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Session 61. Genetic and environmental factors to understand dysbiosis in the GI tract of pigs (with PiGutNet)

Date: Thursday 1 September 2016; 08:30 – 11:30
Chair: P. Trevisi

Theatre Session 61

Poster Session 61

Session 62. Free communications animal nutrition

Date: Thursday 1 September 2016; 08:30 – 11:30
Chair: G. Van Duinkerken

Theatre Session 62

The components of litter size in rabbits: effect of Ano-Genital Distance

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The aim of this work was to test in rabbit of local Algerian population, the effect of Ano-Genital Distance (AGD) before the mating on, sexual behavior, litter size and its components (ovulation rate and prenatal mortality) and sex ratio (ratio of male pups to females pups at birth). In total, 64 multiparous rabbit does were used in this experiment. At the moment of mating, the AGD was measured by three operators and the behavior of the females was noted. At 12 d post coïtum, an endoscopy was realized on the pregnant females in order to measure the ovulation rate (number of non-hemorrhagic corpora lutea) and the number of implanted embryos (live and resorbed). At kindling, the number of pups (live and dead) and their sex were noted. The females with larger AGD were more aggressive (25,4% vs 8%; P<0,01) but presented similar receptivity rate when compared to the females with shorter AGD (82 vs 86%; P>0,05). At 12 d of pregnancy, the effect of the AGD was not significant on the ovulation rate (9,22 vs 9,35; P>0,05). However, the females with larger AGD presented higher early embryonic and fetal mortalities (+45% and +57% respectively; P<0,01). The females with larger AGD gave birth to almost 62% male pups. Conversely, the females with sorter AGD gave birth to about 41% male pups. In conclusion, the AGD in rabbits has influenced the majority of the traits related to reproduction and more investigations are necessary in order to understand more the origin of results obtained.

COST Action FA1401-European network on the factors affecting the gastro-intestinal microbial balance

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COST Actions are an efficient networking instrument for scientists, engineers and scholars to cooperate and coordinate nationally funded research activities. In 2015 the COST Action – FA1401 'European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs (PiGutNet) started. Today, the network involves 49 European intuitions and several companies from 22 European countries. Moreover, partners from Canada, China and Australia collaborate in the network activities. The main objective of PiGutNet is to increase the knowledge about the effect/interaction of environmental and genetic factors on the composition of the microbiota in the gastrointestinal tract of pigs and to improve the risk management associated with antibiotic resistance in pig production. In order to disentangle the factors involved in the gut microbial balance, four main topics were identified: i. Molecular microbiology; ii. Environment, host genetics and epigenetic approach; iii. Feeding strategy; iv. Antibiotic resistance. According to the 4 main research areas above described 5 working groups (WGs) were defined: WG1. Functional and genetic characterization of microbial communities in the gastrointestinal tract of pigs; WG2. Genetic and environmental factors to understand dysbiosis including their interaction (epigenetics); WG3. Feeding strategy to maintain/restore the gut homeostasis; WG4. Antibiotics as a factor of dysbiosis and spread of antibiotic resistance genes; WG5. Knowledge and management exchange. The PiGutNet network is open to accept new partners that aim to share information to progress in the field of the gut microbial balance of pigs. In order to have more information on the network, please, visit the webpage http://www.cost.eu/COST_Actions/fa/Actions/FA1401 and www.pigutnet.eu.

Microbes, diet and host: how do they interact in newborn piglets ?

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Following birth the gastrointestinal tract in pigs is quickly populated with bacteria, viruses and parasites. At the one hand this may induce severe pathological changes in the gut during the first few days after birth, but at the other hand proper gut colonization is also a prerequisite for development of important functions like digestion and innate and adaptive immunity. Gut microbial composition in early life is a reflection of which microbes are present in the local environment as well as factors related to the diet and the host. We have in a long series of experiments studied early life gut colonization in newborn pigs housed individually under very standardized laboratory conditions. Although this represents a much different colonization pattern than seen in commercial pig production, it offers a possibility to study intervention strategies and host responses without the influence of confounding factors. On the host side we have shown that the gut microbiota is influenced by the gestational age at birth, as preterm pigs display a different microbial profile up to one month after birth relative to pigs born at term, given the same diet and housing conditions. Although it is not clear to which extent newborn pigs under farming conditions display characteristics of prematurity, the high litter size and morbidity and mortality in pigs relative to other farm animals, makes it a plausible working hypothesis. Ingestion of milk diets is known to influence gut microbial composition during the first week of life, but we have shown that these diet-induced gut microbial effects may not persist if the diet is changed after the first week. Despite the unstable and transient nature of the gut microbiota in early life, ongoing activities in our lab are now trying to identify if induced differences during the first week of life associates with a persistent change in the epigenetic fingerprint in gut mucosal cells. Further basic and applied research is needed to determine how a proper gut colonization can be secured in early life, and to what extent this influences gut health in later life.

