diets containing 50% waxy maize starch (L-RS) or 50% high-amylose maize starch (H-RS), with TiO_2 as indigestible marker. After 14 days of adaptation to the diets, pigs were fed hourly for 6 h to reach steady state, dissected, and ileal digesta were collected. Ileal starch fermentation was calculated from ^{13}C enrichment of ileal digesta, using the contrast in natural enrichment between starch and non-starch dietary components (Gerrits *et al.* 2012). Data were analysed using a general linear model with diet as fixed effect. Ileal starch digestibility was 25%-units lower for H-RS than L-RS ($P < 0.001$). Carbon from starch incorporated in microbial biomass, which is used to estimate the degree of ileal fermentation, was greater for H-RS than L-RS (10% vs. 3% of starch ingested; $P < 0.001$). In conclusion, small intestinal starch fermentation can be considerable, thereby overestimating glucose yield when based on ileal starch digestibility. Calculations of the proportion of dietary starch that is fermented to produce the microbial carbon yielded unrealistic values and will be further investigated, with emphasis on endogenous carbon recycled from dietary starch.

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Kinetics of thymol and its glucosides along the gastro-intestinal tract of weaned piglets

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Thymol has been proposed as a substitute for antibiotic growth promoters in pig production. To effectuate a microbiota modulating effect, thymol should reach the lower gastro-intestinal tract (GIT). However, due to its lipophilic properties thymol is quickly absorbed from the stomach and upper small intestine. It was hypothesised that absorption of the less lipophilic thymol glucosides: thymol- α -D-glucopyranoside (T α G) and thymol- β -D-glucopyranoside (T β G), would be delayed and subsequently higher concentrations of the aglycon would be available in the lower GIT. Eighteen female piglets (body weight 24 kg \pm 0.59 kg) were randomly allocated to three treatments (n=6) and adapted to meal feeding. On day eight, the animals were fed six meals of the experimental diet containing thymol (T1), T α G (T2) or T β G (T3) at 3.3 mmol/kg feed. Next, animals were euthanized, contents of stomach, three parts of small intestine (SI1: 0-25%, SI2: 25-75%, SI3: 75-100% of total length) and caecum were gathered and analysed for thymol, T α G and T β G concentration by HPLC. Data were analysed by ANOVA using a GLM with factors treatment, gut location and their interaction. The total (free and glycosylated) thymol concentration in stomach was 78, 71 and 47% of the initial feed concentration for T1, T2 and T3, respectively. This indicates that thymol in pure form and from T α G was relatively stable in stomach, while T β G disappeared faster. In SI1, the concentrations in T1, T2 and T3 decreased further, leading to residual concentrations of 23, 35 and 21% relative to the feed content, respectively. The total thymol concentration was numerically highest in SI1, SI2, SI3 and caecum when T α G was fed ($P > 0.05$). In conclusion, we could not demonstrate that higher concentrations of thymol would reach the lower GIT when feeding glucosides compared to free thymol, though the kinetics of the glucosides is not fully understood.

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Sources of variation in individual fecal nutrient digestibility in pigs

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In pig nutrition, nutrient digestibility is an important trait of feed ingredients and complete diets. Improvement in nutrient digestibility is currently mainly achieved by optimizing ingredient composition of the diet, based on digestibility coefficients of the individual ingredients determined during digestibility trials. However, there is variation between pigs in the capability to digest certain nutrients and with the current trend in precision feeding the individual pig becomes increasingly more important. One possible source for this between pig variation is genetics. The present

Abstract

study aimed to evaluate the magnitude of several sources of variation in fecal digestibility in pigs, as a first step to investigate the perspectives for breeding pigs for improved nutrient digestibility. To achieve this, data was collected of nine digestibility trials, containing in total 1150 digestibility records of 416 growing pigs. Considered sources of variance were trial, diet, litter, and individual animal effect for the fecal digestibility of dry matter, ash, organic matter, crude protein, crude fat and non-starch polysaccharides. Due to the design of the experiments, the majority of variance in fecal digestibility was explained by the diet (46-85%), and trial (3-28%). However, within diet and trial, individual animal effect explained 0-10% of the variance in digestibility and litter 2-8%. The within diet and trial repeatability, indicating the percentage of shared variance between repeated observations on the same pig, ranged from 7% for ash to 16% for crude protein. Since the repeatability can be due to both permanent environmental and genetic effects, these results suggests there may be genetic variation between pigs in nutrient digestibility. In conclusion, between pig variation in fecal nutrient digestibility is important when animals are fed the same diet and it might be worthwhile to collect digestibility values on a large scale to further explore the potential to improve digestibility using genetic selection.

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Dietary N-carbamylglutamate modulate amino acid metabolism-related gene expression and liver metabolite profiles in Ningxiang pigs

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This study was conducted to investigate the effects of N-carbamylglutamate (NCG) on the amino acid (AA) metabolism, transport in intestine and liver metabolite profiles in Ningxiang pigs. Thirty-six pigs (43.15 ± 0.23 kg average BW) were randomly divided into two dietary treatments consisting of a basal diet and the basal diet with 0.08% NCG supplementation for a 56 days period with six pens per treatment and three females per pen. At the end of the study, one pig from each pen was sacrificed, the liver and jejunum samples were collected for gene expression or metabolome analysis via liquid chromatography-mass spectrometry. Real-time qPCR assay showed that NCG tended to down-regulate the mRNA expression of AA transporter Slc7a9 ($P = 0.090$) and Slc16a10 ($P = 0.087$) gene in the jejunum, and up-regulate Slc36a2 ($P = 0.039$), Slc1a5 ($P = 0.049$) and Slc16a10 ($P = 0.012$) in the liver. Liquid chromatography-mass spectrometry analysis showed that totally 12 metabolites related to AA metabolism include AA (serine, aspartic acid, ornithine, etc.) and intermediates (phosphohydroxypyruvic acid, N-acetylarlyamine, succinic acid semialdehyde, etc.) were identified in the liver extraction, which involved in four metabolic pathways, such as arginine and proline metabolism, glycine, serine and threonine metabolism, alanine, aspartate and glutamate metabolism, etc. These findings indicate that NCG may alter AA metabolism in Ningxiang pigs by regulating the expressions of AA transporter in the intestine and liver.

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Oral glucose tolerance test in weaned pigs with intrauterine growth retardation (IUGR) syndrome

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IUGR human neonates show high predisposition to develop metabolic syndrome involving obesity and diabetes type 2 in future life but mechanisms still remain unclear. Similarities between pig and human concerning digestive system and energy metabolism make pig an interesting biomedical model in this aspect. The aim of our study was to give more light on carbohydrate and lipid metabolism in weaned IUGR piglets. The criteria for IUGR were as follows: piglets born on term with birth body weight below 1.1 kg and head manifesting characteristic shortening of the facial part. Blood samples from 6 pairs of IUGR piglets and their normal body weight (NBW) littermates were collected 2 weeks after

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weaning. Blood was withdrawn preprandially for hematology and biochemistry analyses followed by oral glucose tolerance test (1.75 g/kg b. wt. of glucose in 100 ml water given orally). Birth body weight of IUGR and NBW neonates was respectively, 0.99 ± 0.07 and 1.55 ± 0.07 kg ($P < 0.001$). On the postnatal day 42, the body weight of IUGR was significantly lower as compared to NBW (13.1 ± 0.97 vs. 16.8 ± 1.16 ; $P < 0.001$). Concerning IUGR fasting hematology and biochemical parameters: thrombocyte number was lower (545 ± 43 vs. $660 \pm 105 \times 10^9/l$; $P < 0.05$) as compared to NBW. The other red and white blood cells parameters were not different between IUGR and NBW piglets. Oral glucose tolerance test showed significant differences between IUGR and NBW piglets. Though basal plasma glucose did not differ between the groups: 64.4 ± 10.6 vs. 72.3 ± 2.7 mg/dl, after 1 h it showed significantly higher concentration in IUGR compared to NBW (247 ± 58 vs. 142 ± 32 mg/dl; $P < 0.05$). After two hours, plasma glucose in IUGR piglets remained elevated (208 ± 77 mg/dl) whereas in NBW it started to return to a basal level (99 ± 17 mg/dl; $P < 0.05$). In conclusion, results of oral glucose tolerance test in weaned IUGR piglets revealed functional alteration of carbohydrate metabolism.

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Compounds of organic acids, cinnamaldehyde and Permeabilizing Complex™ impact on growth performance and modulation of gut microbiome in weaning piglets

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The aim of the project was to examine the ability of a mixture (ACPC) of organic acids (formic, acetic and propionic), cinnamaldehyde and Permeabilizing Complex™ (PC) to improve growth performance and also impact on intestinal microbiome in weaning piglets. Growth performance as well as the pH in the gastro-intestinal tract (GIT), microbial population in the ileum and villus height of jejunum were determined. Ninety-six weaning pigs were assigned to three treatments and fed commercial diets. The negative control group diet contained no feed additives, whereas the positive control group was supplemented with antibiotics of colistin (100 g/t) and chlortetracycline (100 g/t). The trial group was fed ACPC (1 kg/t). Results showed that at the 56th day of trial, body weight, average daily gain and feed intake were higher in the trial group compared with the two control groups. The pH in the stomach of pigs fed the negative and positive control diet was similar (4.28 vs. 4.30), while pH in stomach of pigs fed the diet supplemented with ACPS was significantly ($P < 0.05$) lower compared to the stomach pH of pigs in the negative and positive control groups. Microbial analysis showed that the number of *E. coli* and *Salmonella typhimurium* in the ileum of pigs were reduced ($P < 0.05$) in the groups fed ACPC and antibiotics in comparison with the negative control. Counts of *Lactobacilli* and *Bifidobacteria* in the ileum were higher ($P < 0.05$) in the trial group than in the other two groups. The villus height in the jejunum was greater in the positive control group and in the trial group in comparison with the negative control ($P < 0.05$). These results indicated that the use of organic acids based product improved growth performance in piglets by changing the intestinal micro-ecological environment.

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The impact of diarrhoea treatments on the composition of the gastrointestinal bacterial microbiota of pigs post weaning

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Aminoglycosides and zinc oxide (ZnO) prevent enterotoxigenic *E. coli* (ETEC)-induced post-weaning diarrhoea (PWD), but their non-specific bactericidal action can cause intestinal dysbacteriosis. Bromelain protects pigs from PWD, by inactivating host ETEC receptors, thereby preventing ETEC colonisation. It also inhibits enterotoxin-induced fluid secretion. This study investigated whether a proprietary formulation of bromelain (125mg/4mL) would induce intestinal dysbacteriosis in post-weaned piglets. At weaning (Day 0), 72 piglets were selected from 9 high-health gilt litters. Two

Abstract

piglets from each litter were randomised into four groups (n = 18/group); i) bromelain (oral, D-1 and D6), ii) ZnO (2,500 ppm in feed, D7 to D19), iii) neomycin sulphate (NS; 8mg/kg bw in feed, D7 to D19) or iv) Controls (unmedicated). Faeces from each piglet (Days -1, 6, 19, 39) and colonic mucosa (Day 40) underwent next generation sequencing (Illumina MiSeq) of 16S rRNA (V4) and the composition of bacterial communities were analysed by Qiagen CLC Microbial Genomics (v9.5). Bacterial production of D-Lactate was measured in faecal supernatants. Differences in relative abundance of bacterial groups were determined by unbalanced ANOVA (Genstat 18th edn). There were no differences in bacterial populations between groups pre-weaning, specifically numbers of F4 ETEC. Neomycin and ZnO significantly induced dysbacteriosis relative to Control and bromelain pigs, which displayed similarly clustered bacterial profiles. Neomycin increased the abundance of *Prevotellaceae* and *Enterobacteriaceae*, whereas ZnO reduced *Desulfovibrionaceae* and *Veillonellaceae*, and increased *Streptococcaceae*, *Ruminococcaceae* and *Clostridiaceae* ($P < 0.05$). Two NS-treated pigs died. However, NS and ZnO significantly reduced faecal D-lactate, suggesting reduced inflammation and improved barrier function. On removal of antimicrobials (Day 19), NS and ZnO pigs developed diarrhoea, but by Day 40 their microbiomes had similar bacterial abundances as Controls, except for reduced *Erysipelotrichaceae* and *Veillonellaceae* (butyrate producers) and increased *Prevotellaceae*. In conclusion, the antimicrobials NS and ZnO caused intestinal dysbacteriosis, while bromelain did not adversely disrupt the microbiome.

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Heat stress during late gestation of primiparous sows affects intestinal barrier of their offspring

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Heat stress (HS) during pregnant sows has a long term developmental damage to the offspring. Excessive heat exposure impairs intestinal function. We hypothesized that sows exposed to HS during late gestation would induce the intestinal barrier dysfunction of neonatal piglets. Twelve first-parity Landrace × Large White sows were randomly assigned into thermoneutral (TN) (18-22°C; n=6) or HS (28-32°C; n=6) condition from 85 d of gestation until natural farrowing. Two piglets each litter were selected and sacrificed to harvest the small intestine. Freshly isolated samples were analyzed for intestinal morphology (Hematoxylin eosin), integrity, mucosal barrier function (Alcian blue/periodic acid Schiff), and apoptotic percentage (TUNEL). Compared to the TN group, the length of the small intestine tended to decrease ($P = 0.07$) in HS group. The ratio of villus height to crypt depth in duodenum and the activity of lactase in both jejunum and ileum were decreased ($P < 0.05$) due to HS. Maternal HS increased ($P < 0.05$) the number of goblet cell and MUC2 mRNA expression in the jejunum. There was no difference in serum endotoxin concentration between TN and HS group, while serum diamine oxidase activity was increased ($P < 0.05$) in HS group. Furthermore, maternal HS led to increase ($P < 0.05$) the apoptotic percentage and Fas mRNA expression, and tended to increase ($P = 0.08$) caspase 3 mRNA expression in the duodenum. In conclusion, HS during late gestation of primiparous sows decreased intestinal integrity and caused mucosal barrier dysfunction of neonatal piglets, which partly through inducing the apoptosis in the intestinal mucosa.

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Linking blood metabolite and colonic metabolite and microbiota profiles to sanitary conditions in starter pigs

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Low environmental sanitary conditions can have negative effects on health and performance in pigs. To further study the effects of sanitary conditions on nutrient metabolism and health, we evaluated effects of a contrast in sanitary status of pigs on their blood and colon metabolites and microbiota composition in colon. Pigs were kept under high (HSC) or low sanitary conditions (LSC) from weaning onwards. HSC was induced via applying a strict hygiene protocol, pigs receiving

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a preventative antibiotic injection and vaccinations against a number of pathogens. LSC were obtained by omitting cleaning of pens prior to stocking, not applying a hygiene protocol, and spreading of foreign manure. In addition, LSC pigs did not receive an antibiotic treatment and vaccinations. At week 13 of age, 18 animals from both the HSC and LSC group were sacrificed to obtain blood and colon digesta samples. In these samples metabolite profiles were acquired by employing both Nuclear Magnetic Resonance (NMR) and triple quadrupole mass spectrometry (TQMS). In addition, in colon digesta samples the microbiota composition was determined by sequencing the 16S hypervariable regions V3-V4. NMR analysis showed eight metabolites in blood and 32 metabolites in colon digesta significantly different ($P < 0.05$) between treatments. TQMS showed differences for five different metabolites in both blood and colon digesta. These blood metabolites could be linked to protein accretion, nutrient metabolism, immunity, and responses to stress. The microbiota diversity in colon digesta (Shannon index) was significantly higher in LSC compared to HSC ($p < 0.05$), values being 2.99 and 2.85, respectively. Abundance of 36 significant bacterial genera (FDR < 0.05 and ARC $> 0.01\%$) was mostly lower in colon digesta from HSC compared to LSC pigs. In conclusion, metabolites in blood and colon digesta and microbial groups in colon show a link with the sanitary conditions in which pigs are housed.

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Effects of dietary vitamin E on growth performance, intestinal morphology and functions of weaned piglets

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Vitamin E has reductive, antioxidant and anti-stress properties that may affect intestinal morphology and functions in weaned piglets. This study was conducted to evaluate the effects of dietary vitamin E on growth performance, intestinal morphology and functions of weaned piglets. Thirty piglets (Duroc \times Landrace \times Yorkshire; 6.36 ± 0.55 kg body weight) were weaned at 21 d of age and randomly allocated into 1 of 5 treatments, consisting of 0% (VE0), 100% (VE100), 200% (VE200), 500% (VE500), or 1000% (VE1000) vitamin E recommendations of NRC (2012), for a period of 14 d with 6 pens (1 piglet per pen) per treatment. All piglets were slaughtered and the intestinal samples were collected from mid-jejunum at the end of experiment. Intestinal morphology and cell proliferation was determined using HE stain and Ki-67 immunohistochemistry, respectively. The activities of sucrase, lactase, maltase, and alkaline phosphatase (AKP) in jejunal mucosa were measured by commercial kits. Quantitative RT-PCR was used to analyze the mRNA expression of *Slc1a1*, *Slc5a1*, *Slc6a19*, *Slc7a9*, and *Slc15a1* in jejunal mucosa. The results showed that dietary vitamin E did not affect growth performance and jejunal villus height, while tended to decrease jejunal crypt depth (CD; $P = 0.06$) and villus width (VW; $P = 0.08$) in weaned piglets. Compared with VE0 group, jejunal CD ($P = 0.009$), VW ($P = 0.002$), and the percentage of Ki-67 positive cells in crypt ($P = 0.02$) were significantly decreased in VE500 group. Dietary vitamin E significantly decreased ($P = 0.03$) sucrase activity while it had no significant effects on lactase, maltase, and AKP activities. There were no significant differences in *Slc1a1*, *Slc5a1*, *Slc6a19*, *Slc7a9*, and *Slc15a1* mRNA expression among the treatments. In conclusion, dietary vitamin E affects jejunal morphology and mucosal enzyme activity, and it may partly via affecting the proliferation of crypt epithelial cells in weaned piglets.

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Effects of lauric acid on cell proliferation, differentiation and autophagy of IPEC-J2 cells

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Abstract

Introduction It is well known that medium chain fatty acids (MCFA) are able to fight against pathogen, and more efficiently absorbed by intestinal cells than long-chain fatty acids. Therefore, MCFA are widely used in animal production. However, the effects of MCFA on intestinal epithelial cells are not well understood. The present study was conducted to test the hypothesis that lauric acid (LA), one of the MCFA, would affect proliferation and differentiation of intestinal epithelial cells.

