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Full Length Research

Effect of adding varying eubiotic addictive on digestive tract parameters, serum metabolites and growth performance of growing pigs

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ABSTRACT: The purpose of the study was to determine the combined efficacy of feed additives on growing pigs, performance and digestive tract parameters. The experiment was conducted for 28 days with 48 male piglets allocated to six dietary treatments. Group 1 was offered a diet without eubiotic; 2 – a diet with acids mixture; 3 – phytobiotic, medium-chain fatty acids (MCFA) and yeast; 4 – probiotic, MCFA, and yeast; 5 – phytobiotic, probiotic, acids mixture, and sodium butyrate; 6 – phytobiotic, probiotic, MCFA, and sodium butyrate. The average daily weight gains and feed intake were recorded. Blood samples, digesta samples, and ileal tissue samples were collected for studies. There was no significant difference (p>0.05) in weight gain, feed intake, or FCR among the treatments as well as in the ileal and caecal pH value, microbial content, and total SCFA content in caecal digesta as the level of inclusion increases. However, there was a significant difference in treatment condition 2 to 4. Ammonia content in ileal digesta was significantly higher compared to other groups as caecal digesta was significantly higher in group 6 in comparison with groups 1 and 2. Villi height was significantly higher (p<0.05) in groups 2, 3 and 6 compared to the control. Villi height to crypt depth ratio was significantly higher (p<0.05) in groups 5 and 2, but the most promising effects seem to be from combinations 3 and 4. In comparison with control: in groups 2, 3 and 5 higher Alanine transaminase, glucose and triglyceride; in groups 3, 4 and 5 higher total protein and cholesterol; in group 4 higher albumin and in group 6 higher BUN, were found. Generally, it was concluded that; used eubiotic preparations have significant effect on the gut morphology, growth performance, microbiota and some blood parameters but in smaller ration. It was therefore recommended that eubiotic feeds should be included in pigs feed in smaller ration if the desirable effect is to be determined.

Keywords: Digestive tract, feed additives, performance, pig.

INTRODUCTION

Recently, the correct and rapid development of the gastrointestinal tract in piglet has been a major challenge in pig management (Nowak *et al*., 2018). However, via the process of gastrointestinal development, the proper condition of microflora and villi in the intestine as well as the appropriate pH in its individual parts and also preventing diarrhoea incidents are understood. Due to these findings, an effective strategy for the development of the digestive tract should focus on all these factors and could be realized by using different feed additives.

Natural feed additives as probiotics, phytobiotics, or organic and inorganic acids are commonly used in animal nutrition in Europe and are considered as safe. These products, also called "Eubiotics" (Greek "Eubiosis"), affect microbiota balance in the gastrointestinal tract. The gut microbial ecosystem is fundamental in proper activity of immunological system and also for maintaining homeostasis of the pigs (Brestoff and Artis, 2013). According to current knowledge of the host-microbial relationship, strategies including the use of eubiotics may promote animal health and growth. Supplementation of piglet diets with probiotics (and also prebiotics or synbiotics) can increase microbial diversity, which can help to exclude pathogenic microbes (Hill et al., 2014).