The porcine gut microbiota: composition and links with host's genetics and phenotypes

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Microbiomes and their effects on hosts are emerging as outstanding factors to study in the animal production field. In fact, the microbiome of the intestinal tract may be considered a new host organ that plays a major role in health and well-being. In our laboratory we are developing a research line that targets the pig's gut microbiota with the objective to study the interplay with its host for shaping host's phenotypes. To this end, a cohort of French Large White piglets ranging from 14 to 70 days old was assessed for fecal microbiota composition by pyrosequencing the 16S rRNA gene. All animals were weaned at 28 days and measured for immunity and production traits. Bacteroidetes, Firmicutes, and Proteaobacteria phyla were predominant at all ages, while specific microbial groups (e.g. Lactobacillus) were more represented in the youngest animals. In this sense, a temporal trajectory of bacterial communities in 31 piglets revealed a stratification of piglets in two main groups after weaning that were primarily distinguished by the levels of unclassified Ruminococcaceae and Prevotella, respectively. This results were confirmed in phylogenetic network and clustering analyses in 518 60-days old pigs. A Dominance of Prevotella was positively correlated to increased concentrations of luminal secretory IgA, average daily gain and body weight. In parallel, the genetic parameters of the gut microbiota composition were estimated and, among a set of 63 genera, 7 had low (0.1<h2<0.2), 15=“ 8=“ and=“ h2=“ high=“ medium=“>0.4) heritabilities for abundance variation. Finally, regularized canonical correlations and sparse Partial Least Squares analyses highlighted both positive and negative correlations between health traits (e.g. monocytes, eosinophils, platelets) and genera such as Prevotella, Roseburia and Dialister. Thus, the gut microbiota composition is both influenced by the host's genetics and linked to health and growth traits, which confirms the relevance of this ecosystem for the porcine production.</h2<0.2),>

Delineating spatio-temporal processes in the gut mucosa of pigs

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Biological tissues, like intestine, are highly dynamic and develop in time. To investigate this intestinal development we have used whole genome analyses. Temporal gene expression patterns provide an important characterization of gene function. Identifying such gene expression patterns provide much greater insight into their biological functions and timing of certain biological processes compared to individual genes because they often share similar expression patterns. To get insight into the dynamics of biological processes in the gut mucosa of pigs we used a set of jejunal gene expression data. To identify such reference gene expression pattern of control pigs, data of 9 microarray experiments were combined. In total 98 arrays from 2 platforms were used, measuring expression levels at 17 different time points (range 0-63 days post-natal). A set of 8,069 genes were common across both platforms was used as input to extract nine reference patterns of expression over the time course. In order to identify whether these clusters of genes showed similar functions, a Reactome pathway enrichment analysis was performed for each cluster. Decreasing expression patterns over time were enriched for 'extracellular matrix', 'metabolism', 'platelets' and 'kainate receptors'. Whereas an increasing pattern over time was associated to 'immune system' and 'cell proliferation'. Genes in the relatively flat pattern over the whole time course were related to 'gene expression'. In conclusion, by performing a meta-analysis on jejunal gene expression patterns of (control) piglets from multiple experiments, we obtained insight into the time-dependent fluctuations of biological processes in this tissue. This insight may be exploited to modulate particular processes by changes in management, nutrition, or genetic background.