Material and methods The IPEC-J2 cells were incubated in 37°C humidified air with 5% CO₂ and maintained in DMEM/F12 medium supplemented with 5% fetal bovine serum, 1% ITS, and 5ng/ml EGF. The cell viability, proliferation, and alkaline phosphatase (Alp) activity of LA-treated cells were measured using CCK-8, BrdU and Alp assay kit. Lipid droplets in the cells were assessed by the Oil Red O method. The abundance of Lc3B/A was detected by WB and IF.

Results The cell viability of IPEC-J2 cells was decreased ($P < 0.05$) with the increase of LA concentration (2mM to 7mM) and incubation time (24 h to 72 h). Cell proliferation was significantly decreased ($P < 0.05$) by LA in a dose-dependent manner. However, the Alp activity in IPEC-J2 cell was significantly increased ($P < 0.05$) when incubated with 0.1 or 0.25mM LA for 48h. Moreover, high concentration of LA induced the accumulation of lipid droplets in IPEC-J2 cells. The ratio of Lc3B/Lc3A was also significantly increased ($P < 0.05$) from 0 to 48 h with 7mM LA. However, Lc3B was not co-localized with lipid droplets in cells.

In conclusion, the results demonstrate that LA inhibits cell proliferation, but enhances cell differentiation of IPEC-J2 cells at low concentration. Moreover, high concentration of LA induces lipid deposition in IPEC-J2 cells and autophagy.

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The effects of dietary protein content on intestinal structure and barrier function of ileum and colon in weaned piglets

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High dietary protein content increases the incidence of diarrhea in weaned pigs. The present study was conducted to test the effects of dietary protein content on intestinal structure and barrier function in ileum and colon of weaned piglets. A total of 16 piglets (9.47 ± 0.22 kg body weight) with 35 d of age were randomly assigned into 1 of 2 groups, including 18% crude protein diet (LP) and 26% crude protein diet (HP). Intestinal tissues were collected from each group for analyzing intestinal physiological and metabolic changes using transcriptomics and other technologies after a 12-d feeding period. Compared with the LP diet, ileal villus height and colonic crypt depth in HP diet were significantly decreased ($P < 0.05$). In addition, dietary HP significantly changed ($P < 0.05$) the intestinal barrier function, intestinal immunity responses and diarrheas pathway of piglets. Compared with LP group, the relative abundance of ZO-1 and Occludin protein in ileum and colon of HP treatment was significantly decreased ($P < 0.05$). Moreover, the mRNA expression of genes involved in tight junction protein and intestinal immunity response was mainly reduced in HP diet compared with LP diet. In conclusion, the results indicate that high protein in diet impairs intestinal morphology and results in dysfunction of intestinal barrier, which may be the potential reason for diarrhea of piglets.

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Faecal microbiota transplantation in gestating sows and/or neonatal offspring alters lifetime intestinal microbiota and growth in offspring

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As feed accounts for ~70% of pig production costs, feed efficiency (FE) is critical. Recently, a link was identified between porcine FE and the intestinal microbiota. Therefore, it may be possible to manipulate the intestinal microbiota, in order to improve FE. The aim of this work was to investigate if faecal microbiota transplantation (FMT) in sows and/or neonatal offspring, using inocula derived from highly feed efficient pigs, could improve FE in offspring. Pregnant sows were assigned to control or FMT treatments, and subsequent offspring to control, FMT once (at birth), or FMT four times (between birth and weaning). Offspring were individually housed from weaning to slaughter (at ~155 days of age). Faeces was collected throughout the lifetime, and ileal, caecal and colon digesta was collected at slaughter for microbiota analysis, using 16S rRNA gene sequencing. Data were analysed using SAS 9.3, and significance was set at $P \leq 0.05$. The FMT procedure altered sow faecal and colostrum microbiota composition, and resulted in lighter offspring body weight at days 70 and 155 of age, when administered to sows and/or offspring. This was accompanied by FMT-associated changes within the offspring microbiota, mostly in the ileum. These included changes in bacterial diversity and load, along with numerous compositional differences at the phylum and genus levels (e.g. *Spirochaetes* and *Bacteroidetes* at high relative abundance and mostly *Clostridia* members, respectively), and differences in abundance of predicted bacterial pathways. In addition, intestinal morphology was negatively impacted, duodenal gene expression altered, and serum protein and cholesterol reduced due to FMT. Taken together, results suggest poorer absorptive capacity and intestinal health, most likely explaining the reduced body weight due to FMT. Although FMT, as practiced here, negatively impacted pig growth, it nonetheless demonstrates the enormous impact early life intestinal microbiota has on the lifetime growth in pigs.

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Comparative effect of two dietary regimes on intestinal microbiota of post-weaning pigs

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The use of antibiotics in livestock and the spread of antibiotic resistance require the development of feed alternatives. Since microbiota has been established as a health indicator, characterize a healthy microbiota and in-feed antibiotics effects along animals early-life is still a challenge. The study objective was to define the intestinal microbiota of commercial pigs along first ages and the impact of two dietary regimens. For that, 312 21-day-old weaned piglets distributed in 12 pens (26 animals each) were fed *ad libitum* with two experimental diets until fattening-phase: 1) basal diet supplemented with antimicrobials; and 2) supplemented with an alternative high-fiber content formula without antimicrobials. For the fattening-phase both groups were moved to the same facility and received a common diet. One fecal sample per pen (6/treatment) was taken on 9 (pre-starter), 16 (starter) and 40 (fattening) days post-weaning and microbial DNA extracted. 16S rRNA gene was sequenced using MiSeq[®] technology. Bioinformatics analysis was performed with QIIME and R software. Community structures diverged as animals grew (ANOSIM test, $P = 0.001$), more pronounced in starter animals (highest beta-diversity) but resembled during fattening ($P < 0.05$). Alpha-diversity increased from early-ages to adult animals ($P < 0.01$). However, a predominant 1006 species core remained stable (91.1%) within the three age groups. Regarding dietary effects, alpha and beta-diversities were similar. Nonetheless, a clearer diet-effect was detected at starter-phase with diets clustering separately (ANOSIM test, $P = 0.05$). Concerning microbial populations, at pre-starter and starter periods, microbiota was modified by treatments with an increase in number of sequences of fibrolytic genera (*Psychrobacter*, *Butyrivibrio*, *Shuttleworthia*) with diet 2 and an enhancement of miscellaneous genera (*Sutterella*, *Lactobacillus*, *Streptococcus*) with diet 1. At fattening-phase, no differences were found. In conclusion, the animal age was the most evident factor affecting the microbiota while dietary-regime effect was restricted to early-ages suggesting a resilient endogenous microbiota after removing treatments.

Effect of zinc oxide sources and doses on intestinal bacteria in weaned pigletsJ Michiels¹, S Durosoy², A Roméo², M Majdeddin¹, J Degroote¹, N Van Noten¹, C Van Kerschaver¹, E Van Liefferinge¹ and M Vandaele¹¹Ghent University; ²Animine

Zn is commonly supplemented in piglets diets at nutritional dosage (110 mg/kg Zn) to fulfil animal requirements or at pharmacological dosage (2400 mg/kg Zn) for its growth-promoting effect. High dosages of zinc oxide (ZnO) can improve performance through adjusting intestinal health, but Zn level in animal wastes may lead to environmental concerns. Alternative solutions are consequently under investigation. In this study, a potentiated ZnO source (HiZox[®], Animine), a coated ZnO and the standard ZnO were compared at different doses. A total of 108 piglets, weaned at 21 days, were allocated to 18 pens (6 piglets/pen) and fed 6 experimental corn-soybean based diets during 14 days, with different zinc sources and doses: standard ZnO (110, NC; or 2400 mg/kg Zn, PC), coated ZnO (110 or 220 mg/kg Zn) or potentiated ZnO source (110 or 220 mg/kg Zn). Piglets were weighed individually at d0 and d14 and feed intake was recorded. At the end of the experiment, 3 piglets per pen were selected and sacrificed. Contents from proximal and distal small intestine were collected. Numbers of bacteria (*E. coli*, coliform bacteria) were assessed using selective media. There was no significant difference between the treatments for the growth performance. NC showed the lowest weight gain (1.2 kg) and PC the highest (1.6 kg). In the proximal small intestine, numbers of *E. coli* were significantly ($P \leq 0.05$) higher for NC and for the coated ZnO, compared to PC; the potentiated ZnO obtained intermediate results. In the distal small intestine, similarly, *E. coli* and coliform bacteria populations were significantly ($P \leq 0.05$) higher for the coated ZnO compared to PC. To conclude, populations of *E. coli* and coliforms were significantly reduced by ZnO at pharmacological dosage, whereas the coated ZnO showed no reductions and the potentiated ZnO was intermediate.

The effect of therapeutic levels of dietary zinc oxide on the faecal bacterial community of piglets at weaningK McDermott and H Miller
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Therapeutic levels of dietary zinc oxide (ZnO) are fed to piglets at weaning to reduce the incidence of post-weaning diarrhoea. By 2022, therapeutic levels of ZnO will have been phased out of the piglet's diet, generating the need for a suitable alternative. To do this more effectively it would be helpful if we understood the effect of ZnO on the gut microbiota. Previous studies have explored the effect of ZnO on the gastrointestinal microbiota, however little work has considered the effect on the faecal microbiota (used as a proxy for the distal gut) using next generation sequencing technology. Therefore this study aimed to determine the effect of therapeutic levels of ZnO on faecal bacterial composition. A batch of 240 piglets were assigned to a therapeutic zinc (2500 ppm) or standard zinc diet (100 ppm) for 20 days post-weaning. Twelve replicates were included with 10 piglets per pen. Litter mate piglets within the same replicate, but from different treatments, were selected for faecal sampling ($n = 24$). DNA was extracted using the QIAamp DNA Stool Mini Kit (200 mg; Qiagen) and the V1-V3 region of the 16S rRNA gene was sequenced with 300 bp pair-end reads on the MiSeq platform (Illumina). Sequences were processed in the software Mothur. Alpha diversity (Chao1, Shannon and Simpson), beta-diversity (PERMANOVA) and differential abundance of operational taxonomic units were performed in R with the packages Phyloseq, Vegan and DESeq2. ZnO was found to have a significant effect on beta diversity ($P < 0.001$) with an increase in Bacteroidetes ($P < 0.001$) and genera such as *Roseburia* ($P < 0.001$), *Parabacteroides* ($P < 0.001$) and *Ruminococcaceae* ($P < 0.001$) which have been shown to have a protective effect on the intestinal barrier in humans. In conclusion, ZnO at weaning was found to shift the faecal community composition, favouring the growth of butyrate producing bacteria.

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Effect of *Bacillus subtilis* supplementation on productive performance, intestinal morphology and microbiota of small intestine of heat stressed pigs

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Exposure of pigs to high ambient temperature (AT) provoking heat stress (HS) is associated with changes in intestinal milieu, reduced feed intake and depressed performance. A 21-d experiment was conducted to evaluate the effect of supplementing *Bacillus subtilis* on performance, intestinal morphology and microbiota composition of small intestine of HS pigs. Fourteen individually housed pigs (25 ± 2 kg initial BW) exposed to natural AT variations (26.4 to 35.6°C) and fed a wheat-soybean meal diet ad libitum were divided in 2 groups. One 7-pigs group received *Bacillus subtilis* cultured in liquid LB media containing 8.16×10^{10} cfu divided in two portions, each offered at 0700 and 1900 h every day. The other 7-pigs group received same volume of sterilized LB media at the same time. At the end of experiment, pigs were sacrificed and intestinal content samples were collected to analyze the population of *Bacillus sp.*, *Lactobacillus sp.*, *E. coli* and *Bifidobacterium sp.* using qPCR; also, intestinal segments were collected for histological analysis. Average daily gain (687 gr), daily feed intake (1,162 gr) and feed: gain ratio (1.72) were not affected ($P > 0.10$) by *Bacillus subtilis* supplementation. Pigs supplemented with *Bacillus subtilis* tended to reduce the *E. coli* population ($P = 0.093$), and to increase 15-fold the *Bifidobacterium sp.* population ($P = 0.068$); there were no changes in *Lactobacillus sp.* and *Bacillus sp.* populations ($P > 0.10$) because of *Bacillus subtilis* supplementation. Pigs supplemented with *Bacillus subtilis* had larger villi height in duodenum and ileum ($P \leq 0.002$), and the villi height: crypt depth ratio was greater in ileum of the *Bacillus subtilis* supplemented pigs ($P = 0.009$). In conclusion, although the supplementation with *Bacillus subtilis* did not affect the performance of HS pigs, it increased the population of beneficial microorganisms and appeared to improve the intestinal morphology.

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Influence of dietary prebiotics and arachidonic acid on development of the fecal microbiome of pigs fed milk replacer

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Early life nutrition is a critical determinant of intestinal microbiome development with long-term consequences on growth, development and health. Milk oligosaccharides fuel establishment of the microbiome but their content and composition differs markedly across species. Long-chain polyunsaturated fatty acids also are critically important in neonatal development and may modulate intestinal health. We hypothesized that supplementation of dietary oligosaccharides and arachidonic acid (ARA) would alter fecal microbiome development over the first 3 weeks of life. Day-old pigs were fed one of four milk replacers for 21 days: 1) 0.5% ARA; control, 2) four g/L galactooligosaccharide + four g/L polydextrose (PRE), 3) 2.5% ARA, and 4) PRE + ARA in a factorial arrangement ($n = 24$ per group). Fecal swabs were collected every seven days and microbiome analysis was performed via 16S rDNA Illumina sequencing. Pigs were euthanized on d22 and fatty acid composition of colonic mucosa was analyzed via GC-MS and colonic contents were assayed for volatile fatty acids via GC-FID. Diet did not affect growth ($P > 0.1$). Principal component analysis showed that microbial taxa changed in a time- and diet-dependent manner with diets containing PRE clustering by day seven and becoming progressively more tightly clustered over time ($P < 0.05$). Pigs fed PRE displayed an increase in the Bacteroidetes phylum ($P < 0.05$). Colonic mucosal ARA% (w/w) increased from 3.83 in controls to 6.67 in pigs fed ARA ($P < .001$). Dietary PRE increased ARA and linoleate concentration in colonic mucosa ($P < .002$). Dietary PRE reduced colonic pH associated with increased concentration of VFAs ($P < 0.05$). In conclusion, addition of PRE to milk replacer progressively alters microbial taxa over the first three weeks of life and increases colonic fermentation. Dietary ARA has minimal impact on microbial taxa but

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enriches colonic mucosa. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2014-67017-21750 from NIFA.

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Transfer of dietary essential oils to colostrum is associated with improved performance and potential microbial populations' shifts in weaned pigs

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Dietary essential oils (EO) enhance feed intake and weight gain in post-weaning piglets. These effects have been related to the conditioning of EO transferred to maternal fluids. In addition, EO have antimicrobial properties. However, the contribution of EO present in colostrum to control gut microbiota has remained unexplored. We hypothesized that the intake of EO in colostrum and milk plays a significant role in the improvement of post-weaning piglet development. Twenty Large White sows were assigned to a control (non-EO) diet or a diet containing 1000mg/kg of a mix of 8 EO (lemon ironbark, peppermint gum, nerolina, lemon myrtle, clove, thyme, cinnamon and oregano). Colostrum samples were collected (day 1 post-farrowing) and analysed for EO traces by GC-MS. The EO enriched colostrum was tested *in-vitro* in a kill-time assay against enterotoxigenic *E. coli* (ETEC; O149 F4:F6:LT:Sta) or *Lactobacillus ruminis* (representing pathogenic and beneficial bacteria, respectively). One-hundred and twenty pigs born from non-EO or EO sows were assigned to a non-EO or an EO post-weaning diet in a 2x2 factorial design. Data was analysed with ANOVA in Minitab 16. Significance was set at $P < 0.05$. All EO except lemon myrtle were transferred to colostrum. The inherent antimicrobial properties of milk alone were found to be synergistically augmented with the transfer of EO showing an additional reduction in coliform counts of 5 Log CFU/ml after 8 hours of incubation. Furthermore, the *Lactobacillus* were 32 times more resilient to EO-milk than coliforms. Thus, the presence of EO in milk has the potential of shifting gut microbiota in the piglet gut. Dietary EO in sows significantly improved post-weaning piglet feed intake (15.43%) and weight gain (16.27%) compared to non-EO sows. Our results suggest that the transfer of EO to maternal fluids can improve the performance of weaned pigs through a modulation of the gut microbiota.

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Dietary spray-dried porcine plasma proteins promote a probiotic profile in the fecal microbiota of mice

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Introduction Feeding functional proteins obtained from porcine plasma (spray-dried plasma; SDP) prevents deterioration of the intestinal barrier and defense mechanisms in animals challenged with enterotoxins. However, multiple modes of action (MOA) are hypothesized with SDP. One potential mode of action is via alteration in the microbiome.

Hypothesis The hypothesis was that the MOA of SDP involves changes in the intestinal microbial populations compatible with its anti-inflammatory and homeostatic effects.

Methods Weaned C57BL/6 mice were used. Mice were fed 8% SDP (supplied by APC SLU, Spain) for 14 days and then feces were collected and immediately frozen in liquid nitrogen. Controls were fed an isonitrogenous and isoenergetic diet. Fecal DNA was extracted using a phenol-chloroform method. The genomic study was done

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with the MiSeq Illumina and DNA was analyzed from amplicon sequencing of prokaryotic 16S small subunit rRNA genes.

Statistical analyses For the statistical analysis the False Discovery Rate test was applied. The minimum statistical significance was set at $P \leq 0.05$ and values between 0.05 and 0.1 were considered a significant trend. **Results.** SDP strongly increased the presence of *Lactobacillus* species ($P = 0.004$) that are butyrate and acetate producers, with well-known anti-inflammatory effects; it also stimulated proliferation of Porphyromonadaceae ($P = 0.014$), with species that are negatively correlated with the expression of pro-inflammatory cytokines; and Lachnospiraceae ($P = 0.084$), involved in the regulation of regulatory T-lymphocytes homeostasis and the restoration of mucosal permeability.

Conclusion Feeding SDP for 14 days at weaning promotes a fecal microbiota profile that is compatible with its known effects on gut lymphocyte modulation, reducing gut inflammation and restoring gut integrity.