Probiotics, when used in the appropriate amounts, can prevent microbial imbalance by altering intestinal populations, epithelial lining, and the gut-associated lymphoid tissues (Metzler *et al*., 2005; Santini *et al*., 2010; Liao and Nyachoti, 2017). Moreover, the use of a mixture of several strains of microorganisms increases its efficacy, especially if the bacteria differ in the fermentation profile and prevent the development of different pathogens (Barszcz *et al*., 2016; Piyadeatsoontorn *et al*., 2018). Yeast generally positively affects feed intake and young pig's performance, as well as microflora and ileum structure (Pereira *et al*., 2012; Bael and Roxas, 2013). The phytobiotics, containing the bioactive substances, are commonly used in pharmacology as fragrances and preservatives for foods (Grashorn, 2010; Gheisar and Kim, 2018), but they may also present antibacterial, antiviral and antifungal properties (Vidanarachchi *et al*., 2005). Organic and inorganic acids (fumaric, benzoic, lactic, phosphoric etc.) or their salts could effectively improve environment of digestive tract by reducing pH, which favours development of health promoting microorganisms and nutrients utilization (Suiryanrayna and Ramana, 2015). Organic acids salts, e.g. sodium butyrate can play an important role in maintaining the integrity of intestinal mucosa (Fang *et al*., 2014), and also in improving performance and decreasing diarrhoea incidence in weaned piglets by modulation of intestinal permeability and the bacterial communities in the ileum and colon (Huang *et al*., 2015). Medium chain fatty acids are a source of easily absorbed energy and affect the growth of intestinal villi, improve digestion and absorption of nutrients and the growth of piglets (Hong *et al*., 2012; Chwen *et al*., 2013; Hanczakowska *et al*., 2013; Li *et al*., 2015). The improvement in intestinal environment is beneficial for the nutrient absorption, which could be improved by higher levels of serum triglyceride and glucose and lower levels of nitrogen and blood urea nitrogen (BUN). Fang *et al*. (2014) reported that the declined plasma urea concentration was relevant to the improved efficiency of nitrogen utilization. Moreover, some of the feed additives can positively affect animal performance (Costa *et al*., 2011; Liu *et al*., 2018) and quality of welfare by reducing e.g. ammonia emissions from pig breeding (Vidanarachchi *et al*., 2005; Bartoš *et al*., 2016; Liu *et al*., 2018). According to literature, using more than one feed additive could be more efficient than using one; however, it is highly dependent on the type, composition and form of the administered preparation (Botsoglou *et al*., 2002; Windisch *et al*., 2008; Liu *et al*., 2018). This study attempts to verify the recipes of eubiotic preparations developed in earlier studies (Nowak *et al*., 2017) and enriched with functional ingredients. The aim of the present study was to recognize the efficacy of combined feed additives (probiotic bacteria strains, yeast, phytobiotics or acids and/or their salts) in different combinations on the growth performance of pigs, intestinal mucosa and some blood parameters.

MATERIALS AND METHODS

Site of study

The study was carried out at the Swine Unit of the Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State, Nigeria. It is located in the coastal southern part of the country, lying between latitudes 4°32'N and 5°33'N, and longitudes 7°25'E and 8°25'E. The state is located in the South-South geographical zone, and is bordered on the east by Cross River State, on the west by Abia State, and on the south by Atlantic Ocean and the south-most tip of Cross Rivers State.

Experimental animals and experimental design

A total of 48 castrated pigs of about 10 kg average body weight (BW) were selected for the study. The pigs were allocated to six dietary treatments using simple random sampling according to their body weight (8 replications each) and kept in individual pens on straw bedding. All diets which sum up to 100 thousand naira were offered in mash form according to the experimental design. The basal diet was prepared according to pig requirements (GfE, 2006) (Table 1). The experimental design used in the study as adopted from Nwoke *et al*. (2018) is shown in Table 2.

Preparation of the diets

A multispecies probiotic bacteria preparation (*Leuconostoc mesenteroides*, two strains of *Enterococcus faecium* and *Carnobacterium divergens* at a ratio of 1:1:1:1) with maltodextrin as a protector was dosed in the total amount of 1011 CFU/t of feed. The preparation formula was prepared at Poznań University of Life Sciences and the individual strains were deposited in the Polish Patent Collection of Microorganisms in Wrocław under the Accession Numbers: L. mesenteroides PKM B/00096; E. faecium PKM B/00097; C. divergens PKM B/00099 and E. faecium PKM B/00098. The formulation and dosage of the probiotic preparations were determined based on the results of *in vitro* studies. Probiotic was encapsulated with fatty acids.