The effect of host genetics factors on shaping pig gut microbiota

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Studies on humans suggests that host genotype plays an important role in the diversity of the gut microbial community. Currently, there is a lack of research in pigs regarding the genetic influences on gut microbial community. The aim of this project is to address how host-specific genotype background influences the composition of the pig gut microbiota and how this composition influences growth performance. Colon microbiota of 210 Piétrain sows was characterized by 16S Illumina amplicon sequencing. Sows were genotyped with PorcineSNP60 BeadChip. Phylogenetic analysis assessed using RDP pipeline. Univariate and bivariate genomic mixed linear models were used to estimate genetic parameters (heritabilities and genetic correlations). Next to the random pig effect, a random slaughter day effect and several fixed effects were included. The covariance structure of the pig effect was modelled by a SNP-based genomic relationship matrix. All pigs showed a microbial community similarity of 35%. Animals more colonized with Firmicutes presented a 68% dissimilarity from those more colonized with Bacteroidetes. Phylotypes contributing for this variation were Unc. Clostridium, Lactobacillus amylovorus, Streptococcus alactolyticus. Prevotella showed negative correlations with Clostridium sensu stricto, Clostridium XI and Ruminococcaceae and a positive one with Lachnospiraceae. Spirochaetales was the most highly heritable group of microbes in the pig gut (0.52), followed by the genera Alloprevotella, Blautia, Catenibacterium, Lactobacillus and Spirochaetes (~0.36). Lactobacillus and feed consumption showed the highest negative genetic correlation (-0.5) followed by Alloprevotella (-0.46). The genetic correlations between daily gain and bacteria ranged between -0.26 and 0.19. To conclude, the microbial composition in the gut seems to be a heritable trait of the pig, which might become part of the breeding goal to improve feed efficiency.

FUT1 gene polymorphism: impact on gut microbiota, immune response and metabolomic profile of piglets

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A single guanine-to-adenine mutation at nucleotide 307 in the α-(1,2)-fucosyltransferase (FUT1) gene is determinant for the susceptibility of piglets to Escherichia coli F18-diarrhea. We studied the influence of FUT1 gene variants on the intestinal luminal microbiota, mucosal immune responses, binding of E. coli F18, and plasma metabolomic profile of weaners. Two E. coli F18-sensitive sows (heterozygous, FUT1-M307GA) were mated with a resistant boar (homozygous, FUT1-M307AA). Offspring were raised with their dams until weaning on d 28. Piglets were sacrificed (d 34) and gastrointestinal digesta collected. Distal jejunal tissue was obtained to determine the binding of E. coli F18 to intestinal tissue ex vivo, using a porcine intestinal organ culture model. Mucosa was sampled from the cultured intestinal tissue for IgA and IgM determination. Feces and plasma were collected during the study. Sensitive piglets weighed more than resistant piglets on day 28 (P=0.003) and 34 (P=0.01) of age. The ex vivo adherence of E. coli to intestinal tissue and the numbers in intestinal content were higher (P=0.05) in sensitive piglets than in resistant piglets. No effects on concentration of immunoglobulins in mucosa were measured. In susceptible pigs, numbers of lactic acid bacteria tended (P=0.06) to be higher in faecal samples and at 34 days of age, the fecal number of hemolytic bacteria was higher (P<0.003). A higher number of Enterobacteriaceae (P<0.02) in the distal small intestine, caecum and mid-colon; and of hemolytic bacteria (P=0.02) along the gastrointestinal tract were found in susceptible piglets. The concentration of acetic acid was higher in the colon of sensitive piglets (P=0.01). Minor differences between the genotypes were obtained with regard to the plasma metabolic profile. In conclusion, our results indicate that FUT1 genotype might influence the gastrointestinal colonization of other bacterial groups than E. coli F18, and may also influence growth and exert some minor influence on the metabolism of the host.

