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Effect of Glutamine and/or Probiotic (*Enterococcus faecium*) Feed Supplementation on Piglet Performance, Intestines Structure, and Antibacterial Activity

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ABSTRACT

Hanczakowska E., Świątkiewicz M., Natonek-Wiśniewska M., Okoń K. (2017): **Effect of glutamine and/or probiotic (*Enterococcus faecium*) feed supplementation on piglet performance, intestines structure, and antibacterial activity**. Czech J. Anim. Sci., 62, 313–322.

The effect of glutamine and/or probiotic (*Enterococcus feacium*) supplements on piglet performance, intestines structure, and microbial status was estimated on 181 piglets (16 litters) of Polish Landrace. The piglets were allocated to 2 groups with 8 litters in each, kept in group pens, and fed a standard feed mixture (negative control, group C) or the same mixture supplemented with 2% of glutamine (Group GT). In each group half the animals received Cylactin® added in the amount of 0.35×10^9 CFU per kg feed. The probiotic consists of dehydrated cells of *Enterococcus faecium* strain NCIMB 10415. Feed and water were available *ad libitum.* The piglets were weaned at 28 days of life. At 60 days of life, 6 piglets from each subgroup were slaughtered and their intestines were taken for analysis. Digesta from the digestive tract was removed and the length and weight of particular parts of the intestines were measured. The structure of ileum mucosal epithelium was examined. The acidity of the digesta and the short chain fatty acids (SCFA) content of chyme from the jejunum and caecum were analyzed. *Escherichia coli* and *Clostridium perfringens* counts in these parts of the intestines were also estimated. At the beginning of the experiment, the glutamine significantly improved and the probiotic lowered the piglet body weight gains. Later the probiotic improved but the glutamine lowered weight gains. There was no difference in feed intake or feed utilization. The intestines of the piglets receiving glutamine were lighter and shorter than those of the control ones. The total content of SCFA was significantly higher in the caecum of the piglets fed probiotic than in the control animals. Supplements had no effect on villi height, but both had strong antibacterial activity against *Escherichia coli* and *Clostridium perfringens*. There was no synergy in the effect of glutamine and probiotic.

Keywords: piglet feeding; microbial probiotic; intestinal microbiota; intestine morphology

A few years ago the European Union banned the use of antibiotics as growth promoters for farm animal feeds and thus some replacers have to be found, especially for young animals. Piglets, not having a fully developed digestive tract and immune system, are especially sensitive to the

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adverse impact of the environment. Due to the high demand of growing animals for protein and energy, the proper development and functioning of the gastrointestinal tract are crucial. After weaning, piglets have to adapt to new stressful conditions which are associated with reduced feed consumption, temporary malnutrition, and growth retardation. Feed intake and the functioning of the intestines may be improved by feeding piglets with milk products which are good sources of highly digestible protein and energy.

Another way of preventing intestinal atrophy linked to weaning can be the supplementation of piglet feed with glutamine or glutamate (Ewtushick et al. 2000). Newsholme et al. (2003) have demonstrated that glutamine is "conditionally essential" during the weaning and also under conditions of stress. A positive effect of oral glutamine supplementation on the growth performance and intestinal morphology of weaned piglets was found by Zhong et al. (2011). In our earlier experiment (Hanczakowska et al. 2014), we also found a small improvement of body weight gains of piglets fed the diet supplemented with glutamine.

Piglet performance can be also improved by the supplement of probiotics of bacterial origin (Zeyner and Boldt 2006). The positive effect of the probiotic *Enterococcus faecium* supplement on piglet body weight gains was found also by Mohana Devi and Kim (2014). This probiotic also has antimicrobial activity against *E. coli* (Mallo et al. 2010), but not against *Salmonella* (Kreuzer et al. 2012).

In our earlier experiments we have found a positive effect of the glutamine supplement on piglet performance and their intestines structure (Hanczakowska et al. 2014). The above-cited papers suggest a positive activity of *Enterococcus faecium.* It seemed interesting to check the possibility of obtaining better results using both of them due to possible synergy. Thus the aim of this experiment was to assess the effect of supplementing feed with glutamine and/or *Enterococcus faecium* on piglet performance, intestines structure, and microbiology.