As a phytobiotic, *Oregano vulgaris* and *Thymus vulgaris* water extracts were used in the total amount of 200 g/t of feed (1:1). Water extracts of thyme and oregano used in this experiment were prepared at the Institute of Agricultural and Food Biotechnology, Department of Food Concentrates and Starch Products (Poznań, Poland). For the extraction of biologically active substances, the method of solid-liquid separation was used. The dry herbal material was pre-treated by circulation pump maceration. The obtained extracts were filtrated on plate filters and then concentrated on a vacuum evaporator and spray-

Table 1. Ingredients and nutrients composition of basal diet ingredients composition

Ingredients	Compositions (%)
Soya bean meal	25.35
Corn wheat	30.00
Barley	19.10
Soya bean oil	20.00
Lime stone	0.30
Monocalcium phosphate	1.10
L- lysine (75%)	0.90
Methionine (99%)	0.40
Salt	0.20
Premix*	0.30
Eubiotic	2.00
Total	100.00
Calculated Composition (%)	
ME (MJ/kg)	13.37
crude protein	191.37
Crude fibre	34.60
Lysine	11.80
Methionine + cysteine	7.07
Calcium	7.04
Phosphorus	6.03
Sodium	1.6

*Mineral and vitamin premix contained, per kg: choline chloride 40,000 mg, Fe 15,000 mg, Cu 4,000 mg, Co 60 mg, Mn 6,000 mg, Zn 15,000 mg, I 120 mg, Se 30 mg, antioxidants (butylated hydroxyanisole, butylated hydroxytoluene); 1,500,000 IU vitamin A, 300,000 IU vitamin D3,10,500 mg vitamin E, 220 mg vitamin K3, 220 mg vitamin B1, 600 mg vitamin B2 , 450 mg vitamin B6 , 1,500 mg pantothenic acid, 3,000 mg nicotinic acid, 300 mg folic acid, 3,700 mcg vitamin B12, 15,000 mcg biotin, 260 g Ca.

Table 2. Experimental design.

Groups	Treatments
Group 1	Wheat bran 3%. (control group)
Group 2	0.3% of acid mixture and 2% of wheat bran
Group 3	0.3% of phytobiotic, 0.4% of MCFA, 1% of yeast and 1% of wheat bran
Group 4	0.12% of Phytobiotics, 0.3% of MCFA, 1% yeast and 1% of wheat
Group 5	0.2% of Phytobiotics, 0.12% of probiotics, 0.2% of acid mixture, 0.3% sodium butyrate and 1.2% of wheat bran
Group 6	0.2% of Phytobiotics, 0.12% of probiotics, 0.3% of MCFA, 0.3% sodium butyrate and 1.10% of wheat bran

dried. The dry extracts were standardized and the assays were carried out according to pharmacopoeial methods. The extracts contained, respectively, 0.33% and 0.28% of flavonoids recalculated as hyperoside; 17.80% and 21.50% of polyphenols recalculated as rosemary acid, and 5.07% and 5.80% of tannins recalculated as pyrogallol. The phytobiotics concentration was based on the earlier experiment (Nowak *et al*., 2017).

Yeast Yarrowia lipolytica (Skotan, Chorzów, Poland), medium chain fatty acids – caprylic-caprinic acid (MCFA) (Noack, Poland), organic and inorganic acid mixture containing phosphoric, citric, fumaric, tartaric and malic acids (BARACID, JHJ, Poland) and sodium butyrate (INTEST – PLUS S 95%, Galwet, Poland) were used as the components of the particular eubiotic mixtures. Wheat bran was used as a carrier. The experiment lasted 28 days. The average daily weight gains (ADG) and feed intake (FI) were recorded and at the end the average feed conversion ratio (FCR) was calculated. Blood samples were collected from the auricular vein on the last day of experiment. Serum samples were prepared by centrifugation at 1,500 g for 15 min at 4°C, and they were stored at −40°C for further analyses. Directly after euthanasia (ca. 10 min), ileal tissue samples were collected for morphometric studies. In the ileal and caecal digesta, pH values were measured and also microbial analyses were performed. Digesta were sampled and frozen (−20°C) for three days to determine dry matter and ammonia. A prior feeding trial were carried out on 3 grower pigs for one week before the main study was carried to determine the side effect of the diets.