The A0 blood groups effect on the porcine gut microbiota colonization

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Intestinal epithelium glycocalix plays a role in bacterial-host interaction. The porcine histo-blood group A0 system affected the jejunal mucosa glycomic pattern profile, and thus may influence the gut microbiota colonization events. The present study verified the influence of A and 0 blood groups on the porcine gut microbiota and tested the resilience of the bacterial community after weaning. Two sows with A blood group and two with 0 blood group were selected. Three piglets per sow with blood group identical to the mother were chosen. Fecal samples were collected from the piglets at 7 (tI) and 14 (tII) days after birth and 2 weeks after weaning (tIII). Faeces from the sows were also collected at tI and tII in order to check the microbiota similarity between mother and piglets. Bacterial DNA was extracted with QIAGEN DNA stool mini kit, V3-V4 region of 16S rRNA gene was sequenced on Illumina MiSeq and the resulting data were analyzed in QIIME (v1.9.1) with open-reference OTU strategy using default settings. No significant difference in microbiota composition and diversity were reported between A and 0 genotypes. The dominant phylum was Firmicutes, followed by Bacteroidetes and Proteobacteria, both in sows and in piglets. It is visible a cline in which the Bacteroidetes rise from tI piglets samples (9%) to the mature microbiota (21% in sows samples), whereas Proteobacteria decrease (8% to 3%).The sows showed stable and higher values of alpha diversity, instead microbiota diversity in piglets increased along time, reaching after weaning (tIII) values close to those of sows (mature microbiota), furthermore, a linear correlation between tI and tIII in piglets, for Shannon (r=0.64 P=0.02) and Chao1 (r=0.62 P=0.02) indices, was notable. Results show the absence of A and 0 blood group effect on the porcine gut microbiota and suggest that the manipulation of gut microbiota in the first days of life can influence the microbial community composition of the gastrointestinal tract even after weaning.

Impact of high-wheat bran diet on sows' microbiota, performances and progeny's growth and health

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Finding alternatives to antimicrobial growth promoters is part of the goal of improving sustainability in pig production. Dietary fibres are considered as health-promoting substances acting on pigs' microbiota. This study aimed to investigate whether the enrichment of sows' diet with high levels of wheat bran (WB) could impact the performances of sows and piglets' health. Seven sows were fed a control diet (CON) and 8 sows a WB diet from day 43 of gestation (WB 240 g/kg DM) until the end of the lactation period (WB 140 g/kg DM). Diets were formulated to be iso-energetic and iso-nitrogenous by changing the proportions of some ingredients. Faeces were sampled at different time points (before treatment, during treatment: in gestation and lactation) to determine microbiota composition (sequencing with Illumina MiSeq). Milk was sampled weekly to determine lactose, fat and protein concentration by mid-infrared technology and IgA and IgG contents by ELISA. Before weaning (d26-27), piglets were euthanized, intestinal contents and tissues sampled for further analyses. Zootechnical performances of sows and piglets were recorded. Statistical analyses were performed using the SAS MIXED procedure and repeated measurements. Treatment never impacted piglets' weight (P=0.51). Sows' ingestion during the lactation period was comparable between both treatments until the last 4 days of lactation where the percentage of target ingestion was significantly (P<0.001) lower for the WB (66%) compared to the CON group (89%). No effect on sows' backfat and weight changes was observed. An increased abundance of Lactobacillus spp. in feces of the WB group was observed in gestation before and after diet change (8.8% vs 15.1% of total bacteria). However, for the overall genera changes between treatments, it only seems to occur for minor groups of bacteria. Milk protein, fat, IgG and IgA were not affected by treatment, but a time-effect (P<0.001) was observed while treatment impacted (P<0.05) lactose content. In conclusion, sows' performances were not affected by the high WB diet and more research on the piglets' samples is foreseen.