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Differential effects of dietary copper sulphate and copper(I)oxide on gut microbiota of weaned piglets

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Copper (Cu) is required for normal body functioning of pigs as a co-factor of enzymes in several biochemical pathways. Pharmacological levels of CuSO₄ are commonly used to stimulate growth performance in weaned pigs, but the mechanism has not been fully elucidated. Copper is suggested to have antimicrobial properties that could potentially regulate the gut microbiota, and reduce the negative impact of weaning. Recently, a newly available source, copper(I)oxide (Cu₂O) was shown to be at least as effective as CuSO₄ in stimulating growth performance, in contrast to earlier results with CuO. The aim of the present study was to evaluate the effect of divalent Cu from CuSO₄ and monovalent Cu from Cu₂O on the gut microbiota of weaned piglets. The experiment was carried out with 600 weaned pigs in a 2x3 factorial arrangement, with Cu-source (CuSO₄ and Cu₂O) and level (15, 80 or 160 mg Cu/kg diet) as factors, during 5 weeks. On day 35, 8 pigs/treatment were slaughtered and ileum and colon contents were analysed for gut microbiota composition by quantitative real-time PCR. Copper source and level did not affect ($P > 0.05$) the amount of total bacteria, *Bacteroidetes* and *Firmicutes* in the colon. *Firmicutes* (36-41%) and *Bacteroidetes* (20-23%) were the most abundant phyla. *Lactobacillus* abundance was increased at 80 mg Cu/kg for both Cu sources. Cu₂O reduced *E. coli* abundance compared to CuSO₄ (0.014 vs. 0.047%, $P = 0.053$) and tended to enhance the body weight gain compared to CuSO₄ (final body weight 19.8 vs. 19.3 kg, $P = 0.085$). In conclusion, Cu supplementation improved growth performance of weaned pigs. This effect may be related to an effect on microbiota composition whereas Cu₂O may reduce pathogenic bacteria like *E. coli*, thus being an alternative source of Cu for this purpose. Ileum data and gut microbiota composition by high-throughput sequencing are being processed.

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Effect of controlled cereal fermentation and dietary carbohydrase supplementation on intestinal microbiota in grow-finishing pigs

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Feeding liquid diets where the cereal fraction, only, is fermented (cFLF) has potential to improve intestinal health due to improved microbial quality of feed. It was therefore hypothesized that feeding cFLF ± a carbohydrase complex

Abstract

(Xylanase + β -glucanase, XB) would modulate intestinal microbiota composition. A total of 36 pens of pigs (7 pigs/pen; ~31.0kg) were allocated to 1 of 4 dietary treatments: (1) non-fermented liquid feed (nFLF); (2) cFLF; (3) nFLF + XB; (4) cFLF + XB. *Pediococcus acidilactici* and *Lactobacillus plantarum* were used as inoculants during cereal fermentation. Pigs were fed the liquid diets (28.6% DM) for 55 days and then slaughtered. Samples were collected from ingredients/diets, 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 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. The microbiota profile evolved over time in the fermentation tanks, with *Lactobacillus* and *Pediococcus* predominating at the end of the experiment. *Pediococcus*, *Mycoplasma* and *Campylobacter* were more abundant in the ileum while *Escherichia/Shigella* and *Streptococcus* were less abundant in the caecum of pigs fed cFLF diets ($P < 0.05$). *Lactococcus* and *Pediococcus* were less abundant in the ileum of pigs fed XB ($P < 0.05$). Fifty-seven *Lactobacillus* ASVs were differentially abundant in the ileum; those with higher abundance in nFLF pigs were phylogenetically closer than those found in cFLF + XB pigs ($P < 0.05$). Ileal *Bifidobacterium* abundance was negatively correlated with pig growth ($P < 0.05$). *Lactobacillus* and *Megasphaera* abundance was positively correlated with caecal propionate, butyrate and valerate concentrations. In conclusion, cFLF and dietary inclusion of XB influenced intestinal microbiota composition, a possible mechanism by which these treatments might impact pig health and growth.

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Supplementation of tryptophan alters piglet growth performance, digestibility, and *Lactobacillus* populations

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Weaning is a particularly important transition for the young pig due to a variety of factors. This importance is emphasized with current trends to restrict the use of antibiotics in feed. In an effort to improve weaning transition, two experiments were conducted to examine the effects of additional tryptophan (Trp) in nursery diets on wean pig growth performance, digestibility, and *Lactobacillus* populations. In Exp. 1, 72 weanling pigs were split across two replications and randomly allocated across six pens, totaling six pens per treatment. A two-phase nursery diet was utilized with a control diet (0.22 SID Trp:Lys) or control plus L-Trp (0.025%, Phase 1; 0.02%, Phase 2; 0.24 SID Trp:Lys). In Exp. 2, 72 weanling pigs were randomly allocated across 18 pens and assigned to one of three diets: control and Trp diets (as in exp. 1), or Trp + (0.05%, Phase 1; 0.04%, Phase 2; 0.26 SID Trp:Lys). Diets in Exp. 2 contained titanium dioxide as a marker of digestibility. In both experiments, phase 1 and 2 diets were fed for 2 and 3 wk respectively. Growth performance was recorded weekly and fecal samples were collected at the end of each dietary phase for microbiome analysis. Statistical analysis used Proc Mixed (SAS 9.2) with pen as the experimental unit and wk 0 BW as a covariate. In Exp. 1, no difference was observed in BW, ADG, or ADFI, however, G:F was increased 9% in pigs on the Trp diet across both phases ($P < 0.01$). In Exp. 2, no difference in BW, ADG, ADFI, or G:F was observed. Phase 2 DM and GE digestibility was increased ($P < 0.01$) in Trp pigs, as compared with to control pigs ($P < 0.01$). *Lactobacillus* populations increased with Trp supplementation ($P < 0.05$). Together, increased Trp supplementation may improve nursery pig performance.

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The relationship between fecal nutrient digestibility and microbiota composition in grower-finisher pigs at slaughter weight

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Lately, relationships between feed efficiency and intestinal and fecal microbiota have been investigated. Nutrient digestibility contributes to feed efficiency and is possibly linked to intestinal microbiota composition and activity. The aim of this study was to investigate the association between fecal nutrient digestibility and microbiota composition in commercial grower-finisher pigs. Data on individual feed intake and body weight gain were collected of 160 three-way crossbreed grower-finisher pigs which were either fed a diet based on corn/soybean meal or a diet based on wheat/barley/by-products. Fecal samples of 142 pigs (74 female and 67 male) were collected on the day before slaughter (mean BW 120 kg) and sequenced for the 16S hypervariable region of bacteria (V3/V4) in order to profile the fecal microbiome. Fecal samples of 25 pigs were used to determine the apparent total tract digestibility of dry matter, ash, organic matter, crude fat, crude protein, crude fiber and non-starch polysaccharides. A partial least squares regression was applied to the dataset to investigate links with microbiota composition. Fecal microbiota composition was associated with at least 61% of the variation in fecal nutrient digestibility, which was in a large part due to differences in digestibility between the diets. However, even within diet there was a strong association between fecal microbiota composition and several fecal nutrient digestibilities. In conclusion, results of this study suggest that fecal microbiota composition is strongly related to fecal nutrient digestion, but cause and consequence are not directly obvious. Larger datasets are needed to confirm the relationship between fecal nutrient digestibility and microbiota composition, and investigate the association with feed efficiency.

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An investigation of the intestinal microbiome of pigs divergent in feed efficiency

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Improving feed efficiency is a viable means to improve on farm profitability and reduce the environmental impact of pig production. Due to its inherent importance in gut health and nutrient breakdown the intestinal microbiome is potentially a key driver of feed efficiency. Therefore, the aim of this project was to characterize the influence of the microbiome in determining feed efficiency in pigs identified as being divergent for residual feed intake (RFI). Two trials were conducted to identify pigs that were divergent in feed efficiency with 16 pigs identified in trial 1 (8 high RFI (HRFI), 8 low RFI (LRFI)) and 24 identified in trial 2 (12 HRFI & 12 LRFI). Intestinal microbial diversity, composition and functionality were assessed using 16S rRNA gene sequencing of three regions (Ileum, Caecum, Colon). In the ileum the less efficient HRFI pigs exhibited greater α diversity compared to the LRFI pigs based on the rare operational taxonomic units (OTUs), (Chao1) or richness and evenness (Shannon and Simpson). However, no significant differences for α diversity were identified in the caecum or colon, while β diversity analysis using both bray-curtis and jaccard distance matrices illustrated that samples clustered on sample location rather than RFI group. Analysis of species composition identified changes at the phylum level with HRFI pigs having increased proportions of *Firmicutes* in the caecum (38 vs 23%) while the LRFI pigs had increased *Bacteroidetes* in the same region (70 vs. 55%). While representing less than 1% total species abundance on the colon the HRFI pigs had increased *Lentisphaerae* and *Actinobacteria* compared to the LRFI pigs. At the family level the LRFI pigs had increased *Lactobacillaceae* in the ileum compared to the HRFI group. The results in this study suggest changes in bacterial composition may be important in determining feed efficiency in pigs.

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The effect of early-life galactooligosaccharide intervention on the microbiota composition and the colonic barrier function in suckling piglets

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Prebiotics intervention has been shown to change the gut microbiota composition and improved the intestinal function in piglets. In this study, we want to investigate the effect of early-life galactooligosaccharide (GOS)

Abstract

intervention on the microbiota composition and the colonic barrier function in suckling piglets. Sixty suckling piglets obtained from six sows (10 piglets per litter) were assigned to control group (CON) and GOS group (GOS) in each litter with breastfeeding. From the age of 1 to 7 days, GOS-piglets were given a 10 ml GOS solution (1g/kg body weight per day), and CON-piglets were treated with the same dose of physiological saline. At the age of 8 and 21 days, six piglets from each group were euthanized. Illumina MiSeq Sequencing, gas chromatography, and Western blotting were used in present study. Independent samples t-test was performed using the SPSS software. GOS-piglets on 8th day had a higher relative abundance of Bacteroidetes at the phylum level ($P < 0.05$), with a lower relative abundance of Firmicutes than the CON-piglets ($P < 0.05$). Results of genus level showed that the abundance of *Barnesiella* and *Prevotella* increased significantly in GOS-piglets ($P < 0.05$). GOS-piglets on 21st day had a higher relative abundance of *Parabacteroides*, Unclassified Porphyromonadaceae, and Unclassified Bacteroidales at the genus level than CON-piglets ($P < 0.05$). In addition, the luminal SCFAs concentrations increased significantly in GOS-piglets on 8th day ($P < 0.05$), and had an increased trend on 21st day ($P = 0.064$). The expression of colonic mucosa barrier function proteins (ZO-1 and Occludin) in GOS-piglets was higher than that of CON-piglets ($P < 0.05$). The ratio of phosphorylated AMPK- α (pAMPK/total) and ACC (pACC/total) increased significantly in colonic mucosa in GOS-piglets compared with that in CON-piglets ($P < 0.05$). In conclusion, our study shows that early-life GOS intervention altered colonic mucosal bacterial communities and increased colonic fermentation, then modulated the colonic barrier function by activated the AMPK pathway.

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Difference of gut microbiota establishment between low- and normal-birth-weight piglets during early life

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Low-birth-weight (LBW) piglets have high risks for postnatal growth failure, mortality and metabolic disorders later in life. Early-life microbial exposure is believed to be an effective intervention to the health and metabolism of the host. Yet it is not well elucidated whether the gut microbiota development in LBW piglets is different from the normal littermates, and its possible association with metabolites profiles. In the current study, a combination of the 16S rRNA gene sequencing and metabolomics technique was used to investigate the difference of gut microbial composition and metabolites between LBW and normal piglets during their early life (3, 7, 14, 21 and 35 days of age). Compared to their normal littermates, LBW piglets harbored lower proportions of *Firmicutes* on day 3 and 7, *Faecalibacterium* on day 3, *Alistipes* on day 7, as well as *Lactobacillus*, *Streptococcus* and *Prevotella_2* on day 21. However, the abundances of *Helcococcus* on day 3, *Campylobacter* on day 7 and 21, *Prevotella_2* on day 14, *Moryella* on day 14 and 21 and *Bacteroidetes* on day 21 were significantly higher in LBW piglets than that in normal piglets. Also, results of metabolomic analysis suggested that LBW significantly affected metabolites involved in fatty acid metabolism (e.g., linoleic acid, α -linolenic acid and arachidonic acid), amino acid metabolism (e.g., valine, phenylalanine and glutamic acid), as well as bile acid biosynthesis (e.g., glycocholic acid, 25-hydroxycholesterol and chenodeoxycholic acid). Spearman correlation analysis revealed significant negative association exist between *Alistipes* and α -linolenic acid on day 7, between *Moryella* and linoleic acid on day 14, between *Prevotella_2* and chenodeoxycholic acid on day 21, and between *Howardella* and phenylalanine on day 35, respectively. These findings reveal that LBW could be associated with the aberrant gut bacterial community and metabolism status of piglets, which could further contribute to the impaired growth and development of these LBW neonates.

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Effects of enriched pig housing on gut microbiome and immunology

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A previous study showed that enriched housing increased disease resilience of piglets. The present study assessed whether disease resilience could be associated with housing-induced changes in gut microbiota composition. We studied the microbiota of piglet faeces at weeks 2, 4, 5, 7, and 9 after birth, and of the lumen of jejunum, ileum, and colon at week 9 by 16S rRNA gene sequencing, as well as various immunological parameters, comparing 8 piglets per group in enriched (E) and standard barren (B) housing, respectively. Piglets were weaned after sampling at week 4. Enriched housing provided double space and rooting substrate of peat and wood shavings. Both in faeces and gut luminal contents, and regardless of group or time point, the microbiota of individual piglets was dominated by *Firmicutes*, with *Lactobacillus* being in general the most predominant genus. Groups did not differ in overall community structure and composition in faeces or gut luminal contents. Before weaning, the faecal microbiota of piglets was numerically more diverse in E than in B, and inter-individual distance in microbiota composition within E was larger than within B. After weaning, these differences between groups became smaller. Ileal (week 9) microbiota showed distinct grouping of B and E group animals when using Principal Coordinate Analysis of weighted Unifrac and Bray-Curtis distances ($p < 0.05$). Moreover, inter-individual variation for ileum and colon communities was clearly lower within E than within B ($p < 0.005$). Next step is to link these results to the performance, behavioural, and immunological parameters studied in both groups.

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Dietary sulfur amino acids affects jejunal cell proliferation by affecting antioxidant capacity, Wnt/ β -catenin and mTOR signaling pathways in weaning piglets

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Introduction Sulfur amino acids (SAA) has been suggested to be one of the main factors that affect intestinal function of piglets. We hypothesized that SAA may via affecting intestinal epithelial cells renewal to affect intestinal function.

Material and methods A total of 16 Duroc \times Landrace \times Yorkshire piglets were weaned at 21 d of age and randomly assigned to 1 of 2 groups consisting of diets with low (0.53%) or high (0.85%) levels of SAA for a 7-d period. All the piglets were euthanized for tissue sampling on 7 d post-weaning. Jejunal epithelial cells were sequentially isolated along the crypt-villus axis and yielded 3 “cell fractions” (upper villus, middle villus and crypt cells). Jejunal crypt cell proliferation was determined using Ki-67 immunohistochemistry. The activities of antioxidant enzyme were analyzed using commercial kits and quantitative PCR was conducted for tight junctions and amino acid transporters. Western Blotting was performed for selected signaling pathway.

Results The number of proliferating cells per crypt for piglets fed high SAA diet were lower ($P < 0.05$) than that in low SAA diet. The diet with high SAA increased ($P < 0.05$) the activities of total antioxidant capacity (T-AOC), catalase (CAT) and superoxidodismutase (SOD) compared with low SAA diet. The activities of SOD in middle villus and crypt cells were greater ($P < 0.05$) than upper villus. The mRNA expression of *claudin-1*, *Slc5a1*, and *Slc7a9* in piglets with high SAA diet was lower ($P < 0.05$) than that in low SAA piglets. Moreover, the amount or phosphorylation of proteins related to Wnt/ β -catenin and mTOR signaling pathway were also affected ($P < 0.05$) by SAA along the crypt-villus axis in weaning piglets.

In conclusion, dietary SAA affected jejunal cell proliferation and functions in weaning piglets and it may partly through affecting antioxidant capacity and Wnt/ β -catenin and mTOR signaling pathways.

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Supplementation of *Lactobacillus reuteri* LR1 modulates the colon microbiome and enteroendocrine cells in weaned piglets

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Abstract

Lactobacillus reuteri represents a promising alternative to in-feed antibiotics by improving the intestinal barrier function and immunity of piglets. This study was to test the hypothesis that *Lactobacillus reuteri* might modulate the colon microbiome and the densities of enteroendocrine cells in piglets. A total of 144 weaned piglets at 21-day-old (Duroc × Landrace × Large White) were randomly assigned to three dietary treatments. Each treatment had eight replicates with six piglets per replicate. The piglets were fed either control diet (control) or control diet supplemented with in-feed antibiotics (100 mg/kg olaquinox and 75mg/kg chlortetracycline) or probiotic *Lactobacillus reuteri* LR1 (5×10^{10} CFU/kg). The experiment lasted 6 weeks. The colon microbiome and densities of enteroendocrine cells were analyzed by 16S rRNA high-throughput sequencing and immunohistochemistry, respectively. Our results showed that LR1 supplementation increased ($P < 0.05$) the microbial diversity and community structures of colon contents in weaned piglets when compared to the control. Moreover, LR1 supplementation increased ($P < 0.05$) the class of *Bacilli*, the order *Lactobacillales*, the genus of *Lactobacillus*, the family of *Lactobacillaceae* and *Acidaminococcaceae*, as well as the species of *Lactobacillus amylovorus* in colon contents of piglets. Furthermore, the microbial functional prediction analysis showed that the microbiota in colon contents of piglets fed LR1 supplemented diets had a higher ($P < 0.05$) abundance of methane metabolism. There was no difference ($P > 0.05$) in the densities of 5-hydroxytryptamine (5-HT) positive cells in the colon among three treatment groups. However, LR1 supplementation increased ($P < 0.05$) the number of glucagon-like peptide-1 (GLP-1) positive cells in the colon of piglets when compared to the other two groups. Collectively, these results suggest that LR1 supplementation for 6 weeks modulated the microbial diversity and microbial community as well as the development of GLP-1 cells in the colon of weaned piglets.

