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THE EFFECT OF SUPPLEMENTING SODIUM BUTYRATE CONTAINING FEED WITH GLUTAMINE AND/OR GLUCOSE ON THE STRUCTURE OF THE PIGLET DIGESTIVE TRACT AND SELECTED BLOOD INDICES*

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Abstract

The effect of supplementing the standard piglet diet containing sodium butyrate with glutamine and/or glucose on the structure of the piglet digestive tract and the small intestine epithelium, acidity and volatile fatty acid content of its digesta was investigated. The free amino acids level, insulin and insulin-like growth factor-1 (IGF-1) concentration in the blood were also analysed. The experiment was performed on 156 piglets (15 litters) allocated to 5 experimental groups, 3 litters in each. Group I (C, negative control) received a basal mixture with no supplement. Group II (SB, positive control) was fed the same basal diet containing additionally 3 g of sodium butyrate per kg. Group III and IV, besides sodium butyrate, received additionally 10 g of glutamine (GT) or glucose (GC), respectively. The last group V received all these supplements, i.e. SB+GT+GC (3, 10, 10 g per kg, respectively). At 60 days of age, the piglets (6 animals from each group) were slaughtered and their intestines were measured and weighed. The piglets in group SB+GT+GC receiving all the supplements grew slightly faster than the others, and at the end of the experiment the differences in body weight were significant. The total intestinal mass of the piglets fed with glucose or all the supplements was significantly higher than that of the piglets receiving glutamine but there was no significant difference in the total length of intestines. There was also no significant difference in acidity of chyme along the entire length of the gastrointestinal tract. Digesta in the jejunum of both control groups (C, SB) contained significantly more SCFA than the remaining groups. In the caecum their content in the negative control and the group fed with all supplements was significantly higher when compared to the butyrate and glucose group. In the duodenum villus height was similar in all the groups but in the jejunum it was significantly higher in the group receiving all supplements than in other groups. Free amino acids level was lowest in the piglets receiving glucose but there was no difference between the remaining groups. The lowest level of IGF-1 was found in the same group and this difference was significant when compared with remaining groups, except C. It is concluded that glutamine and glucose, when given together with sodium butyrate, improve the structure of piglet jejunum epithelium and average body weight gains. A supplement of glucose significantly lowers free amino acid content and IGF-1 level in piglet blood.

Key words: piglet feeding, sodium butyrate, glutamine, glucose

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Weaning is the most dangerous moment in a piglet's early life. In a young animal the small intestine and its epithelium are not fully developed which, together with a large demand of the growing organism for protein and energy, may result in malnutrition and growth retardation (Moran et al., 2010). Antibiotic growth promoters were used to protect piglet health but were banned by the EU (Dibner and Richards, 2005), hence some replacers have to be found. The small intestine is the main place of feed digestion and nutrient absorption, thus its proper development is crucial for piglet growth (Tang et al., 1999). It also acts as a protective barrier against bacteria, viruses and other harmful materials (Bailey et al., 2005). In this period protein synthesis decreases in the muscles while it increases in the intestine (Seve et al., 1986), and the small intestine becomes one of the most metabolically-active tissues. Thus it should be supplied with easily digestible protein to sustain substantial amino acid metabolism (Schaart et al., 2005). Another way of improving intestine development is supplementing piglet feed with substances which could be utilised by epithelium cells (enterocytes) as an energy source. These substances may be butyric acid or, for practical reasons, its sodium salt (Lu et al., 2008; Fang et al., 2014) and glutamine (Domeneghini et al., 2006; Zou et al., 2006; Molino et al., 2012). According to Mallet et al. (1986) glucose can provide a similar effect, but in the experiment of Jin et al. (1998) it was no better than other carbohydrates such as lactose or starch. In our experiment (Hanczakowska et al., 2011) we received good results using sodium butyrate and there was no significant improvement of body weight gains during the first 56 days of the piglet's life when glutamine or glucose was given. Such positive effects of sodium butyrate could be the result of changes in the proximate parts of the digestive tract especially improving the structure and function of small intestine epithelium (Miller and Slade, 2006). Intestinal villi are the main site of nutrient absorption and their better development could be the reason for better nutrient utilization (Mekbungwan et al., 2002) resulting in better growth of piglets.

