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DIETARY KAOLIN CLAY IN PRE- AND POST-WEANED PIGLETS AND ITS INFLUENCE ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS, AND INTESTINAL MICROFLORA STATUS*

Dorota Bederska-Łojewska♦, Marek Pieszka

Department of Nutrition Physiology, National Research Institute of Animal Production,
32-083 Balice n. Kraków, Poland
♦Corresponding author: dorota.bederska@izoo.krakow.pl

Abstract

The main aim of the study was to evaluate the effect of two experimental feed additives based on kaolin clay on piglets' health and microbiota status. The experiment was divided into 2 parts – from birth up to weaning (28 d) and later after weaning up to 70 d of age. Eighteen litters of piglets with sows were divided into 3 groups: control, A and B. The animals from experimental group A were supplemented with kaolin clay (100%), while the animals from group B received kaolin clay enriched with dried pomace of chokeberry and fructooligosaccharides. We noted an improvement in blood parameters related to anaemia, which were significantly better in the experimental group B in comparison to the control and A group (haematocrit; 37.67 and 37.40 *vs* 39.65%; P<0.0005). The dietary treatments during pre-weaning time influenced the increase of the *E. coli* strain in the colon and jejunum and had no effect on lowering the population of *Salmonella* and *Shigella* in the colon. However, it affected the quantity of *Salmonella* and *Shigella* in the jejunum in both A and B (-20%) groups. The rise of commensal bacteria *Lactobacillus* (+2.3 and +10%) and *Bacteroides* (+5.82 and +5.11%) was observed in groups A and B in the colon. This effect was not present in the jejunum.

Key words: diarrhoea, chokeberry, fructooligosaccharides, piglets, aluminosilicates

Currently there are many feed additives available on the market to strengthen the proper functioning of the gastrointestinal tract of suckling and weaned piglets (Pluske, 2013). Their ongoing development is translated into improved production rates and lower economic losses due to poor weight of the animals or high mortality. A short period of rearing often does not allow the animals affected by illness to undergo growth compensation. The main economic losses are incurred in pig production as a result of intestinal and respiratory diseases. The periods characterised by the

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highest occurrence of diarrhoea are the postnatal period and the time of change of nutrition to a constant feed mixture, which is connected with the strong stress caused by weaning. This is the cause of intense adverse changes occurring in the piglets that can lead to increased susceptibility to infections by pathogens. The young piglets, with a poorly developed immune system, are not able to overcome them without veterinary involvement (Skrzypek et al., 2010; Grecco et al., 2018). The invasion of pathogenic microorganisms often causes serious damage to the intestinal epithelium. Instead of proliferation and maturation of cells, it begins to dominate the processes of apoptosis leading to villus atrophy, crypt superficiality and reduced epithelium mitotic divisions (Dong and Pluske, 2007). To prevent the negative effects that are caused by pathogenic organisms on the intestinal epithelium, the present study focused on the action of kaolin clay and kaolin in combination with dried pomace of chokeberry and fructooligosaccharides on reducing diarrhoea in piglets.

Geophagy is a phenomenon known since ancient times, common for both animals and humans (Panichev et al., 2013). Birds willingly eat soil with bentonite and smectite (Gilardi et al., 1999), while rats suffering from diarrhoea during a period of stress begin to consume kaolin rich in kaolinite (Morita et al., 1988). Aluminosilicates have very favourable physical and chemical properties with regard to the physiology of the gastrointestinal tract. They show strong sorption properties, reduce the surface tension of the intestinal contents, enhance liver function emulsification of fats, have the ability to bind bacterial toxins and mycotoxins (Knezevich and Tadic, 1994; Magnoli et al., 2008; Trckova et al., 2009; Panichev et al., 2013; Schneider et al., 2017). They also cause a reduction in the emission of toxic gases from the litter (Schneider et al., 2017), limit the bioaccumulation of toxic metals (Yu et al., 2008) and supplement the animal diet with a range of macro- and micro-minerals like iron, magnesium or silicon (Al-Ani et al., 2006).