Effects of dietary protein sources on intestinal and systemic responses of pigs

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Previously we provided evidence that experimental diets prepared with different protein sources and administered for 3 weeks to mice, greatly affected host (immune and metabolic parameters) and gut microbiota. In the present study we used pigs to investigate the effects of five experimental diets each containing one of five different protein sources on a range of physiological and immunological parameters. Pigs aged six weeks were fed for four weeks with experimental diets containing one of the following protein sources providing a dietary protein level of 160 g/kg: soybean meal (SBM), wheat gluten meal (WGM), rapeseed meal (RSM), spray dried plasma protein (SDPP), or black solider fly (BSF). To discern changes in intestinal mucosal gene expression, intestinal microbiota composition and systemic immunity, genome-wide gene expression profiling in jejunal and ileal tissue, 16S rRNA gene sequencing of intestinal microbiota (on-going), and multiplex detection of cytokines and chemokines in serum were used. Gene set enrichment analysis (GSEA) was performed on the transcriptomic data to identify biological pathways and processes affected in small intestine by the various diets. Jejunum showed the highest response in transcriptome analysis and therefore we focused on jejunum. We observed higher (FDR, q<0.05) expression of genes related to barrier function and immune signalling in duodenum of pigs fed with SBM compared to SDPP and BSF, and these response were higher in the former comparison. Moreover, we have observed lower (q<0.05) expression of genes in duodenum of pigs related to metabolism of bio-molecules (xenobiotics, retinol and tryptophan) in SBM compared to RSM and WGM. Further, overall responsiveness to these diets showed no significant (P<0.05) effects on nine measured blood immune parameters. From the knowledge gained from our previous mice study we expect different effects on the composition and/or diversity of small intestinal microbiota. The results of these studies will be presented and discussed with regard to the potential use of alternative protein sources to replace the traditional protein sources in pig diets.

Effect of protected benzoic acid supplementation in nursery diets on piglet growth performance

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Delivering benzoic acid to the intestinal tract by using an embedding technology, improves growth performance of pigs as a result of higher nutrients digestibility, inhibition of pathogenic microorganisms and maintenance of intestinal microecological balance. This study evaluated the effect of protected benzoic acid (Provenia®, Novus International Inc., USA) in nursery diets on growth performance and also assessed the compatibility of its combination with zinc oxide (ZnO) used at therapeutic dose (3,000 ppm). A total of 288 weaned piglets (7.5 kg body weight; 28 d of age) were used and distributed in a 2×2 factorial design, based on the administration or not of two additives, embedded benzoic acid (2.5 kg/t) and ZnO (3,000 ppm). Pigs were fed a pre-starter diet (d 1 to 14) and a starter diet (d 15 to 35), and ZnO was administered only in pre-starter feed in those treatments containing it, while Provenia was added during the whole experimental period. Average daily gain (ADG), feed intake and feed conversion ratio (FCR) were controlled and daily health and faecal score were recorded. There was no interaction between benzoic acid and ZnO dietary supplementation during the experimental period. Benzoic acid supplementation tended to increase ADG in the starter phase (535 vs 510 g/d; P=0.09) and improved FCR in the global nursery period (1.36 vs 1.43 g/g; P=0.01). Zinc oxide did not affect growth performance, both in pre-starter and total nursery period. However, ZnO reduced the incidence of diarrhea compared with benzoic acid. In conclusion, compared to ZnO supplemented at therapeutic dose in the pre-starter phase, supplementation of nursery feed with protected benzoic acid improved growth performance of piglets.

Transcriptome analysis of porcine mesenchymal stem cells subjected to epigenetic modulation

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Up to now, practically little is known about gene expression profile and its regulation in porcine mesenchymal stem cells (MSCs). Moreover, nothing is known about the impact of epigenetical modifications on porcine MSCs transcriptome. Our previous results showed that using epigenetically modified MSC as donor cells for somatic cell cloning in pigs significantly increased the efficacy of the procedure. Therefore, in this initial study, a comparative gene expression analysis was carried out between porcine MSC treated with Histone Deacetylase (HDAC) inhibitor – Trichostatin A (TSA) and control cells, to address the regulation of the epigenomic transformation and its impact on gene expression profile and to define which sets of genes may be responsible for increased usefulness of TSA-modified MSCs. By using high throughput mRNA sequencing (Illumina) we characterized the transcriptome of in vitro cultured pig bone-marrow-derived MSCs, both treated (24 h) and untreated with TSA. The expression of positive and negative surface markers specific for MSC was confirmed by flow cytometry and immunofluorescence analyses. A TopHat/Cufflinks pipeline allowed for identification of 209 differentially expressed genes at a genome wide level ($q<0.05$). Of the genes, 129 were upregulated in TSA-modified cells of which the vast majority was associated with cellular processes, such as: cell communication and cell cycle or primary metabolic processes. Molecular functions of the encoded proteins were mainly associated with antigen binding and transferase, hydrolase or enzyme activity. A large number of the downregulated genes had molecular functions connected with protein and nucleic acid binding and participated in variety of biological processes involving e.g. cell communication, developmental processes and protein metabolism. A deeper analysis of the obtained results will provide more insights into the mechanisms of impact of epigenetic modulation on properties of cultured MSCs, especially their usefulness for different downstream applications including somatic cell nuclear transfer.