MATERIAL AND METHODS

All procedures used in this experiment were approved by the Local Ethics Committee for Experiments on Animals.

Animals and diets. The experiment was performed on 181 piglets (16 litters) descended from Polish Landrace sows and a Polish Landrace boar. The piglets were allocated to 2 groups with 8 litters in each, kept in group pens, and fed a standard feed mixture (negative control, group C) or the same mixture supplemented with 2% of glutamine (Group GT). In each group, half the animals received Cylactin® added in the amount of 0.35×10^9 CFU per kg feed. The probiotic consisted of dehydrated cells of *Enterococcus faecium* NCIMB 10415 (EFSA 2013). The piglets were fed prestarter diets from 7 to 21 days of life and with starter diets from day 22 to the end of the experiment (day 70 of life). Feed and water were available *ad libitum.* The piglets were weaned at day 28 of life. The composition of the feeds is given in Table 1. The piglets were weighed at 1, 7, 28, 56, and 70 days of their life and their mean body weight gains were calculated. Feed intake was measured daily and feed utilization was also calculated on this basis.

At 60 days of life, 6 piglets from each subgroup were slaughtered and their intestines prepared for analysis. Digesta from the digestive tract was removed and the length and weight of particular parts of the intestines were measured. The structure of the jejunum mucosal epithelium was examined. The acidity of the digesta from particular parts of the intestines and the short chain fatty acids content of chyme from the jejunum and caecum were analyzed. *Escherichia coli* and *Clostridium perfringens* counts were also estimated in these parts of the intestines.

Chemical analysis. The composition of feeds was analyzed according to AOAC (2005) methods. Acidity of the digesta of the stomach, duodenum, jejunum, caecum, and colon contents was measured with a CP-411 pH meter (Elmetron, Poland) equipped with a Metron 12-01 electrode (Metron, Poland). Short chain fatty acids in the jejunum and caecum were separated on column CP-Wax 58 (Varian BV, the Netherlands) (25 m, 0.53 mm, 1 m; carrier gas – helium, 6 ml/min), with a column oven temperature program from 90 to 200°C, using a Varian 3400 gas chromatograph (Varian Associates Inc., USA) equipped with a Varian 8200 CX autosampler (200°C), FID detector (260°C), and Star Chromatography Workstation Software.

Histological analysis. Samples of the jejunum epithelium were spread on polystyrene plates and fixed in 10% buffered formalin. The intestinal wall

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was precisely cut and four slides were prepared from each sample. They were stained with haematoxylin and eosin and embedded in paraffin. Villus height and crypt depth were evaluated under a light Zeiss Axioscop microscope (Carl Zeiss Jena GmbH, Germany) and a CDD ZVS-47DE camera (Optronics Inc., USA) connected by RGB line with a graphic card GraBIT PCI (Soft Imaging System GmbH, Germany) installed in a standard PC.

Microbiological analyses. Bacteria from the intestinal chyme were grown in nutrient broth BHI (Sigma Aldrich, USA) at 37°C for 20 h. Bacterial total DNA was extracted with Prep Man Ultra (Applied Biosystems, USA) at 98°C. The DNA obtained was diluted tenfold in nuclease-free water for direct use. The samples were stored at 4°C until analysis. Quantity and quality of isolated DNA were estimated using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Quantitative assessments of *Escherichia coli* were made with a commercial kit (Applied Biosystems) using TaqMan probes. A standard curve was prepared from the genomic DNA of *Escherichia coli* O157. Estimates of *Clostridium perfringens* were also performed with a commercial kit (KogeneBiotech, South Korea) containing DNA for preparing the standard curve. The concentration of bacteria was estimated on the basis of standard curves. The nucleotide sequences of the tested bacterial strains were taken from the NCBI base. On the basis of these values, the number of copies of *E. coli* and *Clostridium perfringens* in particular samples was calculated.