Prebiotics, to which fructooligosaccharides (FOS) belong, are designed to maintain the homeostasis of the gut microflora and thus to preserve its biological activity at the optimum level and prevent the intestines from colonisation by pathogenic strains of bacteria, viruses and protozoa. The last component analysed in our experiment was dried pomace of chokeberry which, through its antioxidant properties (Sójka et al., 2013; Samoticha et al., 2016) may be a factor supporting the work of the gastrointestinal tract, especially protecting it from inflammation. What is more, *Aronia melanocarpa* is able to improve haematological parameters by protecting the red blood cells from damage (Pilaczynska-Szczesniak et al., 2005).

The aim of the study was to determine the effect of additives containing kaolin clay, dried pomace of chokeberry and fructooligosaccharides on microbiota and the health status of suckling and weaned piglets.

Material and methods

Animals and diets

The experiment was approved by the 2nd Local Ethical Review Committee for the Animal Experiments in Kraków, Poland (protocol number 839/2011) and per-

formed in an Experimental Farm of the National Research Institute of Animal Production. The research material consisted of 193 piglets divided into 3 groups, coming from 18 sows of the Polish Large White × Polish Landrace line, being in the second or third reproductive cycle and receiving non-granulated complete mixture for high prolificacy and nursing sows (Table 1). All the sows were covered by one boar of the Duroc × Pietrain line. After mating, the sows were kept in group pens of 2–3 sows each, and then at 80 d of gestation were moved to farrowing pens equipped with nipple drinkers. The sows and piglets were housed, fed and managed according to accepted standards of husbandry, hygiene, nutrition and welfare. The pens had a plastic slatted floor, including a heated section for the piglets. The farrowing house and rooms used were typical for farrowing sows, with flat decks and slatted floors. Each pen measured 2.5 m by 2.0 m (2.0 m by 0.6 m for piglets remaining in a pen with sows). Each flat deck unit was cleaned daily and, when vacant, was thoroughly washed and disinfected. The rooms were lit by a combination of day and artificial light. Each room was automatically ventilated, keeping the temperature between 22°C and 27°C. After weaning, the piglets were housed in pens with completely slatted floors in a climate-controlled weaning room.

After farrowing, the sows with litters were assigned to proper nutrition groups. The animals were divided into 3 groups, each with 6 sows and piglets. Litters in each group were assigned by the analogue method, taking into account their size and the average birth weight of piglets. Each of the piglets was individually marked by placing an ear-ring in the right ear. In the control group the initial number of piglets was 63, in group A – 62 piglets, and in group B – 68 piglets. The nutrition part of the experiment was divided into two stages due to the different feeding type: the first from birth to weaning (1–28 d of age), and the second from weaning to 70 d of age. During the first stage of the experiment the piglets remained with the sows. The weaned piglets were kept in group pens on a layer of straw (each litter in an individual pen). The piglets from the control and experimental groups were fed with a non-granulated complete pre-starter feed compound (4–28 d of age) and the starter (from 29 d to 70 d of age) (Table 1), meeting the requirements for all nutrients. Feed rations were prepared according to Nutrient Requirements of Pigs (1993). In addition, the piglets from birth to weaning had unrestricted access to breast milk. On the 3rd d the piglets were administered with glucose (10–20 mL) and 1 mL of ferric additive Ferran 200, which was repeated at 21 d of age. On the same day, the piglets also received a vaccine against *Mycoplasma* spp. The males were castrated when they were 4–5 d of age. The following additive compounds were delivered: kaolin clay (Lubelskie Zagłębie Węglowe, Poland), aluminosilicates – content 670 g kg^{-1}DM, dried pomace of chokeberry (Polfeed, Poland) and fructooligosaccharides – Orafti P95 (Beneo-Orafti, Tienen, Belgium).

The suckling piglets were administered with the additive in the form of a paste using an applicator in the amount of 6 mL per animal at 5, 12, 19 and 26 d of age. Each of the suckling piglets from the experimental groups obtained 6 mL of the additive (containing: 3 mL of water; 4.2 g of kaolin clay; 0.9 g of FOS and 0.9 g of dried pomace of chokeberry). The control group received a placebo – 6 mL of water administered using an applicator. From the weaning period, levigated additives were

added to the concentrate mixture in an amount of 6 kg/ton of feed. The A and B additives contained the following: additive A – kaolin (100%); additive B – kaolin (70%), dried pomace of chokeberry (15%) and fructooligosaccharides (15%). Doses were established on preliminary experiments carried out in the pig farm (unpublished data). Feed and drinking water were offered to the animals *ad libitum*.