Chemical analysis

The pH of the digesta was measured using a microelectrode and a pH meter (model 301, Hanna Instruments, Vila do Conde, Portugal). Ammonia was extracted and analysed by the spectrometric method using a Nessler reagent (POCh, Gliwice, Poland). The SCFA analysis was performed according to the procedure described by Barszcz *et al*. (2011) on HP 5890 Series II gas chromatograph (Hewlett Packard, Waldbronn, Germany) with a flame-ionization detector and Supelco Nukol-fused silica capillary column (Supelco, Bellafonte, USA; 30 m × 0.25 mm i.d.; 0.25 mm). Helium was used as the carrier gas. Samples of fresh digesta for microbial analysis were prepared by adding 27 ml of buffered peptone water (Oxoid, Hampshire, UK) to 3 g of samples and homogenized for 30 seconds in a laboratory stomacher. Microbial counts were determined using a decimal dilution series of homogenised samples. The total bacteria count was determined by the standard plate method using a Columbia LAB-AGAR + 5% KB Agar (Biocorp, Warsaw, Poland) after a 24 hours incubation at 37°C, and a lactic acid bacteria count using MRS LAB-AGAR (Biocorp, Warsaw, Poland) after a 72 hours incubation at 30°C. The yeast content was calculated using YGC Agar (Oxoid, Hampshire, UK) after incubation at 25°C for 3 to 5 days. Coliform bacteria were determined using McConkey agar (Biocorp, Warsaw, Poland) after a 24 hours incubation at 37°C. Enzyme activity (ALT, AST), total protein, BUN, triglycerides, glucose and total cholesterol concentrations in the blood serum were determined using Alpha Diagnostics (Warsaw, Poland) and Pointe Scientific (Warsaw, Poland) commercial kits. Analyses were performed using Microplate reader synergy 2 (BioTek Instruments, Winooski, USA).

For morphometric analysis, the ileum tissue samples were fixed in 4% formalin, and after that washed and dehydrated in ethyl alcohol of increasing concentration, xylene, and then embedded in paraffin. Sections with a thickness of 10 μm were cut on a microtome Thermo Shandon. They were then stained with periodic acid-Schiff (PAS) method. Preparations were analysed by Optek UB-200 microscope equipped with the ToupCam™ digital camera and the Multiscan 18.03 computer image analysis program (Computer Scanning Systems II, Warsaw). Villi height, villi area and crypt depth were measured. For the measurement of the intestinal villi height, they were randomly selected from the cross section of the 10 villi. Height was measured from the top of the villus to its base at the mouth of the intestinal crypt. Then the surface of the villi was calculated according to the formula of Uni *et al*. (1998).

Statistical analysis

The significance of differences between control and experimental groups were calculated using one-way ANOVA with Duncan's post-hoc test, and an alpha level of $p<0.05$ was used to assess the significance among means. The statistical analysis was performed using SPSS, ver. 20.0.

RESULTS

The result indicated that there that no health problems were encountered in pigs during the trial. There were no significant differences in weight gain, feed intake or FCR among treatments ($p>0.05$) as the level of inclusion increases. However, level of inclusion in group 2 to 4 had significant difference in weight gains, feed intake or FCR among treatments ($p<0.05$) (Table 3). The daily gains ranged between 0.670 and 0.75 kg, feed consumption ranged from 31.60 to 34.30 kg, and FCR from 1.60 to 1.75 kg/kg.

There was no significant difference in ileal and caecal pH value, but significant differences were found in ileal and caecal ammonia concentration (Table 4). Ileal ammonia content was significantly higher in group 6 as compared to other groups. In group 6, ammonia content in caecal digesta was also significantly higher in comparison with groups 1 and 2. No differences were found in microbial counts and total and particular SCFA content in caecal digesta.

There was a significant ($p<0.05$) difference among groups for all the measured blood serum parameters, except of AST (Table 5). Alanine transaminase level was generally higher in all the experimental groups in comparison with group 1, but the difference was significant among groups 2, 3 and 5 in comparison with groups 1 and 6. Albumin concentration in blood serum was higher ($p<0.05$) in group 4 than in group 1. Total protein content level was lower in group 1 in comparison with groups 3, 4 and 5 ($p<0.05$) and also in group 3 protein level was significantly higher than in groups 2 and 6.