Statistical analysis. Statistical analysis of the treatment effects was performed using two-way analysis of variance with comparisons of means by Duncan's multiple range test at $P \leq 0.05$ and

Table 1. Composition of piglet diets (g/kg diet)

Component	Prestarter (days 7–21 of age)		Starter (days 22–70 of age)	
	C	GT	C	GT
Soybean meal	120	120	220	220
Wheat (ground)	450	450	300	300
Barley (ground)	179	159	341	321
Skim milk powder	60	60	50	50
Dried whey	60	60	60	60
Soybean meal HP300	100	100	–	–
Calcium phosphate	10	10	7	7
Calcium carbonate	11	11	11	11
Vitamin-mineral premix[1,2]	5	5	5	5
Salt	3	3	3	3
L-Lysine	2	2	3	3
Glutamine	–	20	–	20
Nutritional value and chemical composition				
Metabolizable energy (MJ)	13.3		12.7	
Dry matter (g)	894		887	
Crude protein (g)	208		184	
Crude fat (g)	17		16	
Crude ash (g)	58		54	
Crude fibre (g)	37		36	

C = control diet, GT = diet supplemented with 2% glutamine

[1]premix provided per kg of diet: vitamins: A 3 000 000 IU, D_3 300 000 IU, E 21.0 g, K_3 0.45 g, B_1 0.45 g, B_2 1.2 g, B_6 0.9 g, B_{12} 0.008 g, pantothenic acid 3.0 g, choline chloride 80 g, folic acid 0.4 g, nicotinic acid 4.0 g; magnesium 10 g, manganese 8.0 g, iodine 0.16 g, zinc 28 g, iron 20 g, copper 32 g, cobalt 0.08 g, selenium 0.04 g, complete limestone to 1000 g

[2]premix provided per kg of diet: vitamins: A 2 400 000 IU, D_3 300 000 IU, E 14.0 g, K_3 0.3 g, B_1 0.3 g, B_2 1.2 g, B_6 0.9 g, B_{12} 0.005 g, pantothenic acid 2.0 g, choline chloride 80 g, folic acid 0.4 g, nicotinic acid 4.0 g; magnesium 10 g, manganese 8.0 g, iodine 0.16 g, zinc 28 g, iron 20 g, copper 32 g, cobalt 0.08 g, selenium 0.04 g, complete limestone to 1000 g

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$P \leq 0.01$ levels of significance using the STATISTICA software package (Version 10, 2011).

Intestinal villi measurements were analyzed in a factorial-hierarchical design using the GLM procedure of the SAS software (Statistical Analysis System, Version 8.0, 1999).

RESULTS

At the beginning of the experiment (days 1–28 of age) and later (days 28–56 of age), the glutamine significantly ($P < 0.01$) increased and the probiotic decreased ($P < 0.01$) piglet body weight gains (Table 2). In days 56–70 of life just the opposite occurred: the probiotic increased ($P < 0.01$) but the glutamine decreased, though not significantly, the weight gains. There was no difference in feed intake or feed utilization during the whole experiment. No interaction between both supplements was stated. Both supplements reduced the piglet mortality.

Glutamine lowered the weight of all parts of the intestines (Table 3) and in the case of the duodenum and caecum this difference was significant ($P < 0.01$). It also reduced the length of the intestines, which was most apparent in the case of the jejunum ($P < 0.01$) and less so in the case of the duodenum ($P < 0.05$). As a result of these changes the intestines of the piglets receiving glutamine