Effects of FUT1 and MUC4 genotypes on microbiota and gene expression in the jejunum of healthy pigs

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The gastrointestinal microbiota is relevant to animal health and performance and is partially affected by host genetic factors. Pigs genotype is related to some E. coli infections. A G/A mutation at 307pb of α(1,2)-fucosyltransferase (FUT1) gene affects the properties or the quantity of the mature enzyme, inducing the expression of the E. coli F18 receptor. The g.8227 G/C mutation of mucin 4 (MUC4) gene is linked with the causative gene for the intestinal susceptibility to E. coli F4ac. The impact of FUT1 and MUC4 genotypes on the gastrointestinal microbiota and on the intestinal expression of some genes related to inflammation were tested. Seventy weaned pigs were reared for six weeks and then gastrointestinal digesta and mucosa tissue from jejunum were collected. FUT1 and MUC4 genotypes were tested by PCR-RFLP. Total bacteria DNA was extracted using the QIAamp DNA Stool Mini Kit and the v3-v4 regions of 16S rRNA gene were sequenced using the Illumina MiSeq. Microbiota sequencing data were analysed using QIIME's with open-reference based on OTU strategy. Total RNA was extracted using the Trizol reagent and reverse transcribed (ImProm-II Reverse Transcription System) and the qPCR of IL8, GPX2 and REG3G was performed in a Roche Light Cycler instrument. The effect of the combinations between FUT1 and MUC4 genotypes on the data was tested using the CONTRAST option of the GLM procedure of SAS. The allele frequencies distribution of FUT1 locus was 0.29 (A) and 0.71 (G), while for MUC4 locus was 0.78 (G) and 0.22 (C). No pig with the genotype AA (FUT1) – CC (MUC4) was found. The pigs with the combination of FUT1 AG genotype with MUC4 GG or CG had more variability in microbiota composition (PD_whole_tree variation index, chao index), more abundant presence of Streptococci and Clostridium, and a higher expression of GPX2, vs GG_GG and GG_CG ($P<0.05$). Contrasts between MUC4 genotypes were not significant. FUT1 genotype may affect the adaptive response to the bacterial colonization in heathy pigs.

Dietary organic acids, prebiotic and probiotic on gut microflora and lymphocyte populations in pigs

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The objective of this study was to evaluate whether a combination of natural feed additives consisted of Enteroccocus faecium 2×1,010 cfu/g, at 35 mg/kg feed, benzoic acid at 5 g/kg feed and mannan-oligosacharides at 1 g/kg feed could affect growth performance, microflora composition and immunocyte populations on fattening pigs. One hundred ninety two crossbred pigs were allocated into two experimental groups from 114 until 165 day of life. Control group was fed a basal diet, whereas the other group received the diet with the combination of feed additives. Intestinal samples were collected from the jejunum, caecum and mid colon. Total aerobes, total anaerobes, Clostridium perfringens, Enterococci, Enterobacteriaceae spp, Lactobacilli spp and Bifidobacteria spp were estimated by conventional microbiological techniques, using selective agar media. Immunocyte population analysis was also performed via four-color flow cytometry using the following monoclonal antibodies: cytoplasmic CD3 (pan-T-cells), CD79a (B-cells), SLA-DR (swine MHC-II), CD4a (T-helper cells) and CD8a (T-cytotoxic cells). The group fed the diet supplemented with the aforementioned additives showed improved ($P<0.05$) body weight on slaughter age, increased ($P<0.05$) counts of Enterococci and Lactobacilli in the jejunum, Enterococci and Bifidobacteria in the caecum, and Enterococci, Lactobacilli and Bifidobacteria in the mid colon, along with decreased counts of Enterobacteriaceae spp. Flow cytometric analysis showed no differences in the total B and T-helper and cytotoxic cell populations. The tested combination of feed additives provides promising results on fattening pigs in the absence of immune challenge, in order to improve growth performance and establish a beneficial microflora.