Butyrate also has other effects on animal physiology. It may increase the concentration of insulin in blood plasma (Gálfi and Bokori, 1990; Fang et al., 2014) and change the amino acid level in blood (Welters et al., 1996). Such effects were found also in the case of glutamine and glucose (Maechler and Wollheim, 1999; Molfino et al., 2010). Assessing research carried out in recent years (Hanczakowska and Szewczyk, 2011) it can be stated that butyrate is one of the most important compounds in the intestinal mucosa metabolism and its activity may be stimulated by presence of glutamine or glucose (Beaulieu et al., 2002).

Therefore the aim of this experiment was to investigate the effect of supplementing the piglet diet containing sodium butyrate with glutamine or/and glucose on the structure of the piglet digestive tract and the small intestine epithelium, acidity and volatile fatty acid content of its digesta. The free amino acids level, insulin and IGF-1 concentration in the blood were also analysed.

Material and methods

Animals and experimental design

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

Table 1. Composition of piglet diets (g kg^{-1})

Component	C	SB	SB+GT	SB+GC	SB +GT+GC
Barley, ground	200	200	200	200	200
Wheat, ground	353	350	335	335	320
Corn meal	100	100	100	100	100
Soybean meal	200	200	200	200	200
Rapeseed press cake	30	30	30	30	30
Skim milk powder	40	40	45	45	50
Dried whey	50	50	50	50	50
Vitamin-mineral premix[1]	5	5	5	5	5
L-lysine	1	1	1	1	1
Salt	2	2	2	2	2
Calcium carbonate	7	7	7	7	7
Dicalcium phosphate	12	12	12	12	12
Glutamine	-	-	10		10
Glucose	-	-	-	10	10
Sodium butyrate	-	3	3	3	3
Calculated:					
metabolizable energy (MJ)	12.9	13.0	12.9	13.0	13.0
crude protein (g)	180	181	180	181	181
Lys (g)	10.1	10.20	10.20	10.20	12.28
Met+Cys (g)	6.2	6.27	6.21	6.21	6.20
Thr (g)	2.18	2.20	2.18	2.18	2.18
Thr (g)	6.73	6.79	6.74	6.74	6.76
Ca (g)	9.50	7.31	7.30	7.30	7.36
P (g)	3.58	3.62	3.59	3.59	3.61
Gross composition (analysed):					
dry matter (g)	884	884	885	874	878
crude protein (g)	173	173	170	174	172
crude fat (g)	35	35	36	36	38
crude ash (g)	56	56	58	52	58
crude fibre (g)	21	21	22	24	22

* Supplied per kilogram of mixture: vitamin: A – 13500 IU; D_3 – 2000 IU; E – 40 mg; K_3 – 2.5 mg; B_1 – 2.5 mg; B_2 – 4 mg; B_6 – 4 mg; B_{12} – 40 mcg; pantothenic acid – 14 mg; choline chloride – 350 mg; folic acid – 1 mg; nicotinic acid – 25 mg; magnesium – 50 mg; manganese – 60 mg; iodine – 0.5 mg; zinc – 150 mg; iron – 100 mg; copper – 160 mg; cobalt – 0.3 mg; selenium – 0.2 mg.

C – control diet. Diets supplemented with: SB sodium butyrate, SB+GT sodium butyrate with glutamine, SB+ GC sodium butyrate with glucose, GT+GC+SB all these supplements.