Table 2. Results of piglet rearing

	Experimental group		*P*-value	Probiotic		*P*-value	SEM	I
	C	GT		0	+			
Born piglets in treatment (*n*)	92	89	–	90	91	–	–	–
Dead and culled piglets (head)	7	4	–	7	4	–	–	–
Dead and culled piglets (%)	7.6	4.5	–	7.8	4.4	–	–	–
Average body weight of piglets (kg)								
Piglets *n*	85	85	–	83	87	–	–	–
Day 1 of age	1.79	1.85	0.060	1.82	1.83	0.591	0.02	0.911
Day 7 of age	2.99^{a}	3.40^{b}	0.040	3.29^{B}	3.10^{A}	0.009	0.06	0.579
Day 28 of age	7.65^{a}	8.59^{b}	0.020	8.70^{B}	7.54^{A}	0.000	0.16	0.082
Day 56 of age	14.32^{A}	16.04^{B}	0.002	16.27^{B}	14.11^{A}	0.000	0.25	0.146
Day 70 of age	22.37^{a}	24.01^{b}	0.050	23.68	22.71	0.077	0.33	0.547
Average body weight gains of piglets (g/day)								
Days 1–28 of age	218^{A}	249^{B}	0.004	255^{B}	211^{A}	0.0000	5.62	0.116
Days 28–56 of age	238^{A}	267^{B}	0.006	270^{B}	234^{A}	0.0007	5.43	0.644
Days 56–70 of age	575	569	0.770	529^{A}	614^{B}	0.0008	12.69	0.499
Days 1–70 of age	298^{a}	321^{b}	0.016	317	302	0.118	4.64	0.581
Feed intake (g/day)								
Days 7–28 of age	20	23	0.332	23	20	0.306	1.70	0.380
Days 28–56 of age	420	445	0.563	464	402	0.162	20.11	0.917
Days 56–70 of age	916	818	0.146	890	843	0.476	31.48	0.623
Days 7–70 of age	385	387	0.951	399	373	0.374	13.27	0.819
Average feed utilization per 1 kg of body weight gain (kg/kg)								
Days 7–28 of age	0.090	0.093	0.796	0.089	0.094	0.692	0.01	0.095
Days 28–56 of age	1.76	1.67	0.325	1.72	1.71	0.539	0.06	0.740
Days 56–70 of age	1.59	1.44	0.175	1.68	1.37	0.059	0.06	0.532
Days 7–70 of age	1.25	1.18	0.112	1.23	1.20	0.853	0.03	0.616

C = control diet (added probiotic (+): 42 piglets, no probiotic (0): 43 piglets), GT = glutamine-supplemented diet (+: 45 piglets, 0: 40 piglets), SEM = standard error of the means, I = interaction

means in the same row with different superscripts differ significantly at $^{A,B}P \leq 0.01$, $^{a,b}P \leq 0.05$

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Table 3. Mass and length of piglet intestines

	Experimental group		*P*-value	Probiotic		*P*-value	SEM	I
	C	GT		0	+			
Live weight (kg)	18.6	18.2	0.524	18.2	18.6	0.508	0.31	0.012*
Age at slaughter (days)	59.7	59.2	0.161	59.3	59.6	0.326	0.17	0.028*
Intestinal mass (g)								
Duodenum	23.6^{B}	19.3^{A}	0.009	16.0^{A}	26.9^{B}	< 0.001	1.35	0.068
Jejunum	799	753	0.139	780	772	0.772	15.70	0.093
Caecum	53.7^{B}	42.9^{A}	0.0007	45.0^{a}	51.6^{b}	0.024	1.89	0.014*
Colon	296	273	0.063	288	281	0.547	6.76	0.011*
Total	1368^{b}	1265^{a}	0.034	1309	1324	0.729	25.94	0.021*
Intestines length (cm)								
Duodenum	26.6^{b}	24.8^{a}	0.012	23.5^{A}	27.9^{B}	0.004	0.90	0.002**
Jejunum	1388^{B}	1257^{A}	0.005	1333	1312	0.521	22.80	0.002**
Caecum	20.6	19.2	0.081	20.8^{b}	19.0^{a}	0.034	0.50	0.003**
Colon	278	262	0.161	274	265	0.431	5.44	0.911
Total	1712^{B}	1562^{A}	0.0001	1652	1622	0.383	24.23	0.001**

C = control diet, GT = glutamine supplemented diet, + = added probiotic, 0 = without probiotic, SEM = standard error of the means, I = interaction

means in the same row with different superscripts differ significantly at $^{A,B}P \leq 0.01$, $^{a,b}P \leq 0.05$

$^{*}P \leq 0.05$, $^{**}P \leq 0.01$

were lighter ($P < 0.05$) and shorter ($P < 0.01$) than those of the control ones. A significant interaction between both supplements in the changes of whole intestines length except that of colon was found. The probiotic had an opposite effect than the glutamine on these parts of the intestines: both the duodenum ($P < 0.01$) and caecum ($P < 0.05$) were heavier than in the control animals. However, these differences had no effect on the total intestine weight.

The only significant ($P < 0.01$) difference in the acidity of the intestinal chyme was the higher pH of the content of the small intestine (jejunum) in the piglets receiving the probiotic (Table 4). There was also no difference in acidity and the short chain fatty acids (SCFA) content in the intestines between the control and glutamine-fed piglets. The jejunum of the piglets receiving the probiotic had a significantly higher content of butyric and valeric acids but the amount of both these acids was very small. The probiotic resulted in a significant ($P < 0.01$) increase of acetic and isobutyric acids content in the caecum and a small reduction ($P < 0.05$) of the isovaleric acid content. Consequently, the total content of SCFA in the caecum of piglets fed the probiotic was significantly ($P < 0.01$) higher than that in the control animals.