Table 1. Composition and nutritional value of feed mixture for late gestation and lactation sows, pre-starter diet for suckling piglets and starter diet for piglets after weaning up to 70 d of life

Item		Sow feed	Piglet feeds	
			Pre-starter	Starter
Barley meal	%	–	11.57	21.71
Wheat meal	%	30.0	43.50	27.10
Maize meal	%	35.0	–	20.00
Wheat bran	%	15.0	–	–
Soybean meal (46% CP)	%	16.0	25.00	21.00
Ground limestone	%	–	0.80	0.90
Milk replacer	%	–	–	4.0
Skimmed milk powder	%	–	9.0	–
Dried whey	%	–	5.0	–
Monocalcium phosphate	%		1.10	0.90
NaCl	%		0.14	0.35
L-Lysine 98%	%		0.27	0.48
L-Threonine 98%	%		–	0.124
DL-Methionine 99%	%		0.12	0.20
L-Tryptophan 98 %	%		–	0.04
Plant oil	%	–	3.0	2.0
Vitamins and minerals	%	4.0*	0.50**	0.50**
Porzyme 9300	%	–	–	0.10
Phyzyme XT	%	–	–	0.001
Experimental additive[2]	%	–	–	0.60
Total	%	100.00	100.00	100.00
Content in 1 kg				
metabolizable energy	MJ	12.80	13.80	13.40
crude protein	g	169.0	205	180
lysine	g	9.08	13.80	13.00
methionine and cystine	g	6.34	7.88	7.25
threonine	g	5.50	7.67	7.75
tryptophan	g	1.76	2.55	2.52
calcium	g	9.10	9.08	8.04
phosphorus	g	7.10	6.71	5.90
sodium	g	2.80	2.0	1.72
potassium	g	6.70	9.22	7.27

*The composition of mineral-vitamin premix in 1 kg: Na – 50 g; Ca – 207 g; P – 48 g; Mg – 50 g; Lysine – 60 g; Methionine – 12 g; Valine – 10 g, Threonine – 21 g; Vitamin A – 380,000 IU; Vitamin D_3 – 50,000 IU; Vitamin E – 3500 mg; Vitamin K_3 – 125 mg; Vitamin B_1 – 57 mg; Vitamin B_2 – 152 mg; Vitamin B_3 – 1,000 mg; Vitamin B_6 – 114 mg; Vitamin B_{12} – 1.2 mg; Niacin – 1,000 mg; Folic acid – 125 mg; Biotin – 7.5 mg; Fe – 3,000 mg; Mg – 1,330 mg; I – 50 mg; Zn – 3,000 mg; Cu – 510 mg; Co – 17 mg; Se – 8.7 mg. Premix used in the mixture for sows: Global Max 4% of LNB (Cargill).

**The composition of vitamin-mineral premix in 1 kg: Na – 50 g; Ca – 208 g; P – 42 g; Mg – 10 g; Lysine – 60 g; Methionine – 12 g; Valine – 10 g, Threonine – 21 g, Vitamin A – 380,000 IU; Vitamin D_3 – 50,000 IU; Vitamin E – 3,500 mg; Vitamin K_3 – 125 mg; Vitamin B_1 – 57 mg; Vitamin B_2 – 152 mg; Vitamin B_3 – 1,000 mg; Vitamin B_6 – 114 mg; Vitamin B_{12} – 1.2 mg; Niacin – 1,000 mg; Folic acid – 125 mg; Biotin – 7.5 mg; Fe – 3,300 mg; Mn – 1,330 mg; I – 50 mg; Zn – 3,010 mg; Cu – 510 mg; Co – 40 mg; Se – 12 mg.

Measurement of blood parameters

At d 25 and 70 blood was collected from 8 piglets from each litter from a jugular vein to perform the following assays: morphology with smear (AVIDIA 2010 analyser, Siemens, Germany), and biochemistry (ALT, AST, creatinine, glucose, urea nitrogen, total protein, bilirubin, cholesterol, triglycerides) was conducted on a Siemens Dimension Xpand Plus (Germany) system.