The experiment was performed on 156 piglets (15 litters) originating from Polish Large White (PLW) sows and a PLW boar. The piglets received experimental diets *ad libitum* from the 7th day of life to weaning (35th day of life), while from weaning to the end of the experiment (60th + 2 days of life) restricted feeding was used. The amount of feed was increased every 7 days by 200 g. The piglets were allocated to

5 groups (3 litters in each group), kept in group pens (each litter in a separate pen), and fed with the same feed mixture prepared according to Grela and Skomiał (2014). Group I (negative control, C) received a basal mixture with no supplement; Group II (positive control, SB) was fed the same basal diet containing additionally 3 g of sodium butyrate per kg; Group III and IV, besides sodium butyrate, received additionally 10 g of glutamine (GT) or glucose (GC), respectively; and Group (V) received all these supplements i.e. SB+GT+GC in amount of 3, 10, 10 g per kg, respectively. All the supplements were provided by Sigma-Aldrich Corporation, St Luis, MO., USA. The composition of the diets is given in Table 1.

Measurements and collection of samples

The piglets were individually weighed at 1, 35 (weaning) and at 60±2 days of life (end of the experiment). Mean body weight gains were calculated on the basis of these results.

Intestines were prepared from six randomly selected piglets from each group (i.e. two piglets from each litter). Intestinal content was removed and its individual parts were measured and weighed. Acidity of digesta in particular parts of the digestive tract and its content of short chain fatty acids (SCFA) were also measured.

Samples of two parts of the small intestine (duodenum and jejunum) were spread on polystyrene plates and fixed in 10% buffered formalin. The intestinal wall 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 (Zeiss GmbH, Germany) and 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.

Blood was collected from the jugular vein of six piglets from each group at slaughter, three hours after feeding.

Chemical analyses

The composition of feed mixtures was analysed according to AOAC (2005) methods. Free amino acids in the blood serum were determined after deproteinisation with 6% sulfosalicylic acid (1:1) and centrifugation. Samples were analysed using the AAA 400 INGOS automatic analyser. Integration of peaks was performed in the Chromulan programme. Amino acids content was determined according to the standards.

Glucose, insulin and IGF-1 (insulin-like growth factor 1) concentration was estimated using the reflectometric method on Vitros s. 950 and 250 analysers (Ortho Clinical Diagnosis and Johnson & Johnson Company), and dry test strips (Kodak Ektachem Clinical Chemistry Slides) according to Ortho-Clinical Diagnostic 2001.

Acidity of the stomach, ileum, caecum and colon contents was measured with a CP-411 pH-meter (Elmetron, Zabrze, Poland), equipped with a Metron 12–01 electrode (Metron, Toruń, Poland). SCFA profile in the jejunum and caecum was separated on column CP-Wax 58 (Varian BV, Middelburg, the Netherlands) (25 m, 0.53 mm, 1 m, carrier gas – helium, 6 ml/min), with a column oven tempera-

ture programme from 90 to 200°C, using a Varian 3400 gas chromatograph (Varian Associates Inc., Walnut Creek, USA) equipped with a Varian 8200 CX autosampler (2000 C), FID detector (2600 C), and Star Chromatography Workstation Software.

Statistical analysis

Statistical analysis of treatment effects was conducted by an one-way analysis of variance with comparisons of means by Duncan's multiple range test at $P \leq 0.05$ and $P \leq 0.01$ levels of significance using the Statistica 10 package (StatSoft, 2011).

Results

The piglets receiving all the supplements grew slightly faster than the others (Table 2) and this difference was significant at $P<0.05$ when compared to the SB, GT and GC and at $P<0.01$ when compared to the control group. At the end of the experiment differences in piglet body weight were similar to these in body weight gains and were significantly ($P<0.05$) higher in the group receiving all supplements than in all other groups.