There was no significant difference in the mucosal epithelium structure of the ileum (Table 5) apart from the deeper crypts in piglets receiving the probiotic and the tendency ($P = 0.076$) to wider villi in animals of the same group.

Both supplements had a strong antibacterial activity against *E. coli* ($P < 0.01$) in the jejunum (significant interaction was stated) but the probiotic was also active against *Clostridium* (Table 6). Both supplements reduced the amount of both bacteria species in the caecum, but the difference was greater in the case of *E. coli* ($P < 0.01$). The glutamine decreased the content of *Clostridium* to a lower degree ($P < 0.05$) than the probiotic ($P < 0.01$).

DISCUSSION

The results of the experiments concerning the use of glutamine in piglet feed are not consistent. In the experiment of Zou et al. (2006) its supplement improved piglet body weight gains, however Hsu et al. (2010) did not find such an improvement. Liu and Pen (1999) found that 1% of glutamine supplement improved piglet body weight gains

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Table 4. Acidity of chyme in the stomach and in various parts of the intestines and volatile fatty acid (VFA) content of piglets' chyme (μmol/g chyme)

	Experimental group		*P*-value	Probiotic		*P*-value	SEM	I
	C	GT		0	+			
Stomach	2.88	2.64	0.085	2.72	2.79	0.592	0.07	0.029*
Duodenum	5.93	5.97	0.817	6.07	5.83	0.122	0.07	0.265
Jejunum	5.84	5.99	0.183	5.75^{A}	6.08^{B}	0.009	0.06	0.670
Caecum	5.39	5.56	0.102	5.44	5.51	0.503	0.05	0.519
Colon	5.94	5.74	0.093	5.74	5.95	0.074	0.06	0.268
VFA content of jejunum chyme								
Propionic	0.21	0.20	0.921	0.13	0.28	0.103	0.04	0.523
Isobutyric	0.73	0.71	0.904	0.83	0.61	0.170	0.07	0.512
Butyric	0.17	0.14	0.519	0.09^{A}	0.22^{B}	0.007	0.02	0.575
Acetic	10.72	9.65	0.556	9.61	10.75	0.531	0.87	0.252
Isovaleric	0.01	0.00	0.254	0.01	0.00	0.254	0.01	0.254
Valeric	0.05	0.08	0.860	0.03^{a}	0.10^{b}	0.034	0.02	0.921
Total	10.88	10.78	0.555	10.71	11.86	0.504	0.89	0.310
VFA content of caecum chyme								
Acetic	58.00	55.40	0.512	40.92^{A}	72.45^{B}	< 0.001	3.87	0.102
Propionic	38.68	39.77	0.718	39.01	39.44	0.885	1.43	0.216
Isobutyric	3.54	4.42	0.328	2.09^{A}	5.88^{B}	< 0.001	0.58	0.629
Butyric	22.02	18.36	0.099	21.05	19.33	0.427	1.12	0.126
Isovaleric	0.28	0.33	0.511	0.39^{b}	0.21^{a}	0.026	0.04	0.803
Valeric	5.29	4.84	0.594	5.54	4.58	0.270	0.44	0.054
Total	127.81	123.09	0.509	109.01^{A}	141.89^{B}	< 0.001	4.73	0.810

C = control diet, GT = glutamine supplemented diet, + = added probiotic, 0 = without probiotic, SEM = standard error of the means, I = interaction

means in the same row with different superscripts differ significantly at $^{A,B}P \leq 0.01$, $^{a,b}P \leq 0.05$

$^{*}P \leq 0.05$

during 2 weeks after weaning at 28 days of age. Our results are partially in accordance with this last opinion. Piglets receiving glutamine grew faster only at the beginning of the experiment. Similar results were found also by Domeneghini et al. (2004), who found improvement in piglet body weight gains at the beginning of the experiment but later this difference was compensated, and at the end piglet body weights did not differ significantly. It is known that after weaning