Intestinal microflora and inflammatory changes

At the weaning, 6 piglets from each group (3 males, 3 females) were euthanised by morbital injection into the heart of 0.3 mL/kg (Biowet, Poland) in order to estimate the composition of the gut microflora in the jejunum and distal colon. Immediately after sectioning, the two selected segments of gut were dissected, the intestinal ends were secured by thread to protect against outflow of content, and then placed on ice and delivered to the microbiology lab. From each segment 1 g of content was placed into 0.9 mL of normal saline to prepare the 1:10 dilution. Then, serial dilutions were made up to 1: 100 000 by the generally applicable Koch plate dilution methods. Next, serial dilutions of the material were transferred to a sterile petri dish, which were poured over the substrates to determine the quantities of the various groups of microorganisms:

a) total amount of bacteria – MPA medium,

b) total number of lactic acid bacteria – Demeter medium,

c) *Salmonella* and *Shigella* bacteria – SS medium,

d) *Bacteroides* bacteria – Schaedler agar medium with blood (anaerobic conditions),

e) *Clostridium perfringens* – BBL *Clostridium difficile* Selective Agar.

The incubation time was 24 h at 37°C. After cultivation, the grown colonies of bacteria were counted for each dilution and the amount converted per 1 g of the content by the formula:

$$L=(\sum\_(i=0)\text{^}n\ xy)/n$$

where:

L – the number of microorganisms in 1 g,

x – the average number of colonies in the dilution,

y – dilution,

n – number of readings.

Escherichia coli was determined by titer, that is the highest dilution of the material which indicates the presence of the tested microorganisms. Broth with lactose and bromocresol purple (LPB) was used as the culture medium. Positive result was determined by a colour change from purple to yellow and the content of the gas in the Durham tube. All positive results were grafted on Endo agar (selective differential medium). The appearance of purple colonies with a metallic sheen confirming the presence of faecal *E. coli* was assumed as positive result. The incubation time was 24 h at 37°C.

Statistical analysis

The data were statistically analysed by one-way analysis of variance ANOVA using Statgraphics Plus 5.1 software (2001). The Tukey's test was used to indicate significant differences between the groups. Differences were considered statistically significant as $P<0.05$.

Results

The piglets in group A (Table 2, 3) showed significantly higher mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) at 25 d than piglets from the control and B groups. The highest level of reticulocytes was observed in the control group in comparison to groups A and B at the end of the experiment ($P<0.0005$). The highest values of haematocrit ($P<0.0005$) and red blood cell level ($P<0.0016$) were shown in group B at 25 d of age. All haematological parameters discussed above were fixed in physiological range for piglets, although MCH and MCHC with very small deviation (Winnicka, 2002; Rahman, 2016; Merck Manual, 2018).

Table 2. Effect of dietary supplementation with kaolin clay (group A) and with kaolin (70%) enriched in dried pomace of chokeberry (15%) and fructooligosaccharides (15%) (group B) on haematological and biochemical blood parameters at 25 d of age