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Effect of different sources of starch on composition and activity of a pig microbiota in a validated, dynamic *in vitro* model of the colon

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Swine feed contains >70 energy% starches, but there is little research on the effect of different starch sources on gut bacteria. The present study aimed to see if different starch sources could change the gut microbiota composition and activity. Fecal inoculum was prepared from finishing pigs (~70 kg). Feces was collected (fresh grab samples collected from 48 pens housing 6 pigs/pen) from pigs fed a commercial diet, with ~70 energy% starch. Feces was pooled, standardized, mixed with glycerol and frozen in liquid nitrogen until inoculation in the *in vitro* model. The microbiome was fed three different starches (from potato, wheat and maize) in a validated, dynamic *in vitro* model of the colon (TIM-2). The microbial composition and production of microbial metabolites were analyzed at three different time points (24, 48, and 72 hours) by sequencing of the V3-V4 region of the 16S ribosomal RNA gene and ion chromatography, respectively. The sources of starches ($P = 0.13$) and time ($P = 0.13$) had no significant effect on alpha diversity (Shannon index), but the microbiota showed distinct grouping on the three starches when using Principal Coordinate Analysis of unweighted Unifrac ($P_{\text{PROMANOVA}} = 0.037$), weighted Unifrac ($P_{\text{PROMANOVA}} < 0.001$) and BrayCurtis Distances ($P_{\text{PROMANOVA}} < 0.001$). The lowest longitudinal changes in beta diversity (compared to the original fecal inoculum) were observed for Maize starch. The microbiota at the phylum level was dominated by Firmicutes (potato starch: 30%, wheat starch: 50%, maize starch: 45%), Bacteroidetes (potato starch: 36%, wheat starch: 27%, maize starch: 17%), and Actinobacteria (potato starch: 30%, wheat starch: 14%, maize starch: 37%). The average cumulative amount of SCFA (acetic acid, propionic acid and butyric acid) produced on potato, wheat, and maize starch was 99.3, 104, and 147 mMol, respectively. The average cumulative amount of BCFA (iso-butyric acid and iso-valeric acid) produced on potato, wheat, and maize starch was 4.7, 7.5, and 15.4 mMol, respectively. In conclusion, different sources of starch changed the gut microbiome composition and showed differences in microbial activity during *in vitro* fermentation. Maize starch may be the best option for simulating pig feeds in the *in vitro* model as the microbiota composition was closest to the original inoculum. It will be interesting for future work to examine the microbial functionalities. The *in vitro* model proved to be an excellent tool to mechanistically study the effect of feed ingredients on the gut microbiota of pigs.

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Effect of rearing environment and diet on gut microbial colonization, immune and functional development in neonatal piglets

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Hyperprolificacy in sows leads to the use of artificial rearing systems with milk formula feeding for piglets. We hypothesized that neonatal environment and diet influence early-life microbial colonization and gut development. Forty-eight new-born piglets were randomly allocated into four groups in a 2x2 arrangement with environment (sow-reared (SOW) or artificial rearing unit/isolator (ISO)) and diet (sow milk (SM) or formula (FO)) as main factors. On days 1, 2, 3, 5, 7, 10 and 14 of life, fecal samples were collected for microbiome analysis. At days 7 and 14, six piglets per group were euthanized for tissue and digesta sampling. Blood immune cell subsets and T-cell polarization were measured by flow cytometry. Jejunal morphology, enzyme activity and colon lactose were determined. Colon short-chain fatty acids (SCFA) and D-/L-lactate were measured. Claudin-3 and ZO-1 expression was determined by real-time q-PCR. Data were evaluated using GLM with environment and diet as main factors in SPSS. Bacterial richness in ISO group did not change significantly over time as compared to SOW piglets. Microbiota-based age prediction of neonatal piglets by random forest regression model revealed a lower age of ISO-SM and ISO-FO piglets as compared to SOW groups. In blood, total CD4⁺, CD8 α ⁺ and CD4/CD8 dp cells did not differ but T-cell lineage differentiation markers revealed a higher abundance of Tbet expressing CD4⁺, and CD4/CD8 dp and gdTCR1⁺ cells in SOW piglets at both time points, indicating enhanced Th₁-cell polarization due to higher exposure to more diverse microbiota. In jejunal epithelium, increased proliferation was associated with lower lactase activity at both time points and higher lactose concentrations in jejunal and colon digesta. High D-/L-lactate and SCFA in the colon of ISO-FO piglets were accompanied by lower expression of Claudin-3 and ZO-1. Both, environment and diet differentially affect the neonatal microbial ecosystem and host response.

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High dietary zinc oxide and chlortetracycline increase antibiotic resistance genes unrelated to chlortetracycline in weaned pigs

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High doses of dietary zinc oxide are used in many countries in piglet nutrition to counteract *E. coli* induced post weaning diarrhea in piglets. Likewise, in-feed antibiotic use is common in Asia and the Americas. The antibiotic effect of dietary ZnO has been shown to increase antibiotic resistance in enterobacteria. This has also been observed for antibiotic use. As both additives are in use in pig nutrition, the aim of this study was to monitor the development of antibiotic resistance- and related genes to investigate possible additive effects of dietary zinc oxide and chlortetracycline. Weaned pigs (n = 10 per group) were either fed diets with 110 mg/kg (low) or 2400 mg/kg (high) of a commercial zinc oxide preparation with or without addition of 300 mg/kg chlortetracycline (CTC). Fecal samples were taken and DNA thereof was amplified by qPCR to quantify enterobacteria and a range of antibiotic resistance genes. Data was analysed by the Kruskal-Wallis test, followed by the Mann-Whitney U test. The enterobacterial tetracycline resistance gene was transiently increased in diets containing high zinc, chlortetracycline and their combinations after four days. Similar transient increases were visible for chloramphenicol (MdtL)- and macrolide (MacB) resistance genes. However, sulfonamide (sul1)-, bacitracin (BacA)-, polymyxin (ArnA) and enterobacterial streptomycin (aph(6)-ID) resistance genes showed higher quantity in diets containing high zinc, chlortetracycline and their combinations even after 14 days. No differences between diets were visible for the Gram-positive streptomycin resistance gene aph(3)-IIIa. Interestingly, CTC containing diets also yielded higher copy numbers for the enterobacterial integrase 1 gene, which is responsible for incorporation of foreign DNA. This study has shown that

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there are additive effects of high dietary ZnO and chlortetracycline regarding several antibiotic resistance genes. Furthermore, there are strong indications that the general spread of antibiotic resistance genes is enhanced by a combination of ZnO and CTC.

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Effects of dietary protease on growth performance and gut microbiota of growing-finishing pigs

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The investigated the effects of dietary protease on growth performance and gut microbiota of growing-finishing pigs. A total of 84 pigs (25 ± 2.2 kg initial BW; equal number of barrows and gilts) were randomly assigned to 3 dietary treatments (4 pigs/replicate; 7 replicates/treatment) in a randomized complete block design (BW and sex as blocks). The dietary treatments were 1) a typical grower-finisher diet to meet or exceed the requirement of crude protein and amino acids as a positive control (PC), 2) PC + 0.015% protease (PCPRO), and 3) a low protein diet (calculated using enzyme matrix value) as a negative control + 0.015% protease (NCPRO). The protease used in this study was a commercial product. Pigs were fed their respective dietary treatments for 11 weeks. Fecal samples were collected by rectal palpation on the last day of experimental period. Measurements were growth performance and gut microbiota by metagenomic analysis with pyrosequencing. The performance and gut microbiota data were analyzed using the PROC GLM procedure of SAS and CLcommunity program, respectively. Pigs fed PCPRO and NCPRO had higher average daily gain (977 and 980 vs. 937 g/d; $P < 0.10$) and gain to feed ratio (0.354 and 0.362 vs. 0.329 g/g; $P < 0.05$) than those fed PC. The PCPRO and NCPRO increased ($P < 0.05$) phylum Firmicutes and genus *Lactobacillus* and decreased ($P < 0.05$) phylum Bacteroidetes and genus *Clostridium* in gut microbiota of growing-finishing pigs compared with PC. In conclusion, dietary protease improved growth rate and efficiency of growing-finishing pigs by modulating their gut microbiota.

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Increased total butyrate concentration from high amylose wheat lines during *in vitro* fermentation by pig faeces

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Reducing the rate of starch digestion in the small intestine, leads to increased resistant starch (RS) entering the large intestine (LI), which has been shown to provide health benefits when fermented by the resident microbiota. Much work has focused on chemically-modified forms of RS. One such method is to increase the amylose (AM) content; however, there is limited research examining the range of AM content from natural wheat lines. Our hypothesis was that wheat lines bred to increase AM in the grain would lead to slower fermentation kinetics and differences in short-chain fatty acid production (SCFA).

In this study, starch extracted from six wheat flour lines (W), with various amylose contents, underwent *in vitro* fermentation in both a cooked and uncooked form. Porcine faecal inoculum was used comprising of faeces collected from five pigs. Gas kinetics were recorded throughout the duration of fermentation, and SCFA and ammonium end-products analysed using Tukey Studentized Range test. It was found that, in comparison to total SCFA production, total butyrate was increased in the uncooked form rather than the cooked form ($P < 0.05$) with a MSD (0.4269). Overall, the highest AM lines of W5 (AM=87%) and W6 (AM=93%) showed significantly increased total butyrate concentrations ($P < 0.05$) from the parent line W1 ((MSD of 1.5526).

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This study provides insight into the effects of amylose variation of wheat lines and the impacts on fermentation kinetics and end-products. Higher AM content did lead to slower fermentation kinetics, however, when cooked, the same starches showed different levels of SCFA production. In this instance, total butyrate production was significantly higher after 72 h from W5 and W6. This has implications in using these high AM lines as a commercial product both in a cooked or uncooked form.

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Benzoic acid and essential oils improve performance in nursery to finish pigs

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Post weaning is a period characterized by diarrhea and drop in performance. Antibiotics were used to minimize these impacts for many years; however, their usage restriction has intensified. The general hypothesis tested in this study is that benzoic acid and essential oils are able to improve performance. The objective was to evaluate the effects of the combination of benzoic acid and essential oils on piglets performance and diarrhea in nursery to finish phase. One hundred and twenty piglets (barrows) were weaned at 23 days (6.40 ± 0.53 kg) and assigned into 3 treatments (10 replicates) in randomized block design: Negative control (CN), without any additive; Positive control (PC), with colistin (200 ppm) in the nursery diets and enramycin (10 and 5 ppm in the growth and finish diets, respectively); and association of benzoic acid and essential oil (BA + EO)(3 g/kg). All piglets were fed *ad libitum*. The animals were weighed at 0, 42, 84 and 132 days of experiment. In the nursery phase, diarrhea occurrences were recorded twice a day. The data of performance were submitted to an analysis of variance and the Tukey test was used to compare the means. For the diarrhea incidence a generalized linear model (binomial analysis) was used using the GenMod procedure (SAS, 2009). At 42 days of the experiment, BA + EO group had better feed conversion (P=0.037) than NC. At 132 days, the final weight (P=0.007) and average daily gain (P=0.019) of BA + EO and PC were higher than NC. In the nursery phase, diarrhea incidence in BA + EO was lower (P=0.001) than PC and NC. In conclusion, the supplementation with BA + EO reduces the diarrhea incidence and improves the feed conversion in the nursery phase resulting in a better performance in the total period.

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Carpal gland secretions as an alternative maternal fluid to evaluate the transfer of dietary volatile compounds in pigs

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Skin secretions have the potential to become a non-invasive easy-to-collect tissue sample alternative to amniotic fluid (AF) and milk when assessing the transfer of maternal food cues to the offspring. However, little is known on how these secretions may reflect dietary volatiles. In addition, the lack of sudoriferous glands in pigs pose additional limitations to transferring odour cues of dietary origin to skin secretions. The existence of carpal glands (CG) on the caudomedial side of the front legs offer an alternative route for volatile compound release, unexplored to date in pigs. We hypothesised that dietary volatile compounds found in CG secretions mimic the transfer profile to AF and colostrum. Eight multiparous Large White sows were assigned to one of two experimental diets from d 107 of gestation until weaning: 1) control diet; 2) control diet supplemented with a mix of essential oils (EO) (thyme, peppermint gum and nerolina) dosed at 300 mg/day/sow (EOS). AF was collected at farrowing and colostrum and CG samples were collected on d 1 post-farrowing. The principal compounds of each EO (thymol, piperitone and linalool) were analysed by GC-MS. A two-way ANOVA (Prism 7, GraphPad Software Inc.) was used for statistical assessment. The three principal compounds were higher (P < 0.05) in AF of EOS compared to control sows. However, no significant transfer (P > 0.05) was detected in colostrum. Relative to the control group, thymol was significantly (P < 0.05) transferred to CG. In the EOS group, thymol concentrations in CG and AF were not significantly different

($P > 0.05$). Thus, our data suggest that CG secretions have the potential to be used as an alternative to AF to monitor the transfer of specific dietary volatile compounds. In addition, our findings support the hypothesis that CG contribute to the post-natal communication between sow and piglets, which warrants further investigation.

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Digestible energy content of *Camelina sativa* co-products for growing pigs

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Camelina sativa is an oilseed cultivated in Europe and its oil is used for biofuel production. Solvent-extracted camelina meal (CM) and expeller-extracted camelina expellers (CE) are the co-products that remain after oil extraction. A study was conducted to evaluate the apparent digestible energy concentration of CM and CE for growing pigs. Thirty-six Pietrain x (Landrace x Large white) barrows of 61.8 ± 2.83 kg body weight were allotted to 6 treatments (6 pigs/treatment). Experimental diets were a basal corn-soybean meal diet without or with 10% or 20% CM and 10%, 20% or 30% CE. The experiment lasted 15 days with 10 days for adaptation and 5 days for separate and total collection of faeces and urine, using ferric oxide to mark the beginning and the end of the faecal collection period. Pigs were fed at 3 times the requirement for maintenance metabolizable energy in two equal meals daily. Apparent digestible and metabolizable energy content of CM and CE was calculated by regression. The CM was greater in protein (419 vs. 381 g/kg dry matter; DM) and fibre (397 vs. 351 g neutral detergent fibre/kg DM) compared with CE. The proportion of soluble and insoluble fibre was not different between CM and CE (6.8g/100g DM soluble and 19.1g/100g insoluble non-starch polysaccharides). However, CE was greater in ether extract (120 vs. 20 g/kg DM) and gross energy (5050 vs. 4639 kcal/kg DM) compared with CM. The apparent digestibility of energy in CM and CE was 0.653 (0.965 CV, $R^2 = 0.86$) and 0.655 (1.21 CV, $R^2 = 0.89$) for CM and CE, respectively. The digestible and metabolizable energy values were 3027 and 2996 kcal/kg DM in CM and 3307 and 3278 kcal/kg DM in CE. In conclusion, the CE had greater energy value than CM, likely due to the greater oil content in CE than in CM.

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Higher boron supplementation of a diet for weaner pigs arrested growth performance whereas no effect on intestinal morphology

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Boron is known for an essential mineral that would affect various physiological and metabolic functions of pigs (Armstrong et al, 2003). The objective of this study was to determine the effect of dietary boron concentrations on growth performance and intestinal morphology of pigs reared under a farm-like conditions for two-week after weaning. A total of 108 male pigs [Duroc x (Yorkshire x Landrace)] weaned at 21 days (d) of age with an initial body weight of 6.5 ± 0.3 kg (mean \pm SEM) were allocated in to one of three dietary treatments (i.e., 0, 5, and 10 ppm boron) and two environments (sanitary vs. poor sanitary). Pigs were weighed individually and feed intake was measured on pen basis weekly. At the end of the experiment, pigs ($n = 36$) were euthanized and collected ileal tissue samples to determination of crypt depth (CD), villous height (VH) and VH:CD ratio. Data were analyzed using completely randomized block design with GLM procedure of two-way ANOVA in SPSS software. Pigs fed diets with 0 and 5 ppm boron showed similar daily gain and feed intake from 1 to 14 days, regardless of environmental conditions. However, lower ($P \leq 0.05$) daily gain and feed intake was observed for pigs fed a diet supplemented with 10 ppm boron compared to those were in the other two dietary treatments from 1 to 14 days. Dietary boron concentrations did not alter the intestinal morphology for pigs regardless of environmental conditions. Sanitary environment showed higher ($P \leq 0.05$) VH but lower ($P \leq 0.05$) VH:CD ratio than poor sanitary condition on day 14.

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In conclusions, pigs fed a diet containing 10 ppm boron had lower growth performance regardless of environmental conditions and found no difference for intestinal morphology on d 14 after weaning.

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Piglets prefer low (25mM) over high (100mM) concentrations of acids without improving the hedonic values for sugar or glutamate (MSG)

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The use dietary acidifiers is common practice to compensate for low HCl secretion in the stomach and improve feed digestibility and gut health in piglets. In a previous study, we observed a robust preference (80%) for citric (CA), phosphoric (PA) and tartaric (TA) acids at low concentrations (3mM, 4mM and 9mM, respectively). In addition, TA and PA were also highly consumed to a similar intake than a high sugar concentration (200mM). So, we tested the hypothesis that high doses of TA and PA would stimulate higher intake than sugar in piglets. Ninety-six pigs (8.8 ± 0.1 kg) were housed in pairs of males or females and offered commercial feed and water *ad libitum*. Pigs were trained on a double choice (DC) procedure offering two stainless steel bowls containing either water or a test solution. The DC consisted of two-minute sessions testing the choice for sucrose or MSG at 100mM (positive controls), and lactic acid (LA), CA, PA and TA at 25, 50, 75, 100mM in 22 piglet pairs each. Preference values were compared to the neutral value (50%) using the Student's t-test. Sucrose and MSG were significantly ($P < 0.01$) preferred over water with 76% and 83% preference. All acids were highly ($P < 0.05$) preferred (79%, 75%, 67% and 72% for CA, PA, LA and TA, respectively) at 25mM. However, preferences for all the acids at higher concentrations declined except for LA which peaked (76% preference) at 75mM. At the highest dose (100mM) only CA was significantly ($P < 0.05$) preferred (70%), a lower value than the positive controls (sugar and MSG). In addition, all acid solutions tested showed a lower ($P < 0.05$) intake than MSG and sugar controls (100mM). We conclude that compared to low doses or MSG or sugar, high doses of acidifiers do not improve the motivation to consume in piglets.

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Differentiating disease versus reduced feed intake on apparent total tract digestibility of porcine reproductive and respiratory syndrome virus-challenged pigs

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most significant pig pathogens worldwide resulting in morbidity and mortality. This study objective was to determine the impact of PRRSV on nursery pig performance and apparent total tract digestibility (ATTD) of dry matter (DM), organic matter (OM), energy, and nitrogen (N). Twenty-four PRRSV naïve gilts were selected (11.70 ± 1.06 kg BW) and allocated across three treatments ($n=8$) based on BW. Treatments were: 1) Ad libitum-fed (Ad), 2) Restrict-fed (RF), and 3) Ad libitum-fed, PRRSV-inoculated (PRRS). RF pigs were pair-fed the daily feed intake of PRRS pigs to account for voluntary reduced feed intake during challenge. Performance was assessed over a 35 day post inoculation (dpi) period and feces were collected over three periods (dpi 5-9, 13-17, and 25-29) to determine ATTD coefficients of energy and nutrients. Data were analyzed as repeated measures. Overall, ADG decreased in PRRS (60%) and RF (55%) pigs compared to Ad pigs ($P < 0.001$). ADFI decreased in PRRS (51%) and RF (49%) pigs compared to Ad pigs ($P < 0.001$). However, G:F did not differ among treatments. For ATTD coefficients, there was no period by treatment interaction. Across period, ATTD of energy ($P < 0.0001$), N ($P < 0.0001$), DM ($P = 0.0004$), and OM ($P < 0.0001$) were higher at dpi 13-17 compared with dpi 5-9 and 25-29. Irrespective of period, RF pigs had increased ATTD of energy ($P = 0.05$) and N ($P = 0.02$) compared to Ad and PRRS pigs. Further, ATTD of OM tended to be increased in the RF pigs compared with Ad and PRRS pigs ($P = 0.07$). In conclusion, voluntary feed intake did not alter ATTD coefficients (PRRS versus

Ad). However, when pair-fed to PRRS pig feed intakes, ATTD coefficients were increased, particularly at dpi 13-17. Therefore, while reduced feed intake increased efficiency of healthy pigs, this was not observed in challenged pigs.