Table 5. Mucosal epithelium structure of the jejunum

	Experimental group		*P*-value	Probiotic		*P*-value	SEM	I
	C	GT		0	+			
Villus height (μm)	347	350	0.939	343	354	0.743	15.80	0.816
Villus width (μm)	171	168	0.788	158	181	0.076	6.12	0.805
Crypt depth (μm)	373	377	0.831	349^{A}	400^{B}	0.007	9.68	0.229
Villus height/crypt depth	0.929	0.934	0.950	0.989	0.875	0.160	0.07	0.746

C = control diet, GT = glutamine supplemented diet, + = added probiotic, 0 = without probiotic, SEM = standard error of the means, I = interaction

means in the same row with different superscripts differ significantly at $^{A,B}P \leq 0.01$

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Table 6. Microbial counts in the small intestine (jejunum) and caecum digesta (log_{10} CFU/1 g chyme)

	Experimental group		*P*-value	Probiotic		*P*-value	SEM	I
	C	GT		0	+			
Jejunum								
E. coli	3.12^{B}	2.29^{A}	0.002	3.23^{B}	2.18^{A}	< 0.001	0.297	< 0.001**
Clostridium perfringens	3.31	3.08	0.394	3.87^{B}	2.52^{A}	< 0.001	0.199	0.556
Caecum								
E. coli	2.87^{B}	2.20^{A}	0.002	2.92^{B}	2.15^{A}	< 0.001	0.208	< 0.001**
Clostridium perfringens	3.41^{b}	2.30^{a}	0.017	3.66^{B}	2.05^{A}	0.001	0.294	0.398

C = control diet, GT = glutamine supplemented diet, + = added probiotic, 0 = without probiotic, SEM = standard error of the means, I = interaction

means in the same row with different superscripts differ significantly at $^{A,B}P \leq 0.01$, $^{a,b}P \leq 0.05$

$^{**}P \leq 0.01$

piglets have to adapt to new stressful conditions which are associated with temporary malnutrition and growth retardation, therefore the effect of glutamine preventing intestinal atrophy is more apparent during this period.

In contrast, the glutamine probiotic at the beginning of the experiment significantly decreased, but after day 56 of the piglets' life it significantly improved their body weight gains. In the experiment of Mohana Devi and Kim (2014), the supplement of *E. faecium* did not improve nitrogen digestibility significantly in the first 2 weeks of the experiment but improved it significantly after 6 weeks of life. Mallo et al. (2010) supplemented the diet for piglets in days 28–56 of age with *E. faecium* and they found a significant improvement of body weight gains. This was probably due to changes in gastrointestinal microbiota by promoting the growth of lactic acid bacteria. Also, Zeyner and Boldt (2006) found better body weight gains even in younger piglets from birth to weaning. According to these authors, this positive effect was due to reducing the diarrhoea incidences that resulted from the direct antagonism of the probiotic against specific pathogenic microbes.

In the present experiment lactobacilli were not estimated, but the different body weight gains could be due to changes of microbiota in the digestive tract, especially in the small intestine. It is also hard to explain the differences between the effect of the glutamine and that of the probiotic during the different periods of the experiment because the bacteria were determined only once on day 60 of the piglets' life. The results suggest that the glutamine is effective in the first period after weaning while the probiotic rather in the later period of piglets rearing. It is also obvious that there is no synergy between these two supplements.

In our previous experiment, an insignificant reduction of the piglet intestine mass (but not length) after supplementing feed with glutamine was also registered (Hanczakowska et al. 2014). This finding differs from that of Wang et al. (2008). In both experiments greater weight of the small intestine, as the result of glutamine supplement, was found. In the experiment of Wang et al. (2008), the piglets were early-weaned (on day 21 of life) and the intestines were measured on day 28 of life, i.e. much earlier than in the present experiment. Therefore, according to Hou et al. (2006), glutamine gives the best results in the first two weeks after weaning, therefore the 60th day of life could be too late to find the effect of this amino acid. A similar method was used in the second mentioned experiment: early weaned piglets (day 21) and early measurement of intestines (day 14 post weaning).