Table 2. Piglets' rearing indices, intestinal mass and length

	C	SB	SB+GT	SB+ GC	SB+ GT+GC	SEM[1]	P-value
No. of piglets born in treatment	32	32	32	29	31	-	-
Dead and culled piglets (%)	12.5	6.2	4.2	3.5	3.2	-	-
Body weight of piglet at 1st day of age (kg)	1.75	1.77	1.76	1.76	1.75	0.03	0.99
Live weight at 62nd day (kg)	14.6 Aa	17.0 ABa	16.2 ABa	17.0 ABa	19.2 Bb	0.39	<0.01
Average body weight gains of piglets (g)	207 Aa	246 ABa	233 ABa	246 ABa	280 Bb	5.16	<0.01
Age at slaughter (days)	62.0	61.7	62.7	62.8	61.7	0.49	0.92
Carcass weight (kg)	11.6	13.1	12.2	13.0	14.9	0.36	0.17
Intestinal mass (g) (based on 6 piglets in group)							
duodeum	24	33	31	38	38	1.73	0.08
jejunum	747 ABa	926 ABb	733 Aa	910 ABab	984 Bb	30.72	0.01
ileum	29	31	24	31	29	1.28	0.49
caecum	42	48	44	57	58	2.41	0.20
colon	334	306	283	387	377	14.66	0.10
Total	1176 ab	1343 abc	1118 a	1422 bc	1485 c	44.45	0.03
Length of the intestines (cm) (based on 6 piglets in group)							
duodeum	24.3	20.5	24.5	26.0	26.0	1.06	0.48
jejunum	1066	1138	1097	1208	1201	25.20	0.29
ileum	18.5 a	24.7 b	17.3 a	21.3 ab	19.0 a	0.84	0.03
caecum	11.5	13.8	12.8	13.2	13.3	0.54	0.75
colon	246	232	255	275	252	6.72	0.37
Total	1366	1428	1406	1544	1511	27.28	0.21

[1]Standard error of the mean.

Mean values in the row with different letters differ: a, b, c – $P \leq 0.05$; A, B – $P \leq 0.01$.

The total intestinal mass of the piglets receiving sodium butyrate and glutamine was significantly (P<0.05) lower than that of the piglets receiving all the supplements, and also than that of the piglets fed with sodium butyrate and glucose. It was the effect of lower weight of all parts of the digestive tract in this group, especially that of the jejunum, which was significantly (P<0.01) lower than that of the animals of the last group (Table 2). Differences in the length of particular parts of the intestines were smaller; the only significant (P<0.05) difference was found in the case of the ileum of piglets receiving sodium butyrate alone, which was longer (P<0.05) than the rest, except for the GC group where this difference was not significant. These differences did not result in a significant difference in the total length of the intestines.

There was no significant difference in the acidity of the chyme over the entire length of the gastrointestinal tract. Its pH ranged from 6.43 (duodenum) to 5.58 (caecum) (results not presented).

Table 3. Short-chain fatty acid (SCFA) content of piglets' jejunum and caecum chyme (μmol/g wet weight)

	C	SB	SB+GT	SB+GC	SB+GT+GC	SEM[1]	P-value
Number of piglets	6	6	6	6	6	-	
Jejunum							
Acetic	13.90 Cd	10.76 Bc	8.43 Ab	6.85 Aa	6.77 Aa	0.64	0.0000
Propionic	0.222 b	0.110 a	0.100 a	0.108 a	0.060 a	0.02	0.03
Isobutyric	0.317 B	0.101 A	0.031 A	0.026 A	0.013 A	0.03	0.0002
Butyric	0.198 B	0.039 A	0.033 A	0.008 A	0.089 A	0.02	0.002
Isovaleric	0.013	0.122	0.052	0.097	0.086	0.01	0.09
Valeric	0.019	0.125	0.037	0.089	0.023	0.02	0.30
Total acids	14.63 Cd	11.26 Bc	8.68 Ab	7.18 Aa	7.04 Aa	0.68	0.0000
Caecum							
Acetic	79.55 b	73.48 b	77.03 b	57.59 a	81.15 b	2.67	0.02
Propionic	43.80 b	32.73 a	34.52 a	30.41 a	38.36 ab	1.54	0.03
Isobutyric	0.934 Aa	1.289 Aab	1.208 Aab	1.468 Ab	2.666 Bc	0.15	0.0000
Butyric	15.49 ab	13.55 ab	12.91 ab	10.27 a	18.75 b	0.98	0.05
Isovaleric	0.530 b	0.550 b	0.400 ab	0.247 a	0.595 b	0.04	0.05
Valeric	2.576	2.612	2.118	2.805	3.293	0.28	0.80
Total acids	142.92 b	124.21 ab	128.17 ab	102.78 a	145.54 b	4.97	0.03