Item	Control group (n=6)	Group A (n=6)	Group B (n=6)	SEM	P-value
Chosen haematological parameters at 25 d of age					
white blood cell (cells/µL)	17.35 a	15.04 b	14.62 b	0.35	0.004
red blood cell (cells/µL)	6.59 ab	6.35 a	6.74 b	0.05	0.002
haemoglobin (g/dL)	10.58	11.13	11.22	0.12	0.109
haematocrit (%)	37.67 a	37.40 a	39.65 b	0.27	0.001
mean corpuscular volume (fL)	57.21	58.94	59.00	0.35	0.109
mean corpuscular haemoglobin (pg)	16.08 a	17.56 b	16.75 ab	0.20	0.012
mean corpuscular haemoglobin concentration (g/dL)	28.07 a	29.73 b	28.32 a	0.25	0.014
platelet count (fL)	511.58	589.02	553.16	15.25	0.135
neutrophils (%)	27.23	29.89	30.91	0.89	0.255
lymphocytes (%)	66.3	63.49	61.53	0.87	0.095
monocytes (%)	3.57	3.88	4.58	0.28	0.350
eosinophils (%)	1.45	1.43	1.37	0.14	0.976
basophils (%)	0.50 ab	0.59 a	0.47 b	0.02	0.062
reticulocytes (cells/L)	310.95	307.16	329.96	11.63	0.678
Biochemical parameters at 25 d of age					
alanine transaminase (U/L)	37.35 a	34.04 a	43.02 b	1.00	0.001
aspartate aminotransferase (U/L)	64.82 a	56.34 b	65.83 a	1.25	0.002
creatinine (µmol/L)	89.27	83.92	93.08	1.69	0.073
urea nitrogen (mmol/L)	2.97 a	1.98 b	3.23 a	0.17	0.003
total protein (g/L)	50.77 a	50.30 a	53.39 b	0.35	0.000
bilirubin (µmol/L)	5.30 a	4.47 ab	3.78 b	0.20	0.008
total cholesterol (mg/dL)	134.26	137.48	132.72	2.50	0.723
triglycerides (mg/dL)	88.96	80.64	77.25	2.72	0.213

a, b – the different letters in a row show values which differ significantly ($P<0.05$).

Blood parameters which indicate potential inflammation process (leukocytes and basophils) in the period from birth to 25 d of age were lower in group B compared to group A, especially in the case of basophils (P<0.0259), The tendency to its lowest value was also maintained in a later period, although the level of white blood cells was significantly lower only in group A (P<0.0080).

The results of biochemical analysis of blood plasma included in the liver function tests: total protein, bilirubin, aspartate aminotransferase (ALT), alanine aminotransferase (AST) in the first and second period of rearing are very diverse and do not indicate the existence of a relationship between the agent used in the experiment and the health of the piglets. The lowest urea-nitrogen was observed in group A at 25 d of age compared to group B (P <0.0035) and the control.

Table 3. Effect of dietary supplementation with kaolin clay (group A) and with kaolin (70%) enriched in dried pomace of chokeberry (15%) and fructooligosaccharides (15%) (group B) on haematological and biochemical blood parameters at 70 d of age

Item	Control group (n=6)	Group A (n=6)	Group B (n=6)	SEM	P-value
Chosen haematological parameters at 70 d of age					
white blood cell (cells/µL)	20.02 ab	14.84 a	22.17 b	0.85	0.008
red blood cell (cells/µL)	6.28	6.31	6.40	0.06	0.699
haemoglobin (g/dL)	10.42	10.91	10.62	0.16	0.551
haematocrit (%)	36.50	36.31	36.70	0.32	0.906
mean corpuscular volume (fL)	58.16	57.56	57.45	2.11	0.487
mean corpuscular haemoglobin (pg)	16.61	17.31	16.66	0.24	0.593
mean corpuscular haemoglobin concentration (g/dL)	28.53	29.96	28.95	0.33	0.346
Platelet count (fL)	430.64	505.60	479.57	16.86	0.246
Neutrophils (%)	42.62	45.14	42.51	1.16	0.717
Lymphocytes (%)	52.59	48.31	48.73	1029	0.335
Monocytes (%)	2.69	4.2	7.9	2.21	0.5578
Eosinophils (%)	1.10	1.3	1.23	0.07	0.507
Basophils (%)	0.35 a	0.86 b	0.39 a	0.05	0.001
Reticulocytes (cells/L)	350.02 a	274.50 b	255.17 b	11.71	0.001
Biochemical parameters at 70 d of age					
alanine transaminase (U/L)	51.30 a	66.07 b	57.30 a	1.3	0.000
aspartate aminotransferase (U/L)	71.70 ab	74.60 a	66.90 b	1.2	0.022
creatinine (µmol/L)	11.33	15.80	14.05	1.27	0.703
urea nitrogen (mmol/L)	7.75	7.44	8.21	0.63	0.879
total protein (g/L)	53.91	52.61	55.27	0.49	0.082
bilirubin (µmol/L)	2.07	1.82	1.58	0.12	0.224
total cholesterol (mg/dL)	75.50	76.88	76.29	1.44	0.930
triglycerides (mg/dL)	36.57	33.20	33.14	1.18	0.403

a, b – the different letters in a row show values which differ significantly (P<0.05).