Triglyceride concentration in blood serum was significantly lower in group 1 than in groups 2, 3, 4 and 5. Total cholesterol concentration in serum was higher in groups 3, 4 and 5 than in group 1 and 6. Glucose concentration was significantly higher in groups 2, 3 and 6 in comparison with groups 1, 4 and 5, and in group 3 was the highest ($p<0.05$). In group 6, BUN concentration in blood serum was significantly higher than in group 1 and 5.

DISCUSSION

The utilization of the mixture of several active substances as feed additive are more efficient than using them separately as revealed in the present study, but according

Table 3. Body mass, body gain, feed intake, and feed utilization of the pigs in the experiment.

Parameters	Levels of inclusion (%)						SEM (±)
	1	2	3	4	5	6	
Initial body weight(kg)	10.40	10.50	10.51	10.40	10.50	10.50	0.12
Final body weight(kg)	29.20[b]	31.50 [a]	30.04 [b]	30.90 [c]	29.50 [d]	29.90	0.37
Daily weight gain(kg)	0.67[b]	0.75 [a]	0.70 [b]	0.73 [a]	0.68 [d]	0.70	0.28
Total feed intake(kg)	31.70[b]	33.70[a]	32.90[c]	34.30[c]	31.50[cd]	33.40	0.23
Feed conversion ratio	1.70 [c]	1.60 [d]	1.70 [b]	1.70 [b]	1.80 [a]	1.72	0.12

[a,b,c,d,e] means along the same row with different superscripts are significantly ($p < 0.05$) different from each ot her, Ave: Average, SEM: Standard error of mean.

Table 4. Ileal and caecal pH, ammonia content and microbial counts in fresh digesta of pigs.

Parameters	Levels of inclusion (%)						SEM (±)
	1	2	3	4	5	6	
Ileal pH	6.50	6.50	6.60	6.52	6.44	0.55	0.55
Caecal pH	5.40[b]	5.52[a]	5.40[b]	5.20[c]	5.50[d]	5.30	0.05
Ileal ammonia (mM/g)	8.13[b]	8.13[a]	7.60[b]	6.80[a]	9.32[d]	12.38[a]	0.59
Caecal ammonia (mM/g)	9.16[bc]	8.14[c]	10.78[bc]	12.25[ab]	12.08[ab]	13.50[ab]	0.03
Total caecal SCFA (mM/g)	71.10[c]	76.20[d]	68.40[b]	6.30[b]	70.70 [a]	66.30	1.74
Microbial group content (LogCFulg)							
Yeast and mould	4.80	5.10[c]	4.40[bc]	4.40[ab]	4.40 [a]	4.60	0.40
Lactic Acid bacteria	8.80[d]	8.90[c]	7.70[c]	8.80[b]	8.80[a]	8.04	0.11
Enterobacteria Core	6.70	6.40	7.30	6.30	7.10	6.32	0.40
Total bacteria count	9.00	9.50	8.60	9.40	9.30	8.50	0.43

[a,b,c,d,e] means along the same row with different superscripts are significantly ($p < 0.05$) different from each ot her, Ave: Average, SEM: Standard error of mean.

Table 5. Serum Biochemistry of pigs fed with combined feed additives.

Parameters	Levels of inclusion (%)						SEM (±)
	1	2	3	4	5	6	
ALT (IU/L)	10.10[c]	16.90[a]	16.80[a]	12.00[abc]	16.30[ab]	11.50	0.80
AST (IU/L)	8.40	11.90	1.00	9.30	9.00	8.00	0.60
Albumin (g/L)	52.10[b]	56.13[ab]	60.70[ab]	62.90[a]	58.60[ab]	59.60[ab]	1.20
Total protein (g/L)	80.30[c]	91.60[bc]	118.40[a]	102.10[ab]	107.50[ab]	92.76[c]	3.10
Triglycerides (mg/L)	529.80[b]	697.60[a]	775.70[a]	732.50[a]	762.50[a]	632.20[ab]	24.20
Total cholesterol (g/L)	875.30[c]	993.10[c]	1348.00[a]	1218.80[ab]	1211.30[ab]	915.40[c]	39.90
Glucose (mg/L)	623.30[c]	886.80[b]	1906.40[a]	721.70[c]	168.30[c]	902.40[b]	38.90
BUN (mg/L)	149.30[b]	187.40[ab]	217.40[ab]	216.70[ab]	175.10[ab]	247.70[a]	10.10