[1]Standard error of the mean.
Mean values in the row with different letters differ: a, b – P≤0.05; A, B – P≤0.01.

All the supplements given separately or together lowered SFCA content (P<0.01) in the jejunum chyme when compared to both control groups (Table 3). It was the result of a lower content of acetic acid which accounted for about 95% of total fatty acids. SCFA level in the caecum was several times higher than that in the jejunum, and this applies to all acids. Besides acetic acid, propionic and butyric acids were present in relatively high amounts. The content of acetic acid in the caecum of animals receiving butyrate and glucose was significantly (P<0.05) lower than in the remaining animals.

Table 4. Epithelium structure of piglet small intestine mucosa

	C	SB	SB+GT	SB+GC	SB+ GT+GC	SEM[1]	P-value
Number of piglets	6	6	6	6	6	-	-
Duodenum							
villus height (μm)	299	302	293	312	332	4.79	0.06
villus width (μm)	160	151	163	151	156	2.53	0.54
crypt depth (μm)	353 a	389 ab	411 b	368 a	355 a	7.36	0.05
villus height/crypt depth	0.85 ab	0.80 a	0.74 a	0.86 ab	0.93 b	0.02	0.03
Jejunum							
villus height (μm)	394 ABa	398 ABa	385 Aa	403 ABa	424 Bb	3.82	0.005
villus width (μm)	139 ab	152 b	152 b	141 ab	133 a	2.33	0.02
crypt depth (μm)	306	293	291	310	297	6.31	0.86
villus height/crypt depth	1.32	1.39	1.33	1.31	1.45	0.03	0.57

[1] Standard error of the mean.
a, b – mean values in the row with different letters differ: P≤0.05.

Table 5. Free amino acids content in piglet blood plasma (μmol/l)

	C	SB	SB+GT	SB+GC	SB+ GT+GC	SEM[1]	P-value
Number of piglets	6	6	6	6	6		
Taurine	209 Bb	177 ABb	72 Aa	90 ABa	203 Bb	15.78	0.004
Aspartic acid	2183 B	2382 B	1952 AB	933 A	1645 AB	137.90	0.003
Threonine	102	93	92	61	115	6.00	0.09
Serine	117	154	197	105	126	11.28	0.09
Asparagine	11 Aa	115 Bb	135 Bb	94 Bb	79 ABb	11.58	0.0009
Glutamic acid	512 ABab	618 ABbc	651 ABc	404 Aa	620 ABbc	23.22	0.001
Glutamine	190 abc	269 bc	292 c	159 ab	134 a	19.54	0.04
Proline	272 ab	373 ab	388 b	258 a	363 ab	18.46	0.05
Glycine	794 b	822 b	882 b	579 a	902 b	33.88	0.03
Alanine	555 ABa	836 Bb	797 Bb	511 Aa	810 Bb	37.89	0.002
Valine	240 ab	311 b	282 b	207 a	302 b	12.07	0.02
Cystine	21	31	32	33	45	2.67	0.09
Methionine	35 Bc	30 ABab	23 ABab	16 Aa	35 Bc	2.13	0.009
Isoleucine	108	137	135	97	133	6.04	0.13
Leucine	156	189	181	128	169	8.23	0.16
Tyrosine	62 ab	61 ab	58 ab	44 a	79 b	3.44	0.04
Phenylalanine	97 b	89 b	79 ab	62 a	87 b	3.93	0.05
Ornithine	154	138	133	73	173	11.37	0.08
Lysine	305	403	270	232	332	25.03	0.24
Histidine	91	89	82	67	93	3.22	0.08
Arginine	165	176	205	179	106	16.36	0.50
Total	6390 B	7496 B	6942 B	4333 A	6540 B	278.75	0.0007