Analysis of the intestinal microflora in the first part of the experiment (1–28 d of age) indicates that there was an increase in groups A and B in the population of pathogenic bacteria from *Salmonella* and *Shigella* and *C. perfringens* strains in the colon (Figure 2) and *E. coli* in both studied sections of the gastrointestinal tract

(Figure 3). However, there was a decrease of *Salmonella* and *Shigella* and *C. perfringens* observed in the jejunum (Figure 1) but no increase of lactic acid bacteria and *Bacteroides* in the piglets receiving kaolin clay and kaolin clay enriched with dried pomace of chokeberry and fructooligosaccharides (Figure 1) in comparison to the control group. The population growth of the mentioned commensal bacteria was recorded only in the second part of the intestines – the distal colon in both experimental groups A and B (Figure 2).

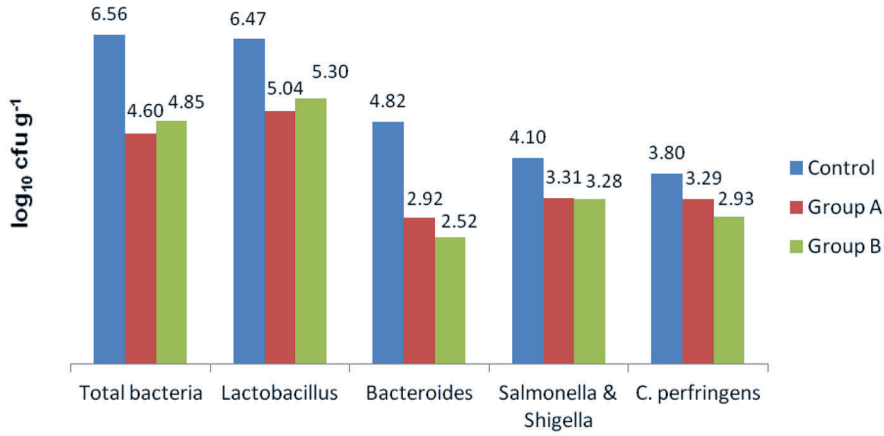

Figure 1. Mean counts of major microbial groups in jejunum of suckling piglets in experimental groups; A – dietary supplementation with additive containing kaolin clay (100%); B – dietary supplementation with additive containing kaolin clay (70%) with dried pomace of chokeberry (15%) and fructooligosaccharides (15%)

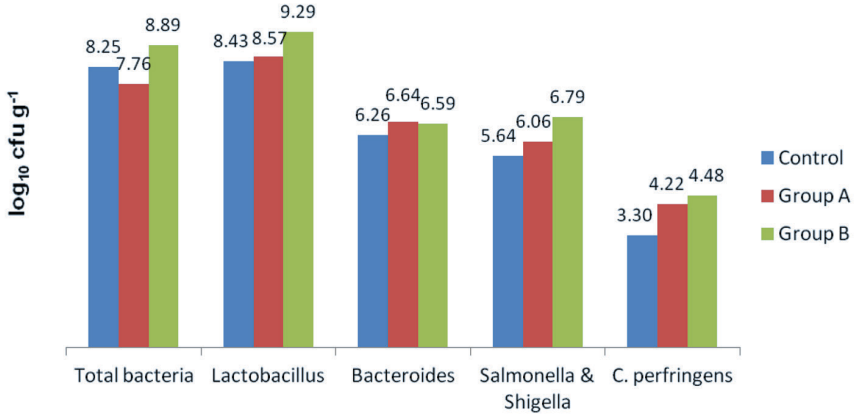

Figure 2. Mean counts of major microbial groups in colon of suckling piglets in experimental groups; A – dietary supplementation with additive containing kaolin clay (100%); B – dietary supplementation with additive containing kaolin clay (70%) with dried pomace of chokeberry (15%) and fructooligosaccharides (15%)