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Variation in pig performance and nutrient digestion among farms seems unrelated to long-term farm health status

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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 affected. Several studies have shown increased requirements for specific amino acids in case of chronic immune stimulation, but the requirements under various on-farm conditions, are unclear. To test the hypothesis that pig performance and immune stimulation are related to farm health status, two farms with high and four with suboptimal health conditions (classified based on 12-month's performance and slaughter-house data) were compared during 15 days. Among farms, management procedures were aligned. Per farm 100 male growing pigs (42 ± 4 kg; 10 pigs/pen) of the same crossbreed, received the experimental diet. Daily gain (DG; 828-1058g/day, $P < 0.001$), feed conversion ratio (FCR; 1.85-2.27, $P < 0.001$), dry matter digestibility (83.7-85.1%, $P < 0.001$), N-digestibility (78.1-81.2%, $P < 0.001$), and various parameters of the immune system measured in blood (ISP; acute phase proteins, neutrophils, lymphocytes, anti-KLH-IgG, anti-KLH-IgM; $n = 60$ pigs) varied among farms, but were not related to a-priori health classification. Trends for negative correlations between ADG and blood-concentrations of the acute phase proteins C-reactive-protein (-0.096 , $P = 0.09$) and PiGMap (-0.110 , $P = 0.09$) and the nutritional-acute-phase-index (-1.38 , $P = 0.10$), and a positive correlation between FCR and PigMAP (0.18 , $P = 0.002$) were found. N-digestibility was negatively correlated with blood-concentrations of neutrophils (-1.145 , $P = 0.026$) and positively with concentrations of lymphocytes (0.053 , $P = 0.005$). All correlations varied between farms ($P < 0.001$). Other associations between ISP and performance or digestibility were absent. Substantial variation exists in performance and digestibility among farms, even when diets, management, and genetics are aligned. These differences, observed during a 3-week period, seem unrelated to farm health scores over a 12-month period. Correlations between ISP and performance, appeared weak at best. We conclude that variation in performance among farms, likely related to variation in subclinical health, deserves further exploration.

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Does the source of citrus pulp affect digestion in pigs?

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Agro-industrial by-products used in animal feed, such as citrus pulp, can be an interesting alternative to improve swine production sustainability. However, there is lack of knowledge on the effect of the different processing methods applied to the citrus pulp management in the Mediterranean regions that can modify its composition and therefore its nutrients digestibility. With this aim, a study of the inclusion of dehydrated citrus pulp (DCP) and silage dried citrus pulp (SDCP) in pig diets and its effects on nutrients digestibility was performed using 24 pigs of 62.3 ± 2.8 kg BW. Three experimental feeds were designed; a basal diet and another two diets produced by substituting 500 g/kg of the basal diet with either DCP or SDCP. Total faeces and urine excretion were collected using metabolic cages and

analysed for the determination by difference of the apparent total tract digestibility (CTTAD) of nutrients. The data set was analysed in a one factor ANOVA, with citrus pulp source as the main effect. Results showed that diets with DCP, characterized by higher sugars and soluble fibre content than SDCP (274 g/kg DM and 287 g/kg DM vs 101 g/kg DM and 271 g/kg DM respectively), lead to higher CTTAD ($P < 0.05$) for dry matter, ash, crude protein (0.75 ± 0.061 vs 0.70 ± 0.063), neutral detergent fibre, soluble fibre, cellulose and gross energy (0.85 ± 0.022 vs 0.82 ± 0.033) compared to diets with SDCP. Calculated digestible energy from DCP and SDCP were 3287 and 2999 kcal/kg DM respectively, whereas apparent digestible protein ranged from 35.68 to 31.68 for DCP and SDCP. Overall, we can conclude that the silage and dried process of citrus pulp generates a lower quality by-product in terms of nutrients digestibility and energy source being the DCP more suitable for its inclusion in pig diets.

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Comparison of sow urine production measured by total collection and para-aminohippuric acid infusion

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Urine production measured by *para*-aminohippuric acid (pAH) infusion method is rather precise for short-term studies but continuous infusion of pAH is required and therefore not suitable in practice. The present study aimed to compare sow urine production measured by total collection (TC) for 6 hours with that measured by pAH infusion method. Ten sows were surgically implanted with mammary vein catheter for infusion of pAH on 0 (during farrowing), 7 and 17 day in milk (DIM). Insertion of urinary catheter and infusion of pAH was done at least 2 hours before the first sampling and the bladder was emptied at 0, 2, 4 and 6 hours on 0, 7 and 17 DIM. The collected urine at 2, 4 and 6 hours were weighed and subsampled for pAH analysis. Urine production measured by pAH method was calculated as infusion rate of pAH/concentration of pAH in the urine. Urine measured by TC and pAH methods were analyzed using the MIXED procedure of SAS including sampling time, DIM and their interaction as fixed effect and sow as random component. Correlation of urine measured by the two methods was assessed using the CORR procedure of SAS. Results were reported as mean \pm SEM. Average urine production measured by TC and pAH methods were 163 ± 19 and 170 ± 17 g/h, respectively, and these two methods were highly correlated ($r = 0.84$; $P < 0.001$). Irrespective of the methods, urine production was decreased as sampling time progressed ($P = 0.002$), but was similar across DIM. The linear regression of TC method on pAH method had an intercept which was close to zero (-0.30 ± 139 ; $P = 0.99$) and slope close to one (0.96 ± 0.12 ; $P < 0.001$). In conclusion, urine production measured by pAH method and TC for 6 hours gives comparable results and neither of these two methods seems to be superior in terms of precision.

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Flavour variety increases the acceptability of feed in nursery pigs

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Diets formulated in pig production are in accordance with animal's nutrient requirements. Nevertheless, sensory specific satiety (SSS) could negatively affect intake even if adequate ingredients are used. The hypothesis of the present experiment was that flavour variety within or between meals increases nursery pig's consumption. Thirty-two nursery pigs (42 days old, 13.2 ± 1.2 kg) allocated into 16 pens (2 pigs/pen) were tested in two consecutive trials. In Trial 1 pens were either tested with three different flavours added to their starter commercial diets (0.075%) (one flavour/feeder: lemon, coffee and cherry) at the same time (Floramatic; Santiago, Chile) ($n = 5$), with the same flavour (lemon) in three feeders ($n = 5$), or with the mix of the three flavours in three feeders ($n = 6$) during 90 minutes. On days 2 and 3 pens received a different treatment to counterbalance the experimental design in a 3x3 Latin square. A similar procedure was conducted in Trial 2, but feeders were rotated every 30 minutes to complete a total 90 minutes period and flavours used were orange, chocolate and grape (Floramatic; Santiago, Chile). Data was analysed with an ANOVA procedure by

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using the statistical software SAS[®]. No intake differences were observed in trial 1 or 2 according to treatments ($P=0.256$ and $P=0.414$, respectively). Nevertheless, an interaction between treatments and day was observed in both trials ($P=0.032$ and $P=0.034$) respectively, were animals that received three different flavours at the same time or each 30 minutes presented a higher intake at the second and third day but not at the first day. Flavour neophobia could prevented the reduction of SSS at the first day of exposure. However, a variety of flavours given simultaneously or in order could increase feed intake in nursery pigs when animals have a previous experience with those flavours.

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Intestinal function and integrity responses to the use of in-feed sub-therapeutic antibiotics

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In many countries sub-therapeutic antibiotic growth promotant (AGP) use is illegal due to concerns over antimicrobial resistance. With the loss of this technology, alternatives to AGP use in nursery pigs are needed to amend losses in pig growth and feed efficiency. However, the mechanism of action by which AGPs work is poorly understood. Therefore, the objective of this study was to determine the mechanisms of action by which AGPs increase nursery pig performance. We hypothesized that sub-therapeutic in-feed chlortetracycline (CTC) would improve markers of intestinal function and integrity in nursery pigs and these improvements would contribute to the performance gains associated with AGP use. Over two replicates, a total of 24 weaned pigs (6.75 ± 0.75 kg BW) were randomly allocated to either control (CON, $n=12$) or sub-therapeutic antibiotic (AB, $n=12$; 40 ppm in-feed CTC) treatments and individually housed. Pigs were ad-libitum fed corn-soybean meal-based diets in two-phases for five weeks. Weekly body weight and feed disappearance were recorded and individual pig ADG, ADFI, and G:F calculated. All pigs were euthanized on day 35, cecum contents collected for volatile fatty acids (VFA) analysis, and ileum and colon tissues collected for assessment of *ex vivo* intestinal function and integrity using modified Ussing chambers and mRNA abundance. Overall, the AB pigs had increased ADG (134%, $P<0.01$) and ADFI (130%, $P<0.01$) compared with the CON pigs. Feed efficiency and cecum concentrations of VFA did not differ. No differences in ileum active glucose and glutamine transport or associated nutrient transporter mRNA abundances were observed. Further, ileum and colon transepithelial resistance, tight junction protein and inflammatory gene mRNA abundances were not different between treatments. These data suggest that the mode of action by which sub-therapeutic CTC improves nursery pig performance may not be directly related to intestinal function and integrity.

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The variety of whole grain cereal differentially impacts the gut microbiota

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Whole grain foods from wheat and rye are typically consumed in large quantities in the Nordic countries and are rich sources of dietary fibers and other bioactive compounds. Dietary fibers has consistently been associated with reduced risk of developing metabolic disorders such as type 2 diabetes and cardiovascular disease, and the protective mechanism is partly linked to the intestinal microbiota. Dietary fiber provides substrates for intestinal microbes but the amount and composition of dietary fiber is different between whole grain wheat and rye and may therefore exert different effects in vivo. The aim with the present study was to increase the knowledge of mechanisms behind health aspects related to whole grain products and therefore we investigated how whole grain rye bread (WGRB), whole grain wheat bread (WGWB) and refined wheat bread (WB) influenced the gut microbiota composition in a pig model. Five 10-weeks-old female growing pigs (Swedish Landrace \times Yorkshire) were used in the study. The pigs were kept individually in pens and the experiment was performed as a change-over experiment with five pigs, three diets and

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three periods, each lasting for seven days. Three types of breads, WGRB, WGWB and WB were used in the study. Fecal samples were collected from each pig immediately after defecation in the end (d6 and d7) of each experimental period. The microbiota was analysed from 16S rRNA gene amplicons generated from the collected samples and the results were evaluated using principal coordinate analysis and procedure Mixed in SAS. The analysis revealed clear differences in microbiota composition, linked with type of bread, with the most profound differences between the WGRB and the WB diets. Several microbial taxa had significantly different relative abundance between diet types, with Ruminococcaceae ($P=0.012$) and Lachnospiraceae ($P=0.012$) as the most evident examples which illustrate that cereal type influence microbiota composition.

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Effects of birth weight on nitrogen digestion and utilization in grower pigs

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A low foetal growth and birth weight leads to a lower number of muscle fibres formed prenatally and a lower body protein content in low birth weight (LBW) piglets. Therefore, LBW piglets may show a lower performance and nutrient efficiency later in life. The aim of the study was to evaluate effects of BW on N-digestion and N-utilization in growing pigs. Two groups of 20 piglets with a low (LBW, 1.11 kg) and high birth weight (HBW, 1.79 kg), balanced for litter were selected and subjected to N-balance measurement using urine and faeces collection over 5 d periods. Pigs were fed a protein sufficient (NCP; CP 15.5%; 100%) or a protein restricted (RCP; CP 11.8%, 70% of sufficient) diet in a change-over design starting at 14 weeks of age. Diets were fed at 2.8xM over two meals per day. LBW and HBW pigs weighed 44 and 55 and 54 and 65 kg, respectively, at the start of the sequential balance periods. Faecal digestibility of DM, N and energy were not affected by BW, while faecal N digestibility was higher for the NCP compared to the RCP diet (91.4 vs 88.8%, $P<0.001$). N-retention was 27.7 and 19.0 g/d for the LBW pigs on the NCP and RCP diets, respectively, and 32.0 and 21.8 g/d for the HBW pigs ($P<0.001$). Both N-efficiency (N-retention/N-intake; 0.629 vs 0.635, respectively) and marginal N-efficiency (0.68 and 0.69, respectively) did not differ between LBW and HBW pigs. IGF-1 (210 vs 265 $\mu\text{g/l}$) and insulin (11.0 vs 17.1 uU/ml) concentrations in plasma were lower in LBW compared to HBW pigs ($P<0.05$). Birth weight is a determinant for BW gain and N-retention later in life, however, N-efficiency at later age is not affected by birth weight.

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Effects of oral monosodium glutamate administration on serum metabolomics of suckling piglets

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Introduction Metabolomics has immeasurable potential for the development and application in animal nutrition. This study was conducted to determine the effects of monosodium glutamate (MSG) on serum metabolomics of suckling piglets by ¹H NMR spectroscopy.

Material and methods Forty-eight healthy piglets (Duroc \times Landrace \times Large Yorkshire; 1.55 ± 0.20 kg body weight (BW)) were obtained from 6 sows (8 piglets per sow). The piglets from each sow were randomly assigned into 1 of 4 treatments (2 piglets per treatment), including oral administration of 0.18 g/kg BW of sodium chloride (CN group), 0.06 g/kg BW monosodium glutamate (LMG group), 0.50 g/kg BW monosodium glutamate (MMG group) or 1.00 g/kg BW monosodium glutamate (HMG group) twice a day. Blood samples were collected on 7 d and 21 d of age, and serum was recovered for hormones and ¹H NMR spectroscopy analysis.

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Results On 7 d, oral administration of MSG significantly reduced ($P < 0.05$) serum citrate content, and the HMG group had greater ($P < 0.05$) serum trimethylamine content but lower ($P < 0.05$) unsaturated fat content than other groups. On 21 d, the HMG group had greater ($P < 0.05$) serum glutamine, trimethylamine, albumin, choline and urea nitrogen contents, while had lower ($P < 0.05$) serum creatine content than other groups. The CN and LMG groups had greater ($P < 0.05$) serum CCK content than MMG and HMG groups, while MMG group had greater ($P < 0.05$) serum insulin content than other groups on 7 d of age. Moreover, the HMG group had greater ($P < 0.05$) serum GLP-1 and lower ($P < 0.05$) leptin contents than other groups on 21 d of age.

In conclusion, oral administration of MSG affects the fatty acids and amino acids metabolism via serum hormones in suckling piglets.

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Weaning stress affects protein degradation in intestinal upper villus epithelial cells

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Weaning stress leads to structural and functional changes in the small intestine of piglets, such as villous atrophy and crypt hyperplasia. The intestinal upper villus epithelial cells represent the differentiated epithelial cells, and play key roles in digesting and absorbing luminal nutrients. The present study was conducted to test the hypothesis that intestinal dysfunction and villus atrophy of weaning piglets may result from protein degradation in upper villus epithelial cells. A total of 30 piglets were weaned at 21 d of age and 6 piglets were randomly slaughtered at 0 d, 1 d, 3 d, 7 d, and 14 d post-weaning. The jejunal upper villus epithelial cells were isolated using a sequential isolation method, and protein synthesis was analyzed using the isobaric tags for relative and absolute quantification. The mRNA expression of genes related to protein degradation and amino acids abundance were measured using real-time quantitative PCR and high-performance liquid chromatography, respectively. The results showed that the synthesis of proteins involved in the protein catabolic process was mainly up-regulated. The mRNA expression of cathepsin C, cathepsin H, and cathepsin L was up-regulated during the early period (0 d-3 d) post-weaning, while no significant differences in *PSMA1*, *PSMA2*, *PSMA3*, and *PSMA4* mRNA expression were observed. Moreover, the abundance of 20 amino acids in upper villus epithelial cells was altered ($P < 0.05$) during the post-weaning period. These results indicated that weaning stress may partly via enhancing protein degradation in villus epithelial cells to affect intestinal morphology and function in piglet.

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Effects of dietary protease on growth performance, nutrient digestibility, and gut microbiota of growing pigs

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This study was conducted to investigate effects of dietary protease on growth performance, nutrient digestibility, and gut microbiota of growing pigs. A total of 84 growing pigs (25.3 ± 2.16 kg BW) were randomly assigned to three dietary treatments (4 pigs/replicate; 7 replicates/treatment) in a randomized complete block design (BW and sex as blocks). The dietary treatments were 1) a typical grower diet to meet or exceed the requirement of crude protein and amino acids as a positive control (PC), 2) PC + 0.015% protease (PCPRO), and 3) a low protein diet as a negative control + 0.015% protease (NCPRO). Pigs were fed respective dietary treatments for 6 weeks, the last week of the experimental period, pigs were fed respective dietary treatments containing 0% chromic oxide as an indigestible marker. Fecal samples were collected by rectal palpation for the last 3 days after 4-day ad period. Measurements were growth performance, nutrient digestibility by an index method, and gut microbiota by metagenomic analysis with

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pyrosequencing. The performance and digestibility data were analyzed using the PROC GLM procedure of SAS. The gut microbiota data were analyzed using CLcommunity program. Pigs fed PCPRO and NCPRO had higher average daily gain (953 and 943 vs. 920 g/d; $P \leq 0.10$), gain to feed ratio (0.451 and 0.462 vs. 0.432 g/g; $P \leq 0.10$), apparent total tract digestibility of crude protein (76.15 and 77.72 vs. 69.59%; $P < 0.05$) than those fed PC. In addition, PCPRO and NCPRO increased ($P < 0.05$) phylum Firmicutes and genus *Lactobacillus* and decreased ($P < 0.05$) genus *Clostridium* in gut microbiota of growing pigs compared with PC. In conclusion, dietary protease improved growth rate and efficiency of growing pigs by increasing their nutrient digestibility and modulating their gut microbiota.