[a,b,c,d,e] means along the same row with different superscripts are significantly ($p < 0.05$) different from each ot her, Ave: Average, SEM: Standard error of mean.

to previous data, it is highly dependent on the type, composition, dosage and form of the administered preparation (Botsoglou *et al*., 2002; Namkung *et al*., 2004; Windisch *et al*., 2008; Nowak *et al*., 2017). Multi-eubiotic composition used in the previous research stimulated digestive tract microflora, but the other observed changes were not beneficial in comparison with separately administered additives (Nowak *et al*., 2017). Thus, in the current research, different eubiotic combinations were used. The strategy of proposed mixtures of eubiotics was based on their complementary action in the gastrointestinal tract, including: (1) development of microflora (probiotic, yeast, phytobiotics); (2) development of intestine villi (MCFA or sodium butyrate); (3) pH regulation (acids mixture, MCFA); and (4) diarrhoea incidents reduction (phytobiotics, probiotic, acids mixture). In group 2, an effective, commercial acidifier was used as a kind of comparative group. Phytobiotic and probiotic additives were used in these same concentrations as in the research of Nowak *et al*. (2017). Both were found as effective feed additives which improved pig performance, and positively affected microbiota and fermentation parameters of pigs. Feeding Lactobacillus derived from the pig intestine as probiotics reduced the abundance of Enterobacteriaceae including pathogenic *E. coli*, reduced incidence of diarrhoea, enhanced immune response during infection and increased weight gain (Fouhse *et al*., 2016). Phytobiotics containing thyme and oregano did not improve feed intake, but the pig performance results were satisfactory because daily gains in pigs offered phytobiotics were higher, which was a result of better feed utilisation (Nowak *et al*., 2017). These herbs are also recognised as digestion stimulants and they enhance the synthesis of bile acids in the liver which has a beneficial effect on the digestion and absorption of lipids (Han *et al*., 2017). Moreover, plant spices stimulate the functioning of pancreatic enzymes and increase the activity of the digestive enzymes of gastric mucosa (Costa *et al*., 2011).

In the current research, used feed additives also did not improve pigs' performance. Numerous factors, such as the environment, management practices, nutrition, additive type and dosage, and animal characteristics (age, species, stage of production) can affect the response to feed additives. Therefore, the non-significant effects of additives in the current study could be attributed to the above mentioned factors. This is especially evident in group 2, where commercial acidifier, well known for its affectivity, was administered in the dosage suggested by the producer. The farm conditions must be considered as an important factor. Generally, it has been suggested that beneficial effects of most additives are clearer in suboptimal and stressful conditions, such as a disease condition, a high stocking density, and bad management practices. The scientific study demands to keep animals according to welfare that generates good environmental and zootechnical conditions and, on the other hand, it makes difficult to demonstrate the effectiveness of some experimental factors. Under favourable rearing conditions without any disease or stress, dietary supplementation with a probiotic had no beneficial effects on growth performance (Houshmand *et al*., 2011).

The main rule of eubiotics is affecting microbiota balance in the gastrointestinal tract. None of the additives changed the composition of the microflora, which was also confirmed by similar total SCFA content in caecal digesta. In groups 4, 5 and 6 where probiotic bacteria were administered, no higher content of lactic acid bacteria or lower content of Enterobacteriaceae was observed (Fouhse *et al*., 2016). Moreover, in groups 5 and 6, where both probiotic and phytobiotics were present in the eubiotic mixture, the results were worse than in groups 3 and 4 where these additives were used separately. This is in the agreement with previous study of Nowak *et al*. (2017). It is possible that phytobiotic and probiotic used in these both studies, irrespective of the form of administration (coated or not coated), affected negatively their mutual activity. It could be also confirmed by similar as in the other groups content of lactic acid bacteria, whereas in groups 4, 5 and 6, higher number of these bacteria was expected. This could be caused by the antagonistic action of phytobiotic for probiotic bacteria, although in the *in vitro* study, they showed poor activity against isolated probiotic strains (Grajek *et al*., 2016). They are also possible other antagonistic activities of other components.