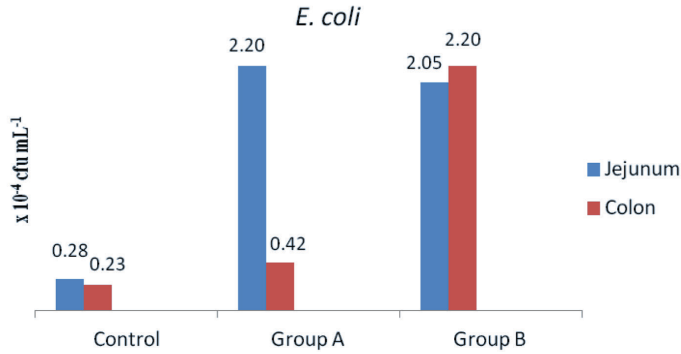

Figure 3. Mean counts of *E.coli* in jejunum and colon of suckling piglets in experimental groups; A – dietary supplementation with additive containing kaolin clay (100%); B – dietary supplementation with additive containing kaolin clay (70%) with dried pomace of chokeberry (15%) and fructooligosaccharides (15%)

Discussion

The observation of the rearing parameters may indicate a positive influence of the kaolin clay supplemented with dried pomace of chokeberry and fructooligosaccharides on animals. The obtained results did not show a beneficial effect on the piglets' growth parameters that were confirmed statistically. In groups A and B a tendency to higher weight gain was observed compared with the control treatment. Average daily gain was also higher in both experimental groups in comparison to the control between 1–28 and 1–70 days of age. Whereas daily feed intake did not differ, the feed conversion ratio was lower in the experimental groups (29–70 days of age) (Bederska-Łojewska et al., 2016). The positive effect of kaolin clay on the proper functioning of the intestines, reconstruction of the epithelium and improving digestibility of feed were observed in other experiments on piglets (Tortuero and Rioperez, 1993; Xia et al., 2005; Alexopoulos et al., 2007; Trckova et al., 2009). It is believed that kaolin clay slows the processes of the intestinal passage, which promotes better assimilation of nutrients and increased resorption of water (Papaioannou et al., 2005). Another mechanism described in the literature is to mitigate the effects of weaning through protecting the pancreatic enzyme from proteolysis. Some authors attribute the improvement in body weight gain to the increased levels of α-amylase, α-chymotrypsin and lipase due to kaolin clay (Cabezas et al., 1991; Parisini et al., 1999; Papaioannou et al., 2005). Aluminosilicates selectively bind toxic substances present in the intestine, thus reducing the potential adverse consequences of their action (Knezević and Tadić, 1994; Phillips, 2004; Abdel-Wahhab et al., 1999; Hassen et al., 2003; Dominy et al., 2004). In the studies of Pieszka et al. (2010), Estrada et al. (2001), Houdijk et al. (2002) and Modesto et al. (2009) the results were similar. However, both chokeberry, due to its antioxidant properties, and oligosaccharides have the properties of stimulating commensal microorganisms which can improve

the functioning of the gastrointestinal tract, providing protection against diarrhoea (Modesto et al., 2009; Li and Kim, 2013).

The lower parameters MCH ($P<0.0120$) and MCHC ($P<0.0141$) observed in the control group could be caused by anaemia associated with iron deficiency. This may indicate the fact that a supplementation of kaolin, which is a natural source of iron (*ca.* 1%), may have a positive effect on the prevention of anaemia in young piglets. A similar relationship was observed by analysing the level of reticulocytes. Their increased level in the control group could be caused by increased erythropoiesis in the bone marrow, which could be a consequence of iron deficiency in an earlier period. The geophagy phenomenon was confirmed as a method to align a low level of iron in some animals during the period of anaemia, and in experiments on newborn rats coming from mothers with a decrease in the amount of red blood cells (Trckova et al., 2009; Patterson and Staszak, 1977). However, it should be noted that not all authors confirm this effect in their studies (Wiles et al., 2004; Alexopoulos et al., 2007). The highest values of haematocrit ($P<0.0005$) and red blood cell level ($P<0.0016$) was shown in group B. This may be attributed to the antioxidant properties of chokeberry. Supplementing the diet with chokeberry juice produced a positive effect in humans, increasing the number of red blood cells by reducing their oxidative damage induced by exercise in a group of studied rowers (Pilaczynska-Szczesniak et al., 2005).