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Heart rate as predictor of heat production at different reproductive stages in second parity free-ranging sows

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The objectives were to investigate relationships between sow heart rate (**HR**) and heat production (**HP**) in confined sows (Experiment-1) and to apply these relationships to estimate HP of free-ranging sows (Experiment-2). The mixed procedure (SAS 9.3) was applied to analyze the two datasets. Sow HR was recorded and HP was estimated in eight second parity sows during gestation (day 30, 60, 80 and 104) and during lactation (day 10, 17 and 24) using indirect calorimetry (RQ-method, Experiment-1). The relationships between HR and HP were: Sow HP, MJ/d ($R^2 = 0.62$) = $0.323 (\pm 0.025; P < 0.001) \times \text{HR, bpm} - 2.4 (\pm 2.3; P = 0.33)$ during gestation and: HP, MJ/d ($R^2 = 0.25$) = $0.118 (\pm 0.034; P = 0.003) \times \text{HR, bpm} + 26.7 (\pm 3.4; P < 0.001)$ during lactation. In experiment-2, two groups of free-ranging second parity sows ($n = 41$) were used to record HR and distance covered on day 60 and 100 of gestation and on day 5, 20 and 40 of lactation using activity sensors. Daily HR was calculated as an overall mean of each day and HR during inactivity was obtained as an average of HR when distance = 0 meter/minute. The 24-hour HR was then estimated as a weighted average of the daily HR (sunrise to sunset) and HR during the night (sunset to sunrise represented by HR during inactivity). The HP of free-ranging sows was calculated from the established equations and found to increase from 27 to 30 MJ/day from day 60 to 100 in gestation ($P < 0.001$) and further to 38 MJ/day on day 5 in lactation ($P < 0.001$), whereas it remained rather stable during the remaining lactation period (39-40 MJ/day). In conclusion, HR was linearly related to HP, and HP increased by 48% from mid-gestation to late-lactation in free-ranging sows.

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Net portal appearance of proteinogenic amino acids in Iberian pigs fed betaine and conjugated linoleic acid supplemented diets

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Betaine and conjugated linoleic acid (CLA) have a synergistic effect on growth and nutrient partitioning in pigs. However, the mechanism of action remains unclear. The objective of this study was to determine if the use of betaine and/or CLA in the diet affects the net portal appearance (NPA) of amino acids (AA) in Iberian pigs. Sixteen 30 kg BW Iberian barrows were randomly assigned to four diets (control, supplemented or not with 0.5% betaine, 1% CLA or 0.5% betaine + 1% CLA) and surgically fitted with three chronic indwelling catheters: in portal and ileal veins, and carotid artery. Blood samples were taken simultaneously from carotid artery and portal vein at 0, 0.5, 1, 1.5, 2, 3 and 6 h after feeding 1.2 kg of the diet. Blood flow was determined by the indicator dilution method using para-aminohippuric acid infused into the ileal vein, and NPA ($\mu\text{mol}/\text{min}$) of AA was estimated according to the Fick principle. Data were subjected to multivariate ANOVA analysis using the PROC MIXED of SAS for repeated measures. NPA of sum of

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essential and non-essential AA was greatest ($P < 0.05$) for betaine followed by control, betaine + CLA, and CLA diet. Amongst essential AA Arg, His, Leu, Lys, Phe and Val the NPA was the greatest ($P < 0.05$) for betaine diet during the 6 h postprandial period. NPA of non-essential AA Ala, Ser and Tyr was the greatest ($P < 0.001$) for betaine diet. NPA of Gln was the lowest ($P < 0.001$) for betaine and CLA diet. Supplementation with betaine increased NPA of AA with respect to the control diet. This effect was not enhanced when CLA, or betaine and CLA were supplemented together. Supplementation with betaine could explain divergences in metabolism of portal drained-viscera in pigs fed betaine and to improve the availability of AA for body tissues.

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Net portal appearance of amino acids in Iberian compared to Landrace pigs fed two diets of different protein content

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Compared to modern breeds, Iberian pigs have lower rates of muscle protein deposition. Factors that limit growth performance of Iberian pigs are still unknown. We hypothesized that differences in net portal appearance (NPA) of amino acids (AA) might partially explain the lower growth rate reported in Iberian pigs compared to modern breeds. NPA of AA was measured in six Iberian and six Landrace gilts (28 kg BW) fitted with chronic catheters in portal vein (PV), mesenteric vein (MV) and carotid artery (CA). Blood samples from PV and CA were taken at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 6 h after feeding 25% of the daily intake of two isoenergetic diets (14-14.5 MJ ME/kg DM) with different CP (13 vs. 16%, LCP and HCP, respectively) content in a crossover design. NPA of AA was calculated by multiplying portal-arterial plasma AA concentration difference by PV plasma flow estimated by an indicator-dilution technique infusing para-aminohippuric acid into the MV. Data were subjected to ANOVA using the MIXED procedure. The overall NPA of the essential AA His, Leu, Phe and Trp, and non-essential AA Ala, Asn, Cys, Glu, Gly, Hpro, Pro, Ser, Tau and Tyr was lower ($P < 0.05$) for Iberian than Landrace pigs (except for Orn) during the 6-h postprandial period. When pigs consumed HCP diet the overall NPA of the essential AA Arg, His, Leu, Lys, Met, Phe, Trp and Val, and non-essential AA Ala, Asn, Glu, Gly, Ser, Tau and Tyr was greatest ($P < 0.05$). Differences in NPA of AA may partially explain the lower growth capacity of Iberian compared to modern genotypes. This may be the result of a higher utilization of AA by the portal-drained viscera in the Iberian pigs compare to Landrace.

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The effects of super-dosing phytase on finisher pig performance, inositol phosphate degradation and myo-inositol production

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Microbial phytase supplementation has been shown to improve pig growth performance when added to phosphorus adequate diets at doses above the current industry standard. The mode of action for this response requires elucidation, however, it has been suggested that the end product of phytate hydrolysis, *myo*-inositol, is important. This study set out to determine the effect of different doses of phytase on finisher pig performance, ileal phytate degradation, *myo*-inositol generation and jejunal nutrient transporter gene expression. A total of 288 crossbred pigs (mean BW 36.7 kg) were fed 1 of 6 diets *ad libitum* for 28 days. The diets comprised a nutritionally adequate diet (PC) with no added phytase, a similar diet but with Ca and P reduced by 1.6 and 1.24 g/kg respectively (NC), and this NC + 500, 1,000, 2,000 or 8,000 FTU/kg. At the end of the trial 10 pigs/treatment, each from different pens, were euthanised for the collection of ileal digesta, jejunal mucosa and plasma. Orthogonal polynomial contrasts were used to test for linear and quadratic responses to phytase supplementation using the GLM procedure of SPSS. Phytase improved the

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growth rate ($P < 0.05$) and FCR ($P < 0.001$) of NC fed pigs to a level similar to the PC; however, there was no difference between standard and high phytase treatments. Moreover, phytase increased ileal InsP₆ degradation ($P < 0.01$) in a linear manner with increasing dose. This was met with linear increases in ileal *myo*-inositol concentration ($P < 0.001$), as well as both portal and peripheral plasma *myo*-inositol concentrations ($P < 0.05$). Despite elevated plasma *myo*-inositol concentrations, there were no changes in relative jejunal SMIT2 gene expression. In conclusion, higher doses of phytase were associated with greater phytate hydrolysis and *myo*-inositol bioavailability in finisher pigs; however, this did not translate to improved growth performance.

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Standardized blend of capsicum and turmeric oleoresins given during late gestation improves performance of sows vaccinated against *E. Coli*

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Research has demonstrated that a standardized blend of capsicum and turmeric oleoresins (XT, XTRACT[®] Nature, Pancosma, Switzerland) had an immune-modulating effect in poultry. The objective of this trial was to evaluate if the supplementation of XT in late gestation could improve performance of sows vaccinated against *E. coli* during farrowing and lactation. A total of 1531 sows vaccinated against *E. coli* at 80 days of gestation and regrouped in 3 successive bands were involved. Sows in bands 1 and 3 were fed a basal diet (CT, N = 529 and 329 respectively), animals in band 2 were provided the same basal diet with 200 g/t of XT (N = 673) from 80 until 110 days of gestation. In terms of sow performance the proportion of piglets born/litter was recorded at farrowing as well as piglets' mortality during lactation. Colostrum was collected at birth for analysis of total protein, albumin and globulins. Following parameters were analysed by analysis of variance: treatment effect, colostrum quality, Parity (P) and their interaction on performance outcomes. Results show that XT increased the levels of total proteins +5.0% in colostrum ($P = 0.04$), and this was mainly driven by higher globulins concentration (+5.0%, $P = 0.08$). XT enhanced the proportion of piglets born alive per litter (92.6% vs 90.7% respectively for XT and CT, $P < 0.01$). This effect was more pronounced in sows of P1 or P2 (+2.2%, $P = 0.02$) than in sows of P3 or more (+1.9%, $P = 0.06$). The treatment did not affect piglets' mortality during suckling, but there was a treatment*P effect on this outcome: litters in P1 and P2 sows had reduced mortality when fed XT compared to CT (10.1 vs 12.5%, $P = 0.01$). Above results suggest that XT fed vaccinated sows during late gestation have the potential to improve nutrient supply to the progeny, litter performance at farrowing and litter size at weaning.

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Sows in mid-gestation have reduced digestibility and retention of calcium and phosphorus compared with growing pigs

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Values for digestibility of Ca and P obtained in growing pigs are usually also applied to gestating sows, but data to demonstrate that digestibility values from growing pigs are also representative for sows are limited. Therefore, 2 experiments were conducted. In Exp. 1, effects of feed intake and physiological state on the apparent total tract digestibility (ATTD) of Ca and P were determined. Forty-eight multiparous gestating sows (BW = 226.1 kg) were fed 1.5 or 3.5 times the maintenance requirement for metabolizable energy (ME) and 24 gilts (BW = 51.5 kg) were fed 3.5 times the requirement for ME. Diets were based on corn, soybean meal, and full-fat or defatted rice bran. The level of feed intake did not affect ATTD of Ca and P in sows, but sows had reduced ($P < 0.001$) ATTD of Ca and P compared with gilts. Experiment 2 was conducted to compare basal endogenous loss (BEL), standardized total tract digestibility (STTD), and retention of Ca and P by sows and growing pigs. Forty-eight multiparous

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gestating sows (BW = 245.9 kg) and 48 barrows (BW = 19.8 kg) were fed 4 diets that differed in concentrations of phytate and microbial phytase. Diets were based on corn, soybean meal, and full-fat rice bran. A Ca-free and a P-free diet were also formulated. Results indicated that the BEL of Ca and P from sows was greater ($P < 0.05$) than from growing pigs. Supplementation of phytase increased the STTD and retention of Ca and P if growing pigs were fed a high-phytate diet, but in sows, phytase increased the ATTD of Ca and P only in a low-phytate diet (3-way interactions, $P < 0.01$). Sows had less ($P < 0.001$) STTD and retention of Ca and P compared with growing pigs. In conclusion, gestating sows had greater BEL and reduced digestibility and retention of Ca and P compared with growing pigs.

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In vitro degradation of plant cell wall analogues using porcine faeces

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Dietary fibres (DF), originating from the primary cell walls (PCW) of grains, are a common component of pig feeds. Grain DF is a complex matrix mainly comprised of cellulose and hemicelluloses such as arabinoxylan (AX). To date, there is still limited understanding about the interactions between different components of PCW and how they are degraded in the porcine large intestine (LI). Due to the experimental restrictions to extract intact and structurally well-defined polymers from PCW, cellulose-based composites were assembled as an alternative using the *Komagataeibacter xylinus* model system. Our aim was to compare the composites of bacterial cellulose (BC) and AX, with a physical combination of these two components, in terms of their *in vitro* fermentability. We hypothesized that the composite material would be more rapidly fermentable. In the study, the produced BC, the composite (BCAX) and the physical mixture (BC&AX) were subjected to *in vitro* fermentation for 72 h with porcine faeces as inoculum. Gas production of each substrate was tested during the fermentation, together with sampling at specific time points for solid state ^{13}C CP/MAS NMR analyses. Cumulative gas profiles (Figure 1) showed that BC was the slowest to ferment ($P < 0.05$), while the two profiles for BCAX and BC&AX were not significantly different ($P = 0.38$). These results indicate that there is only a limited effect of pre-forming the cellulose and AX composite prior to fermentation, compared with the mixed substrate (BC&AX). Furthermore, there was no significant difference ($P > 0.05$) in the %crystallinity of cellulose between BCAX and BC&AX (Table 1) based on the results of solid NMR spectra and curve fitting. These results suggest that the crystalline and non-crystalline regions of cellulose in BCAX and BC&AX are probably degraded in the same ratio, suggesting that crystalline may not be a major determinant of cellulose fermentability.

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Stomach capacity of newborn piglets

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The increasing litter size of hyperprolific sows have led to a significant number of piglets showing signs of intra-uterine growth restriction (IUGR). It has been estimated that approximately 48% of the piglets that die before weaning have empty stomachs, and with IUGR piglets during the suckling period, they are at increased risk. The aim of this study was to test the hypothesis that IUGR piglets have a smaller stomach capacity at birth, and therefore require separate recommendations in regards to colostrum supplementation compared to a normal birth weight piglet. The study included 72 piglets, collected within 24 hours of farrowing, and piglets were classified as “normal” or “IUGR” based on head morphology. Bodyweight, empty stomach weight, stomach length and stomach capacity were measured. Overall, there was an influence of the piglets’ classification, with normal piglets having a greater bodyweight and stomach weight ($P < 0.001$), a greater stomach length ($P < 0.001$) and a stomach capacity ($P < 0.001$).

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Furthermore, the stomach capacity was greater in normal piglets compared with IUGR piglets ($P < 0.001$), and there was a positive correlation between piglet bodyweight and stomach capacity at birth ($P < 0.001$).

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“Super diet” beats antibiotic treatments

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Diarrhoea in weaners is commonly treated with antibiotics. It is desirable to lower antibiotic use to reduce the risk of antibiotic resistance. Feed composition has been shown to reduce diarrhoea, but can a cost-competitive “super diet” compete with antibiotic treatments in a commercial herd controlling diarrhoea. The test period started two weeks post-weaning when the pigs switched from a diet with 2500 ppm zinc to a weaner diet. The test comprised 540 pigs from 9 to 33 kg and 36 pens in each of the two groups: 1. Standard diet and group treatment for diarrhoea with deoxycycline when an outbreak was observed. 2. Feeding the pigs specially designed diet (super diet) for 10 days combined with pen wise treatment initiated when 25% of the pigs within a pen showed diarrhoea. The super diet was composed of ingredients known to positively affect gut health: 1.0% calcium formiate and 0.5% benzoic acid; 2% fish meal; 4.5% blood plasma; 17% milk products and heat-treated grain. The standard diet consisted of: 47% wheat; 27% barley; 6% fish meal; 17% soybean meal and 2% fat. All diets were meal feed and optimized according to the Danish nutritional standards. There was no difference in daily gain (596 vs 590 g/day) and mortality (3.1 vs 3.5%) for pigs treated with deoxycycline or fed Super diet respectively. However, the number of treatments per pig and number of treatments per pig and number of pen-wise treatments was lower in the pens fed the Super diet compared to batch treatment of all pigs 0.88 vs 1.17 treatment days per pig ($p < 0.001$). In conclusion, feeding the super diet lowered the number of diarrhoea treatments compared to the batch treatment with deoxycycline and this did not have a negative effect on daily weight gain and mortality.

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Effects of dietary protease on growth performance, nutrient digestibility, and gut microbiota of finishing pigs

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This study was conducted to investigate effects of dietary protease on growth performance, nutrient digestibility, and gut microbiota of pigs. A total of 84 finishing pigs (64.8 ± 4.03 kg BW) were randomly assigned to three dietary treatments (4 pigs/replicate; 7 replicates/treatment) in a randomized complete block design (BW and sex as blocks). The dietary treatments were 1) a typical diet to meet or exceed the requirement of crude protein and amino acids as a positive control (PC), 2) PC + 0.015% protease (PCPRO), and 3) a low protein diet as a negative control + 0.015% protease (NCPRO). Pigs were fed respective dietary treatments 5 weeks. The last week of the experimental period, pigs were fed respective dietary treatments containing 0% chromic oxide as an indigestible marker. Fecal samples were collected by rectal palpation for the last 3 days after the 4-day ad period. Measurements were growth performance, nutrient digestibility by an index method, and gut microbiota by metagenomic analysis with pyrosequencing. The performance and digestibility data were analyzed using the PROC GLM procedure of SAS. The gut microbiota data were analyzed using CLcommunity program. Pigs fed PCPRO and NCPRO had higher average daily gain (1.01 and 1.02 vs. 0.96 kg/d; $P < 0.05$), gain to feed ratio (0.285 and 0.292 vs. 0.257 kg/kg; $P < 0.05$), apparent total tract digestibility of crude protein (83.61 and 83.39 vs. 74.54%; $P < 0.05$) than those fed PC. In addition, PCPRO and NCPRO increased ($P < 0.05$) phylum Firmicutes and genus *Lactobacillus* and decreased ($P < 0.05$) genus *Clostridium* in gut microbiota of finishing pigs compared with PC. In conclusion, dietary

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protease improved growth rate and efficiency of finishing pigs by increasing their nutrient digestibility and modulating their gut microbiota.

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Dietary oleic acid (C18:1) is efficiently transferred to pork loin fat particularly when finisher pigs are fed a corn-soybean diet

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The distinctive sensory profile of Iberian pork has been related to a unique diet profile including acorns, which results in high oleic –C18:1 and low linoleic –C18:2- acids in carcass fat. In order to test the efficiency of the transfer of C18:1 from feed to carcass in a commercial pig breed a 3x2 factorial design consisting of 3 iso-energetic and iso-proteic feed profiles (corn/soybean (diet 1), wheat/canola (diet 2), and sorghum/lupin (diet 3) and 2 levels of C18:1 (high vs. low) was used. The manipulation of C18:1 levels was achieved by using Australian macadamia oil (62.25% C18:1) or corn oil (39.48% C18:1). Thirty-six finisher pigs (Large White x Landrace) with a starting weight of 50 ± 2.54 kg and a slaughter weight of 107 ± 6.83 kg, were housed individually and offered one of the six diets *ad libitum*. After slaughter *Longissimus dorsi* (loin) samples were collected and stored at -20°C . Fatty acid profiles were obtained by HPLC analysis. Macadamia compared to corn oil resulted in higher levels of C18:1 (25, 45 and 27%) and lower C18:2 (30, 35 and 28 %) for diets 1, 2 and 3, respectively. Feeding macadamia oil resulted in loin fat samples with higher C18:1 levels (38.70, 38.75 and 36.33%) than feeding corn oil (33.63, 36.81 and 35.71%) for the three diets, respectively. The differences were statistically ($P < 0.01$) significant for diet 1. The MUFA/PUFA ratios were higher in all the pigs fed macadamia compared to corn oil. The efficiency of C18:1 transfer from feed to loin fat was dependent on diet type. For each 1% increase in feed C18:1 the increase in loin fat was 14.9, 2.9 and 1.3 % for diets 1, 2 and 3, respectively. Thus, the corn/soybean (diet 1) facilitated a more efficient transfer of C18:1 from feed to pork loin fat.