In the current study, the presence of acids mixture in feed (groups 2 and 5) did not lower pH of digesta, which could establish more favourable conditions for bacterial growth (Suiryanrayna and Ramana, 2015). One of the most important reasons is the buffering capacity of the dietary feedstuffs, the presence of other antimicrobial compounds, acid type and concentration, composition of the diet, and experimental environment (Houshmand *et al*., 2011). Ammonia and SCFA content in digesta are indicators of microbial activity. Ammonia is produced by intestinal bacteria in the digestion of proteins in the intestine. It is transported from the gut to the liver, where it is processed into urea and glutamine (the so-called urea cycle), and then it is removed from the body by the kidneys, and further along with the urine (Van der Meulen and Jansman, 1997). When ammonia is not properly metabolized and removed from the body, it accumulates in the blood. SCFA content did not differ among groups but ammonia content in digesta was significantly higher in group 6. In this group also, BUN was higher in comparison with control, which could be the reason of higher ammonia absorption. Some eubiotics could also affect histological parameters of piglet ileum. Villi height to crypt depth ratio indicates development of intestinal epithelium and is related to absorptive area (Metges, 2010). In the current research, this parameter was higher in groups 2 and 5 and villi area also in group 6. In groups 5 and 6, sodium butyrate was added. This substance has been found to improve the growth performance of weaned piglets; inhibit the growth of harmful intestinal bacteria; and promote the nutrient digestion, absorption and gut barrier function of piglets and morphometric parameters of ileum (Fang *et al*., 2014). Butyric acid is the main energy source for the epithelial cells of the large intestine and is considered to be effective for promoting epithelial growth. Also, other acids mixtures can improve intestinal parameters of growing pigs, which is in agreement with current research (Long *et al*., 2018). Increased epithelial cell proliferation has been observed when short chain fatty acids are given orally or by intravenous injection or gastro-intestinal infusion. Many eubiotic additives have a positive effect on health, metabolism and absorption of nutrients in animals, which is reflected in the blood biochemistry. The improvement in intestinal environment could be observed as higher levels of serum triglyceride and glucose and lower levels of nitrogen in serum. In the current results in the blood serum of animals from groups 3 and 4 (and also 5), higher level of total protein, triglyceride but also of cholesterol was found in comparison with control.

Protein concentration in the blood serum depends on many factors, including the amount of food rich in protein and its synthesis in specialized tissues (primarily in the liver) and the degree of loss of this substance through the digestive system, urinary system, lungs and skin (Muñoz *et al*., 2012). The higher cholesterol level is generally connected with the supply and management of lipids in the body and especially with liver activity (Rauw *et al*., 2007). In group 6, BUN and glucose level was higher in comparison with control, which could be the reason of higher ammonia absorption. Circulating serum concentrations of glucose, lipoproteins, cholesterol, and triglycerides are the result of the uptake and production by lipogenic tissues and therefore any diet (and also feed intake, feed efficiency and feed intake behaviour of pigs) or genetic-related changes in their levels (Rauw *et al*., 2007; Muñoz *et al*., 2012). Some of the values of biochemical blood parameters in experimental groups (albumin, total protein, cholesterol, BUN) but also in control (total cholesterol, total protein) were higher than the values recommended for pigs (Winnicka, 2011). It also should be mentioned that the method of sample collection or storage before analyses can affect values of some parameters.

Conclusions

It is concluded that, the use of combinations of eubiotics did not affect gut microflora, growth of animals and feed utilization. In contrast, they positively affected gut morphology and some blood parameters. From the experimental groups, the most significant positive effect level of inclusion seems to be a combination where phytobiotic or probiotic, respectively added to medium-chain fatty acids (MCFA) and yeast. This however implies that the addition of phtobiotic or probiotic feed additives with medium fatty acids as well as yeast will increase the palatability of animal feeds specifically in pigs.

CONFLICT OF INTERESTS

Author declares not conflict of interest.

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