The lowest leukocytes and basophils level in the period from birth to 25 d of age in group B can indicate a limitation of inflammatory processes in the intestine. Both additives reduced the occurrence of diarrhoea. However, additive B was more effective in this case (Bederska-Łojewska et al., 2016).

Significantly lower levels of urea-nitrogen in group A compared to group B ($P<0.0035$) and the control may result from the impact of the kaolin clay on a higher retention of protein (Parisini et al., 1999). Proteins are absorbed primarily in the duodenum and jejunum. However, their disintegration begins in the stomach where, due to the low pH, the denaturation process is induced. In the duodenum follows further degradation of proteins and peptides into amino acids due to proteolytic enzymes of pancreatic juice. Kaolin, by increasing their availability and protecting them against proteolysis, enhances the digestion of proteins, allowing for their better use (Trckova et al., 2009). The second mechanism which may result in better protein retention may be the slower passage that prolonged their contact with pancreatic enzymes. This longer time allows for their better digestion and utilisation in the animal's body (Trckova et al., 2009).

Growth of *Lactobacillus* was observed in animals receiving kaolin clay in the Li and Kim (2013) study; they also reported a reduced number of *E. coli* strains in the faeces of piglets receiving aluminosilicates. Similar observations were made by Trckova et al. (2009). Alvarez et al. (2008) conducted an experiment investigating the *in vitro* antibacterial activity of flavonoids, of which chokeberry is an extremely valuable resource. The obtained results showed the bactericidal action of these compounds against pathogens such as *E. coli* and *Staphylococcus aureus*. Dried pomace of chokeberry may be helpful through having a positive impact on the health status of the digestive tract by creating friendly environmental conditions for the population of commensal bacteria.

Fructooligosaccharides are attributed to having strong prebiotic properties (Macfarlane et al., 2006; Li and Kim, 2013). However, *in vivo* studies have given contradictory information about its ability to improve the intestinal microflora. There was no difference in the number of commensal organisms (*Bifidobacteria*, *Lactobacillus* and *Enterobacteriaceae*) present in the faeces and gastrointestinal tract of animals receiving fructooligosaccharides in the Mikkelsen et al. (2003) study. However, Li and Kim (2013) reported different results, observing an increase of the population of microorganisms of the *Lactobacillus* in the faeces. A similar effect was observed in the small intestine and colon of the animals receiving a solution containing 5 g/L FOS in the experiment conducted by Oli et al. (1998). Modesto et al. (2009) showed that the fructooligosaccharides derived from beetroot administered to piglets at 40 g/kg raised *Bifidobacteria* populations, however without an effect on weight gains of the animals. In the experiment conducted by Mountzouris et al. (2006), FOS did not alter the desired number of intestinal bacteria. Although many studies describe the impact of non-digestible oligosaccharides to improve the composition of biocenosis in the intestine, there is no conclusive evidence of the positive effect of fructooligosaccharides on a significant increase in the population of commensal microorganisms. The authors' own experiment also seems to confirm this fact. Neither the ability of applied feed additives to reduce the population of pathogenic bacteria nor the growth of commensal bacteria has been demonstrated. The cause of varied results obtained in different experiments may be the fact that the fructooligosaccharides used came from different sources with varying composition. As reported by more recent studies, the best prebiotic effect of FOS is exerted by those that increase the degree of polymerization as it can be better used by the commensal bacteria (Perrin et al., 2002; Biedrzycka and Bielecka, 2004).

Conclusions

In conclusion, both additive A (kaolin clay) and additive B (kaolin clay enriched with dried pomace of chokeberry and fructooligosaccharides) reduced the inflammatory changes, which was reflected in WBC level at 25 d of age. The use of kaolin clay and clay with fructooligosaccharides and dried pomace of chokeberry significantly improves the haematological indicators of blood associated with anaemia (high reticulocytes count in the control group at 70 d of age and higher MCH and MCHC parameters in group A at 25 d of age), caused by iron deficiency in young piglets. The obtained results confirm the ability of the additives A and B to eliminate *Salmonella*, *Shigella* and *C. perfringens* in the jejunum. In the case of using both additives A and B, an increase in bacteria from the *E. coli* group was noted.

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