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Identification of novel bitter compounds and their effect on performance parameters in finisher pigs

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Dietary bitter compounds have the potential to control excessive feed intake and reduce fat deposition in finishing pigs. However, it is difficult to extrapolate bitter taste in humans to pigs. Thus specific “*in vivo*” trials are required to identify bitterants in pigs. We hypothesize that a number of natural plant extracts have a significant bitter taste component, which, in turn, will affect performance in finishing pigs. To test the hypothesis, sixteen compounds known to be bitter to humans were tested in a dose-response manner in 48 piglets using a 2 minute double-choice (DC) model particularly developed to assess bitterness in pigs. Based on the DC results, one of the compounds was selected and tested in a commercial finishing pig trial. The DC showed that the strongest responses ($P < 0.01$) were elicited by quinine and denatonium benzoate both causing aversion in piglets at 0.1mM or less. Additionally, the most effective natural bitter compound was olive leaf extract (OLE) ($P < 0.01$). OLE was selected and tested in a commercial facility (SunPork Solutions, Westbrook, QLD) at four different concentrations (0, 0.05%, 0.1%, and 0.2%) involving 288 male finishing pigs at 15 weeks of age with average initial body weight of 61.1kg (four batches of 72 animals in 6 pens of 12 pigs) and *ad libitum* access to feed and water. Performance parameters were recorded weekly and back fat measured at abattoir. The results showed that OLE did not significantly ($P > 0.05$) affect regardless of the concentration. The variation of age at abattoir (since pigs were slaughtered based on body weight) may have masked potential effects.

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Fermentation end-products at four areas of the porcine large intestine change in response to dietary fruit (mango)

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Dietary fibre (DF) is important for the monogastric diet, so there is focus on the forms and structures of whole plant foods and their impact on gut processes (digestion and fermentation). Our hypothesis was that added fruit (mango) to the pig diet, would lead to a beneficial shift in large intestinal (LI) fermentation end-products.

Mango pulp was used to model whole fruit. Pigs fed this diet were compared with those fed a Control Diet (no fruit). We examined digesta from four areas of the LI (caecum-Cae; proximal-PC; mid-MC and distal colon-DC) for fermentation end-products including SCFA (including branched-chain ratio-BCR) and NH₃. Digesta samples, were collected from 20 pigs (10 pigs/diet) which were euthanized after consuming their test diets for three weeks.

Differences according to diet and GIT site were tested using Tukey's studentized range test of multiple comparisons using PROC GLM within SAS. There was considerable variation between pigs. However, proximally, the Cae and PC digesta showed significantly higher SCFA, predominantly due to acetic acid, which decreased distally with lowest values by DC. NH₃, on the other hand, was significantly lower at these two sites, but increased distally. The Mango diet-fed pigs had a higher SCFA, mainly due to acetate, while NH₃ was lower, as was the BCR (both indicators of protein vs carbohydrate fermentation).

Results indicate that added whole fruit pulp (mango) to the diet, can improve the fermentation end-product profile in the LI, particularly proximally. It is recommended to test further whole plant foods both singly and in combination.

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Fermentation end-products in the porcine small intestine change in response to fruit (mango) in the diet

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There is increasing interest in the role of fruit and vegetable dietary fibre (DF) in pig nutrition. Generally, it is assumed that the mammalian small intestine (SI) is the major site of digestion, and the large intestine (LI) is the area for fermentation. The SI is considered difficult to colonise, due to shorter transit times, effects of host surfactants and enzymes, and higher oxygen concentrations compared with the LI. Our hypothesis was that the SI is a site of significant fermentation, and that dietary mango, would lead to changes in fermentation end-products there.

Twenty grower pigs (10/diet) were fed Control or Mango diets for three weeks before euthanasia. Digesta was collected from the stomach and the second half of the SI, which was split in two (representing jejunum and ileum). They were analysed for lactate, short-chain fatty acids (SCFA) and NH₃. Differences were tested using Tukey's studentized range test of multiple comparisons within PROC GLM of SAS.

There were significant differences due to Diet and GIT Site, for almost all parameters, though not always as expected. There was a significant difference for total SCFA between diets, where Control was higher, and no difference for lactate. The Mango diet did show lower NH₃ concentrations and pH. In addition, Mango had a lower DM%. Results for the GIT site were as expected: DM% decreased along the tract, SCFA increased, NH₃ decreased, and lactate increased.

Abstract

Fermentation of both carbohydrates and protein occurred, particularly at the ileum, and in terms of total SCFA and lactate, were not increased in the presence of mango. Mango was used as a model for fruits, and these results are relevant for fruit/vegetable-rich feeds which could be fed to pigs.

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Feeding sows a gestation, a transition or a lactation diet around parturition

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During the last week of gestation sows are commonly fed a lactation diet intended to meet nutrient requirements for sow milk production. The objective of the current study was to investigate whether a gestation diet, a lactation diet or a 50/50 mix (transition diet) is most appropriate for sows around parturition. It was hypothesised that the 50/50 mix would improve performance during the transition period (farrowing duration, piglet stillborn rate and colostrum production). A total of 35 late-gestating sows, from first to fourth parity, were studied in the transition period from one week before parturition until three days after. Sows were stratified for parity and randomly allocated to one of the three treatments, of which the gestation and lactation diets were formulated in accordance with the Danish recommendations for nutrients for gestating and lactating sows, respectively. Data was regarded a randomized design and analysed using the GLM procedure in SAS except for stillborn rate, which was analysed as binomial data using the GENMOD procedure. On average, sows weighed 236 kg with a back fat thickness of 14 mm, they had an average feed intake of 3 kg and gave birth to 21.3 piglets. Results indicated an improved farrowing performance with a reduced farrowing duration for sows fed the mixed diet (mean 222 min; $P=0.01$, quadratic $P<0.01$) as compared with either gestation diet (375 min) or lactation diet (380 min). The stillborn rate showed the same pattern as farrowing duration, although it did not differ significantly (quadratic $P = 0.11$; 6.8%, 3.4% and 5.6% for gestation, 50/50-mix and lactation diets, respectively). No differences among treatments for colostrum yield (6.7 kg), colostrum intake (358 g/piglet) and nutrient output in colostrum were observed. In conclusion, a transition diet may be more optimal for sows around parturition than a common gestation or lactation diet.

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Fat soluble vitamins values in serum of wild boar (SUS SCROFA)

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Blood chemistry variables are considered useful tools to assess nutritional condition, presence of disease, and the overall health status of wild animals. Fat soluble vitamins (FSV) are important micronutrients in pigs for normal physiological functions related with vision, immunity, bone integrity, and antioxidant properties (McDowell, 2000). However, from our knowledge, reference values for FSV in blood from wild boar has not been investigated. Thus, the aim of the present study was to examine the content of FSV values in serum of wild boar to use as biomarkers of health status. Blood samples were obtained from a total of 40 juvenile and adult wild boars (20 males and 20 females) hunted in Spain from September 2016 to February 2017. Serum FSV (A, E and metabolites of vitamin D) values were determined by HPLC (A and E) and LC-MS/MS. Age and gender differences within wild boars were statistically analyzed by one-way ANOVA. Wild boar showed similar values of vitamins A and E compared to domestic pig. However, vitamin D metabolites, were much lower than domestic pigs (Arnold et al. 2015), with the exception of 25-OH Vit D2, probably due to the inclusion of mushroom on the wild boar's diet. Females showed higher 24,25-(OH)₂ D3 ($p<0.01$), 25OHD3 ($p<0.05$), 3-epi-25-OHD3 ($p<0.05$) and Total 25-Hydroxyvitamin D3 ($p<0.05$) concentration than males wild boar. Higher 24,25-(OH)₂ D3 ($p<0.01$) and 25OHD3 ($p<0.01$) concentration were observed in adults compared to juveniles. No significant

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differences were found in vitamins A and E related to age or gender of wild boars. Our results reveal differences on vitamin D metabolism between genders of wild boar and, also compared to domestic pig data. More data are needed to establish reference intervals for interpreting the vitamin D regulatory role in calcium homeostasis of wild boar.

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Targeting taste and phytonutrient sensors to improve performance in piglets

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PANCOSMA SA

Post-weaning feed intake, intestinal health, and consequent growth present a major challenge in piglet rearing. In addition, the industry faces increasing pressure due to decreased antibiotics use in feed. Feed additives represent a strategy for addressing such challenges. The study objective was to determine if a feed additive targeting sweet-taste and phytonutrient receptors could improve performance of piglets fed an antibiotic-free diet. Piglets (N=560, age 20 d, 10 pens of 28 piglets/trt) were assigned to two treatments for 28 d separated into two phases (P1: 0-14 d; P2: 14-28 d): an un-supplemented diet (CONTROL) or supplementation with an additive containing artificial sweetener and phytonutrient (TAKTIK[®] X-IN, Pancosma; SUC-ANET) at 150 ppm. Both diets included zinc oxide (ZnO) and acidifier. Body weight (LW) and feed intake was measured weekly. Feed to gain (F:G), average daily gain (ADG) and average daily feed intake (ADFI) were calculated per phase. Data was analyzed using the MIXED procedure of SAS[®] with pen as the experimental unit. The model included treatment, gender and their interactions as fixed effects; block and pen within block as random effects. Treatment differences were significant at $P \leq 0.05$. Initial LW was similar between treatments. During P1, SUC-ANET piglets had higher LW than CONTROL (8.85 vs. 8.46 kg, $P \leq 0.05$) and also reduced F:G (0.93 vs. 0.98, respectively; $P \leq 0.05$). Treatment did not affect ADFI. During P2, LW was higher for SUC-ANET piglets than CONTROL (19.42 vs. 18.78 kg; $P \leq 0.05$) and ADG was also higher (474 vs. 459 g, respectively; $P \leq 0.05$). ADFI and F:G during P2 were similar between treatments. SUC-ANET increased weight gain of piglets fed an antibiotic-free diet and therefore it represents a strategy for improving piglet performance. Additional research is warranted to confirm efficiency across a variety of dietary and management contexts.

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The effect of different concentrations of dietary fibre and fat in odour offensiveness and feed conversion efficiency in finishing pigs

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Possibly the greatest challenge facing the expansion of the global pork industry is the odour emissions produced from pig production. This study aimed to investigate if varying levels of dietary fibre and fat levels would influence odour emissions from finishing boars. Six diets with different concentrations of dietary fibre and fat were tested to assess their effect on odour emissions and growth performance. It was hypothesised that diets containing digestible fibre and fat would have a more offensive odour than those with lower fat levels, as is the case with humans. Pigs (n=36; six per diet) were fed their respective diets for three weeks prior to entry to the metabolism chambers, to allow the gut microbiota to adapt to a different diet. Each week, six pigs entered the metabolism chambers (one per chamber) on a Monday, and gases produced were recorded during a 24 hour period (ammonia, methane, nitrous oxide, oxygen and carbon dioxide; recorded between Wednesday and Thursday). Pigs were removed from the chamber on a Friday and the slurry from each chamber was retained for future analysis of odour and gas excretion. Odour compounds were identified using GC-MS, and odour offensiveness was analysed by olfactometry. It was found that the diet with the high indigestible fibre gave the worst odour offensiveness, followed by the high digestible fibre and no fat diet. The diet with the lowest odour offensiveness was also that with the best feed conversion rate, which was the high fibre diet using digestible fibre sources

Abstract

and fat. Following on from this work, the four best diets for growth performance and low odour offensiveness will be fed on a commercial basis to further assess the optimum diet for reducing gas and odour emissions from finisher pigs.

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Effects of isoquinoline alkaloids on nutrient absorption and growth performance of weanling pigs fed corn-soybean meal diets

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An experiment using a 3-phase feeding program was conducted to determine effects of isoquinoline alkaloids (IQ) in diets for weanling pigs. A total of 160 pigs (initial BW: 6.33 ± 0.61 kg) were allotted to four treatments, four pigs per pen, and 10 replicate pens per treatment. Phase 1, 2, and 3 diets were provided during week 1, weeks 2 and 3, and weeks 4 and 5, respectively. Within each phase, the four corn-soybean meal based diets were identical with the exception that they contained either 0, 90, 180, or 360 mg/kg IQ. Data were analyzed by ANOVA using the Proc MIXED of SAS. There were no differences among treatments in growth performance over the entire experimental period; however, in phase 1, ADFI, ADG, and gain:feed (G:F) quadratically decreased ($P < 0.05$) with IQ inclusion with the least values observed for the diet with 180 mg/kg of IQ. In phase 2, ADFI linearly decreased ($P < 0.05$), whereas G:F increased (quadratic, $P < 0.05$) in phase 3 as IQ was added to the diet with the greatest G:F obtained in diets containing 90 or 180 mg/kg IQ. A quadratic increase ($P < 0.05$) of total protein (TP) in plasma was observed in phase 1, with the highest value in plasma of pigs fed the 180 mg/kg IQ diet, and there was a tendency for plasma urea nitrogen (PUN) to increase linearly in phases 2 and 3 ($P = 0.07$) if IQ was added to the diet. In conclusion, IQ supplementation had no effect on overall pig growth rate although G:F was improved in phase 3 if 90, 180, or 360 mg/kg IQ was added to the diet. Results indicate that absorption of amino acids was improved by inclusion of IQ in the diets because PUN and TP values were increased.

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Influence of naturally-produced dietary deoxynivalenol on performance and organ accumulation of growing pigs

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The objective was to investigate influences of dietary deoxynivalenol (DON) on growth performance, and organ DON accumulation in pigs. Forty-eight barrows with an initial body weight (BW) of 10.4 ± 1.7 kg were grouped into six blocks by initial BW and allotted to two dietary treatments in a randomized complete block design. There were six replicate pens per dietary treatment with four pigs per pen. A control diet contained mainly corn and soybean meal. A contaminated diet was formulated to contain 30% of corn distiller's dried grains with solubles naturally contaminated with DON. During a 56-d experimental period, individual BW and pen feed disappearance were recorded every 14 d. On d 14, 28, 42, and 56, a pig from each pen was euthanized for collecting organ and longissimus muscle samples. Orthogonal polynomial contrasts were used to test diet effect, linear and quadratic responses of feeding period, and their interactions. The control diet contained $0.28 \mu\text{g/kg}$ of DON and contaminated diet contained $1.80 \mu\text{g/kg}$ of DON. Pigs fed the contaminated diet for 56 d were tended to have less ($P = 0.058$) gain:feed ratio compared with pigs fed the control diet. There was no interaction between diet and feeding period for organ DON concentrations. The DON concentration and the amount of DON in the kidneys of pigs fed the DON-contaminated diet was greater ($P < 0.05$) compared with the control group. The amounts of DON in the liver and kidneys linearly increased ($P < 0.01$) with longer feeding period. In conclusion, naturally-produced deoxynivalenol in swine diets decreases growth performance, and dietary deoxynivalenol accumulates in the liver and kidneys in a time-dependent manner.

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A longer adaptation period is required as pigs consume less amount of feed in total tract digestibility experimentsA Son and B Kim
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The objective was to identify the minimum adaptation period in total tract digestibility experiments using index method depending on feed intake (FI). Ten barrows with an initial body weight of 64.6 ± 5.7 kg were housed in metabolism crates. The pigs were randomly assigned to a crossover design consisted of two levels of FI. An experimental diet contained 74.2% ground corn, 22.7% soybean meal, 0.5% chromic oxide, and 0.5% titanium dioxide. During a 6-d adaptation period, cornstarch was included at the expense of 1% index compounds. The animals were fed experimental diets at two different levels of FI: 1.5 and 3.0 times the estimated metabolizable energy (ME) requirement for maintenance. Following the adaptation period, fecal samples were collected for 8 d. Feces were collected separately for 24 h on each day from 0800. As collection days passed, the daily Cr concentration in feces linearly and quadratically increased ($P < 0.001$) in both FI levels of 1.5 times and 3 times the ME requirement for maintenance. As collection days passed, the daily Ti concentration in feces also linearly and quadratically increased ($P < 0.001$) in both FI levels. One-slope broken-line analysis showed that breakpoints of Cr concentration in feces were d 5.17 and 3.42 in pigs fed at FI levels of 1.5 and 3 times the ME requirement for maintenance, respectively, and breakpoints of Ti concentration in feces were d 4.71 and 3.34 in pigs fed at FI levels of 1.5 and 3 times the ME requirement for maintenance, respectively. In conclusion, at least 3 d of adaptation period is required before initiating fecal collection in total tract digestibility experiments with feed intake of 3 times the maintenance energy requirement using chromium oxide or titanium dioxide as an indigestible index, and the adaptation period should be extended when feed intake is reduced.

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Reduced phosphorus supply during pregnancy and lactation affects protein uptake, digestion, net absorption and utilization in the young sowK Sørensen and H Poulsen
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Reduced dietary phosphorus (P) affects protein ($N \times 6.25$) intake, net absorption and P:N retention in growing-finishing pigs showing that P supply not only affects P but also protein metabolism. This study addresses the effects of reduced dietary P supply on N (protein) digestion, utilization and balance in young gilts during early pregnancy and late lactation. Eighteen gilts from six litters were randomly distributed to three groups fed T1 = a diet fulfilling the Danish P recommendations (Pregnancy/Lactation 4.54/5.69 g/kg), T2 = T1 diet with reduced dietary P (Pregnancy/Lactation 3.81/5.11 g/kg), and T3 = T1 with low dietary P (Pregnancy/Lactation 3.10/4.53 g/kg). Dietary protein content was 148 in pregnancy and 188 g/kg DM in lactation. The gilts were fed the different diets from 50 kg BW until weaning of the first litter. Two balances were performed: Balance A in early pregnancy (week 6) and Balance B in late lactation (week 4). Statistical analysis were performed by the MIXED procedure in SAS including diet (T1, T2, T3) and balance (A,B) and diet x balance interaction as fixed effects and animal within balance was used as repeated measurement. No interaction between diet and balance was found for the N balance, however, for all balance parameters (ingested, excreted in faeces and urine, retained, ATTD and utilisation) an increase was shown from early pregnancy to late lactation (P). The ATTD of N was 5-10 %-units higher in lactation than in pregnancy. In contrast, no significant effect of reduced dietary P supply from 100 to about 70% was observed on the ATTD of N, N balance and utilization, and P:N retention in young gilts

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during early pregnancy and late lactation. In conclusion, a reduction in dietary P supply from 100 to about 70% of requirement did not significantly affect N digestion and N balance in young gilts during early pregnancy and late lactation.