Data in the literature concerning changes in the mass or length of the intestines under the influence of probiotics are rare. In an experiment on piglets Lee et al. (2011) found a slight increase of total intestinal length after supplementing feed with yeasts and *Bacillus* spores. In this experiment, the probiotic significantly increased mass and length of some parts of the intestines, especially the duodenum. It is unclear because, according to Kotunia et al. (2004), the quick flow of chyme through this part of the digestive tract limits possible anatomical changes. According to Gancarcikova et al. (2009) the increased small intestine length may be a compensatory response

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to direct competition with the microbiota for dietary nutrients.

A slightly lower acidity (higher pH) of chyme in the further part of the small intestine (not in the duodenum) in piglets receiving probiotic could also be due to changes in microbial composition, which is not the same in particular parts of the intestines. Veizaj et al. (2008) found slightly higher pH of digesta in the duodenum but lower in the ileum of piglets receiving probiotic made up of *E. feacium* and *Lactobacilli*. On the other hand, Strompfova et al. (2006) found a lowering effect of *E. feacium* only in the piglet duodenum but not in further parts of the digestive tract.

It is known that the amount of short chain fatty acids in the digestive tract increases from its proximal to distal parts. In a study of Nyachoti et al. (2006) with early weaned pigs, the concentration of acetic acid changed from 0.907 mmol/l in the duodenum to 70.29 mmol/l in the ileum. In the present experiment the concentration of SCFA in the jejunum was many times lower than that in the caecum, which is a main place of degradation of undigested nutrients. Glutamine had no effect on the SCFA content in distal parts of the intestines (caecum) which is in accordance with our earlier results (Hanczakowska et al. 2014). In contrast to glutamine, the probiotic significantly raised acetic and isobutyric acids in caecum which suggests a more intensive fermentation processes. The higher level of isobutyric acid probably resulted from protein degradation because this acid is produced from the amino acid valine by bacterial deamination (Arkowitz et al. 1994).

None of the supplements changed epithelium villi height or width. Intestinal villi are the main site of nutrient absorption and their better development could be the reason for a higher nutrient utilization (Mekbungwan et al. 2002), resulting in a better growth of piglets. Such interdependence was found earlier in the case of glutamine by Wu et al. (1996) and in our previous experiment (Hanczakowska and Swiatkiewicz 2012). On the other hand, in the experiment of Domeneghini et al. (2004) positive changes in the epithelium structure had no effect on piglet growth. Perhaps these differences were due to some other factors, e.g. feed composition or piglet age. No difference in ileum villus sizes under the influence of *E. faecium* supplement was also found by Reiter et al. (2006) and the differences in crypt sizes were not significant. These authors did not measure body weight gains, thus in this case the correlation between the size of the villi and piglet performance cannot be determined. In this experiment, the significant improvement of body weight gains at the time when the intestine samples were taken was not correlated with a slight but not significant increase in villi size.

According to Zhao et al. (2009) the addition of 1% of glutamine to diets for weaning piglets improves intestinal microflora and reduces the amount of pathogenic bacteria. Similar results were obtained in the present experiment, especially in the case of *E. coli*. Perhaps glutamine modulates the bacterial metabolism of the amino acids mainly of the arginine family and reduces the catabolism of most amino acids, including these nutritionally essential. The probiotic was highly effective against both tested species of bacteria in both parts of the digestive tract, while in the experiment of Mallo et al. (2010) the supplement of *E. faecium* significantly reduced the number of *E. coli* in the ileum but not in the caecum. Strompfova et al. (2006) did not find a lower number of *E. coli* in any part of the piglet intestines after administration of this probiotic. The results of Bednorz et al. (2013) suggest a minor influence of *E. faecium* on the overall population of *E. coli* in healthy piglets but it has a profound effect on mucosa-adherent bacteria. The inhibitory effect of *E. faecium* found in this experiment was observed also by Szaboova et al. (2008), who found a decrease of *E. coli* and *Clostridium sp.* in rabbits.

In conclusion, it can be stated that the supplement of glutamine has a positive effect on piglet performance in the first days after weaning and *E. faecium* probiotic in the later period of rearing. Glutamine increased the weight and length of the intestines but the probiotic did not cause differences. The supplements had no effect on villi height but both had strong antibacterial activity against *Escherichia coli* and *Clostridium perfringens*. There was no synergy in the effect of the glutamine and probiotic.

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