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Grass intake of sows quantified by plasma metabolites

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Organic sows ingest grass during the summer, but the digestibility and intake of grass is unknown. Currently, feed for organic sows is therefore formulated based on nutrient recommendations for conventional sows while ignoring the nutrients that are supplied from the grass. To improve feeding of organic sows, it is important to quantify the grass intake of sows and quantify the nutrient digestibility of the grass. A total of sixteen multiparous dry sows (Danish Landrace x Danish Yorkshire) were housed in metabolism cages and total collection of urine and feces was performed during two balance periods of five days. The dietary treatments were daily rations of 0, 2, 4, or 6 kg of fresh grass three weeks post cut. Sows were fed similar amounts of net energy by providing a commercial sow diet. Blood samples were collected from the jugular vein at the last day of feeding, and analysed for plasma metabolites using a non-targeted liquid chromatography-mass spectrometry (LC-MS) metabolomics approach. Analysed plasma metabolites were screened for correlation between grass intake and plasma metabolite concentrations measured as area under the curve (in arbitrary units) from the LCMS analyses. Data on nutrient digestibility and plasma metabolites were analysed using a MIXED procedure while accounting for repeated measurements. Digestibility of dry matter, organic matter, nitrogen and energy was 72%, 64%, 71% and 68%, respectively, using the regression method. The grass intake was found to be highly correlated with plasma pipercolic acid ($Y = 0.93X + 0.29$; $r = 0.95$; $P < 0.001$) and with a metabolite tentatively identified as plasma bisnorbiotin ($Y = 5921X - 232$; $r = 0.95$; $P < 0.001$). In conclusion, digestibility of dry matter, organic matter, nitrogen and energy in grass was rather high (64 to 72%), and the grass intake of sows correlated well to plasma pipercolic acid or plasma bisnorbiotin.

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Identification of fecal hyodeoxycholic acid as a marker of Tylosin-elicited microbiome modification in pigs

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Much effort has been devoted to studying the effects of antimicrobial growth promoters on the microbiome of production animals, but limited information is available on their influences on the metabolome. The objective of this study is to extend the identification of affected microbes to altered metabolites in nursery pigs treated with Tylosin. The feeding trial was performed in a commercial farm using two pens of pigs with and without Tylosin treatment (40 ppm in the diet) during 10-22 weeks of age, respectively. Fecal samples were collected from ten pigs per pen at weeks 10, 13, 16, 19, and 22 of age, and then examined by liquid chromatography-mass spectrometry (LC-MS) analysis. The multivariate models on the LC-MS data indicated that the time-dependent changes in fecal metabolome occurred in both control and Tylosin-treated pigs. More importantly, the metabolomic profiles were similar between the treatment and control groups in weeks 10 and 22, but diverged during weeks 13-19. Analyses of the fecal metabolites contributing to the separation of two groups of pigs indicated that Tylosin treatment not only led to the presence of Tylosin and its metabolites in feces, but also altered the levels of many other metabolites in time-dependent patterns. Among these metabolites, hyodeoxycholic acid, a secondary bile acid generated by microbial metabolism, was greatly increased during weeks 13-19 ($P < 0.05$) in the Tylosin treated group, but not affected in the control group. The integration of current metabolomics data and the microbiome data from a previous study showed the consistence of these two datasets on revealing Tylosin-elicited changes, and further confirmed our previous hypothesis that Tylosin promotes earlier maturation of the microbiome.

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These observations warrant further investigations on the causative relationships between the changes in the metabolome and the microbiome as well as their roles in antimicrobial growth promotion.

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Variable fermentability of dietary fibres from selected vegetables

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Vegetables are rich sources of dietary fibres (DF), but have not been extensively studied in terms of DF fermentability. The aim of this research was to study the *in vitro* fermentability of DF isolated from selected vegetables using porcine faecal inoculum. Our hypothesis was that DF obtained from diverse vegetables would have different rates of fermentation in terms of end-products such as short chain fatty acids (SCFA) which are beneficial for digestive health. DF from spinach, celery and carrot were isolated using physical processes prior to *in vitro* batch fermentation for 48h using porcine faeces.

Data showed that both vegetable and fermentation time affected amounts and proportions of acetate, propionate, and butyrate significantly ($P < 0.001$). Spinach leaves resulted in the lowest total SCFA, while carrots had the highest. In agreement with these results, which are most likely indicative of carbohydrate fermentation, parameters indicative of protein fermentation (Branched-Chain Proportion and ammonia), showed that spinach had the highest Branched-Chain Proportion, and ammonia concentrations, while carrot was the lowest. Not surprisingly, all end-products generally increased in time. These results indicate that while these vegetable DF are all fermentable, it was to a variable extent, suggesting varying potential as contributors to long-term GIT health when consumed regularly.

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Effects of dietary electrolyte balance on growth performance, blood parameters and gastrointestinal tract pH of weaning piglets

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The results of studies that conducted to test the effects of dietary electrolyte balance (dEB, Na + + K + - Cl-) on the performance of weaning piglets were inconsistent, which may be because of the interaction between dEB and other factors. The present study was conducted to test the hypothesis that the effects of dEB on growth performance and physiology of weaning piglets may be affected by dietary supplementation with acidifier. Fifty-six piglets (6.26 ± 0.64 kg body weight) were weaned at 21 d of age and randomly assigned into 1 of 8 treatments for a 14-d period with 7 pens (1 piglet per pen) per treatment. Eight experimental diets were prepared in 2×4 factorial arrangement with citric acid (0, 0.3%) and dEB (-50, 100, 250, 400 mEq/kg). The piglets were sampled and analyzed at the end of trial. The interaction effect between dEB and citric acid was significant ($P < 0.05$) on the fecal score, but no significant effects were observed on growth performance of weaning piglets. Blood partial pressure of CO₂ was greater ($P < 0.05$) in piglets receiving 400 mEq/kg diet than that in piglets receiving 400 mEq/kg diet with 0.3% citric acid. Additionally, the blood Ca²⁺ concentration was lower ($P < 0.05$) in piglets fed 250 mEq/kg diet than that in -50 mEq/kg piglets. Dietary supplemented with citric acid significantly increased ($P < 0.05$) plasma total protein, albumin, creatinine, and total cholesterol concentrations. Compared with other groups, 250 mEq/kg group significantly increased ($P < 0.05$) blood urea nitrogen concentration and decreased ($P < 0.05$) the concentrations of glucose and amylase. Colon pH and ileum pH significantly decreased ($P < 0.05$) in piglets fed 250 mEq/kg diet compared with other diets. In conclusion, dietary dEB influences fecal score, plasma biochemistry parameters and gastrointestinal PH of weaning piglets. Moreover, dietary dEB and citric acid have interaction on fecal score and blood gas.

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Effects of glutamate on intestinal morphology and luminal short-chain fatty acids contents in suckling pigletsX Tan¹, J Zhang², Y Yin² and H Yang¹¹*Animal Nutrition and Human Health Laboratory, School of Life Sciences, Hunan Normal University;* ²*Chinese Academy of Science, Institute of Subtropical Agriculture*

Glutamate (Glu) is considered to be a functional amino acid for human and animals, including pig. However, little is known about the effects of Glu on intestinal morphology and luminal metabolites of suckling piglets. Therefore, this study was performed to determine the effect of oral administration of monosodium glutamate (MSG) on intestinal morphology and luminal short-chain fatty acids (SCFAs) contents in suckling piglets. A total of 48 Duroc × Large White × Landrace newborn piglets were selected from 6 sows. The piglets from each sow were randomly divided into 1 of 4 groups and the piglets were received oral administration of 0.18 g/kg body weight (BW) of sodium chloride (CN group), 0.06 g/kg BW MSG (LMG group), 0.50 g/kg BW MSG (MMG group) or 1.00 g/kg BW MSG (HMG group) twice a day. The piglets were fed on sow milk freely during the whole trial period. On 7 and 21 d of age, 6 piglets (1 piglets per sow) were randomly selected from each group for tissue sampling. The morphology of jejunum and ileum, and SCFAs contents in caecum and colon were measured. The results showed that oral administration of MSG significantly increased ($P < 0.05$) jejunal villus height and crypt depth on d 7 and d21. Ileal villus height was also increased ($P < 0.05$) by MSG on 7 d of age. The contents of acetic acid, propanoic acid, isobutyric acid, butyric acid, isopentanoic acid, and pentanoic acid in caecum and colon of MMG group were greater ($P < 0.05$) than those in CN group on 21 d of age. In conclusion, oral administration of MSG improves intestinal morphology and increases luminal SCFAs contents in caecum and colon of suckling piglets.

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CircRNA profile of longissimus muscle between Ningxiang pigs and Large White pigsY Yang¹, L Lei¹, Y Yin² and H Yang¹¹*Animal Nutrition and Human Health Laboratory, School of Life Sciences, Hunan Normal University;* ²*Chinese Academy of Science, Institute of Subtropical Agriculture*

Intramuscle fat (IMF) deposition is a main factor determining juiciness and consumer acceptability of pork. Circular RNA (circ RNA) is critical for most biological processes. However, the profile of circ RNA in regulating IMF deposition remains unclear in mammals. We analysed the profile of circ RNA in longissimus dorsi muscle of Ningxiang (NX) and Large white (LW) pigs, which contain high IMF and low IMF, respectively. The expression of 274 circ RNAs showed statistical significance ($P < 0.05$) between the two breeds, of which 102 circ RNAs exhibited a greater expression in NX than LW pigs. Eight circ RNAs (circRNA_03372, circRNA_04750, circRNA_04849, circRNA_05780, circRNA_09577, circRNA_10573, circRNA_12442, and circRNA_14928) may directly involve in lipid storage in muscle because their host genes are enriched in fatty acid import, fatty acid biosynthetic process, lipid biosynthetic process, or lipid catabolic process pathway. Circ RNA releases target gene from miRNA through competitively binding miRNA. Therefore, we predicted the function of circ RNA according to the function of target miRNA. By analysing circRNA-miRNA mutual effects, we additionally found out that 2 circ RNAs (circRNA_12737 and circRNA_03425) negatively and 5 circ RNAs (circRNA_10231, circRNA_13828, circRNA_12707, circRNA_03512, and circRNA_14992) positively associated with IMF deposition, and the expression of these circ RNAs was verified by qPCR. In conclusion, we identified 15 circ RNAs that potentially take part in affecting IMF deposition in longissimus dorsi muscle of pig, which provided a molecular basis for improving meat quality.

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Lipid metabolism of pigs fed with fresh or oxidized fish oil

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The present study was conducted to determine the effects of fresh or oxidized fish oil on growth performance and lipid metabolism in pigs. A total of healthy thirty Duroc × Landrace × Yorkshire (DLY) pigs with similar body weight (9.23 ± 0.21 kg BW) divided into three groups (n = 10), receiving diets including 2% fish oil (LFO), 8% fish oil (HFO) and 8% oxidized fish oil (OFO; 250 meqO₂/kg) for 120 days, respectively. At the end of the feeding trail, HFO and OFO groups showed significant decrease in average daily gain, average daily feed intake and final weight compared with LFO group, and those two groups had no difference in feed:gain and fat percentage. The adipocyte diameter of the backfat was strongly increased after HFO and OFO supplementation. Furthermore, HFO group had significantly higher low-density lipoprotein and oxidized low density lipoprotein in serum compared with LFO group. HFO and OFO groups both showed lower n-6/n-3 ratio in *longissimus dorsi* muscle, backfat, visceral fat and abdominal fat and lower polyunsaturated fatty acid in *longissimus dorsi* muscle, backfat and abdominal fat compared with LFO group, but OFO group showed higher n-6/n-3 ratio than HFO group in the tissues. CCAAT/enhancer binding protein alpha, hormone-sensitive lipase and adipose triglyceride lipase mRNA expression level in adipose tissue was all decreased with the dietary treatment of HFO or OFO, and 3-hydroxy-3-methyl glutaryl coenzyme A reductase mRNA expression level in backfat tissue was increased in HFO group compared with OFO group. In conclusion, our data suggested that the pigs fed high fish oil diet possess poor growth performance and disordered lipid metabolism. However, dietary oxidized fish oil did not negatively affect the growth performance and lipid metabolism of the pigs relative to that of fresh fish oil.

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Effect of maternal supplementation with L-Cysteine during late gestation on productive performance and plasma biochemical index of sows

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Sulphur plays an essential role in life processes as a constituent of proteins, vitamins or/and other necessary molecules. Sulphur amino acids are involved in the synthesis of intracellular antioxidants such as glutathione and N-acetyl cysteine. This research aims to evaluate the effects of different L-Cysteine level on the performance and plasma biochemical index of sows during late gestation stage. According to a completely randomized design, a total of thirty pregnant (Large White × Landrace) sows which had similar parity (2-3) were randomly assigned into 3 treatment groups (n=10): 1) control group fed with a basal diet (contain 0.30% Cys); 2) 0.40% Cys group fed with a basal diet + 0.10% Cys (contain 0.40% Met); 3) 0.50% Cys group fed with a basal diet + 0.20% Cys (contain 0.50% Cys). The experiment started on day 90 of gestation, blood samples were collected during the parturition, and the reproductive performance was recorded. The results showed that the birth weight of 0.40% Cys group was higher than

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Abstract

that in the control group ($P < 0.05$), while the birth weight of 0.50% Cys group did not showed any difference to control group or the 0.40% Cys group. The TP level in the 0.40% Cys group was higher than that in the 0.50% Cys group ($P < 0.05$), but the TP in the control group did not show any difference to that in the 0.40% or 0.50% Cys group. The AST in the 0.40% Cys group was higher than that in the control group ($P < 0.05$), but in the 0.50% Cys group, it did not show any difference to the other two groups. The results indicated Cys may modulate the metabolism of the sows and 0.40% dietary Cys level resulted in the best performance in the birth weight of the piglets.

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Effect of maternal supplementation with L-Cysteine during late gestation and lactation on productive performance and serum biochemical index of piglets

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Cysteine (Cys), although classified as a non-essential amino acid, may be essential for infants or newly born animals. In this research, we analyzed the effect of different dietary Cys level of sows on the on the productive performance and biochemical indicator in plasma of piglets. According to a completely randomized design, a total of thirty pregnant (Large White × Landrace) sows which had similar parity (2-3) were randomly assigned into 3 treatment groups (n = 10): 1) control group fed with a basal diet (contain 0.30% Cys); 2) 0.40% Cys group fed with a basal diet + 0.10% Cys (contain 0.40% Met); 3) 0.50% Cys group fed with a basal diet + 0.20% Cys (contain 0.50% Cys). The experiment started on day 90 of gestation, birth weight and weaning weight of the piglets were recorded during the lactation, and one piglet per pen was selected to collect the blood (8 pens/diet) on day 21 of lactation. The results showed the average weight gain of the piglets was higher in the control group than that in the 0.50% Cys group ($P < 0.05$), while the average weight gain in the 0.40% Cys group did not differ to that in the other two groups. The levels of P and IRON2 in the 0.40% Cys group were lower than the control group ($P < 0.05$), while P and IRON2 did not differ to those in the other two groups. The BUN in the 0.40% Cys group were lower than the other two groups ($P < 0.05$), while no difference was found in BUN between the control group and the 0.50% Cys group. These results indicated dietary supplementation with 0.50% Cys during late gestation and lactation decreased the productive performance of piglets and altered the serum component.

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Foetal development of the GIT was constrained by plasma levels of amino acids in sows fed standard gestation diets

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A balanced amino acid (AA) supply have been associated with foetal development in mammals. However, developmental studies focusing on foetal nutrient requirements are scarce in pigs. The objective of the current study was to investigate if plasma AA profiles in gestating sows are correlated with foetal development. Caesarean sections were performed on six Large-White sows (105 ± 8 days of gestation) at the UQ-Herston Medical Research Centre. Samples from maternal blood and amniotic fluid were collected and analysed for essential and non-essential AA contents (except

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for tryptophan). In addition, body weight (BW) and organ (brain, lungs, liver, spleen, pancreas, kidneys, heart, and gastrointestinal tract) weights, as well as umbilical cord length were recorded for 55 of the foetuses (10 per sow when available). The correlations between parameters were calculated by stepwise regression analysis. Body weight was found to be positively correlated ($P < 0.05$) with all the individual organ weights, particularly with liver ($r = 0.93$) and the gastrointestinal tract (GIT) ($r = 0.85$). The length of the umbilical cord was also significantly correlated with BW ($P = 0.04$), and the weight of lungs ($P = 0.01$) and pancreas ($P = 0.01$). The analysis of AA in plasma showed that Histidine (HIS) was the AA with the lowest concentration supplied to the foetus. In addition, plasma HIS levels were very highly correlated with the development of all the organs studied (significance at $P < 0.001$ for all except spleen - $P = 0.0293$ -). In addition to HIS, the development of the GIT was found to be linked to threonine (THR) and aspartic acid (ASP) ($P < 0.01$), and glycine (GLY) and glutamine (GLN) ($P < 0.05$). In conclusion, foetal development was associated with the maternal supply of amino acids, particularly HIS, and the umbilical cord length. In addition to HIS, the optimization of pre-natal GIT development may require additional THR, ASP, GLY and GLN as well.

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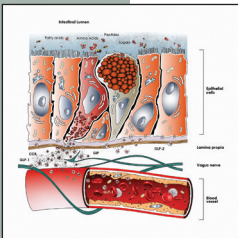
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Digestive Physiology of Pigs (DPP)



The DPP is an international scientific group developed to promote research and communication in the area of digestive physiology of pigs. The International Steering Committee of the DPP, coordinates the celebration of triannual scientific meetings which have become recognised as the definitive international scientific forum on the area. Prior to the DPP2018, the Symposium series started in Shinfield (Reading, UK) in 1979, and continued on a triennially sequence, to France, Denmark, Poland, the Netherlands, Germany, France, Sweden, Canada, Denmark, Spain, USA, and Poland.

The proceedings in this special issue compile all the peer-reviewed abstracts presented at the 14th International Symposium on Digestive Physiology of Pigs held in Brisbane (Australia) between the 21st and the 24th of August 2018 (DPP2018). More than 60 scientist helped reviewed the more than 320 abstracts submitted. The organizers would like to acknowledge their contribution and a full list of reviewers have been added to one of the front pages of this special issue. The DPP2018 Program committee (listed below) coordinated the scientific program including abstract evaluation.

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