[1]Standard error of the mean.
Mean values in the row with different letters differ: a, b – P≤0.05; A, B – P≤0.01.

There was no significant difference in villi height in the piglets' duodenum epithelium, though those found in animals receiving all the supplements were apparent-

ly the highest (Table 4). There were, however, significant differences in crypt depth which resulted in the highest villi height to crypt depth ratio in the last experimental group. In the jejunum villi were significantly (P<0.05) highest in the group receiving all the supplements. This difference was greater (P<0.01) when compared to the group receiving glutamine. There were no significant differences in crypt depth and crypt depth to villi height ratio in this part of the digestive tract.

The blood plasma of the piglets receiving glucose contained a significantly (P<0.01) lower total amount of free amino acids than that of the piglets in the other groups (Table 5). When compared to the positive control group (SB), this reduction was significant in the cases of glycine, alanine, valine and phenylalanine but no significant decrease was found in the content of almost all the remaining amino acids.

Table 6. Glucose, insulin and IGF-1 content in piglet blood plasma

	C	SB	SB+GT	SB+GC	SB+GT+GC	SEM[1]	P-value
Number of piglets	6	6	6	6	6	-	
Glucose (mmol/l)	4.60	5.40	5.90	5.42	4.92	0.26	0.60
Insulin (uU/ml)	8.04 a	10.70 ab	16.60 b	11.68 ab	11.94 ab	0.89	0.03
IGF-1 (µg/ml)	99.00 ab	106.42 b	108.68 b	71.82 a	117.26 b	5.05	0.049

[1]Standard error of the mean.
Mean values in the row with different letters differ: a, b – P≤0.05; A, B – P≤0.01.

The highest level of insulin was found in the blood plasma of piglets receiving butyrate and glutamine (P<0.05) but this difference was significant only when compared to the negative control group (Table 6). The level of IGF-1 in blood was lowest (P<0.05) in the group receiving SB +GC and was only comparable with the negative control group.

Discussion

At the end of the experiment significant differences in the mass of the intestines were found, especially in the proximal part, i.e. the jejunum. The absence of such changes in the duodenum could be the result of a quick flow of chyme through this part of the digestive tract (Kotunia et al., 2004). No significant difference in the distal parts of digestive tract could be due to small doses of supplements which were probably readily absorbed in the jejunum. A higher mass of the jejunum in groups receiving SB (and apart from this also receiving glutamine) is in accordance with the results of Fang et al. (2014), which suggest that SB plays an important role in maintaining the integrity of intestinal mucosa. Also, according to Claus et al. (2007) SB has a specific effect on enterocyte mitosis which in turn leads to an increased plica size by crypt fission. It is known that small intestine mucosa is the main place of nutrients absorption (Pácha, 2000), which could result in better growth and weight of the jejunum. Adeola and King (2006) found that a significant increase in the weight of the mucosa up to the ninth week of piglet age was associated with an increase in the weight of the small intestine tissues.

It is known that glutamine also has a positive effect on the piglet digestive tract (Wu et al., 1996; Hsu et al., 2010). On the other hand, according to Hou et al. (2006) glutamine gives better results in the first two weeks after weaning while butyrate is active in the later period. However, in this experiment glutamine supplement significantly ($P<0.05$) lowered jejunum mass compared to the group receiving SB alone or all of the supplements. This suggests some antagonism in butyrate and glutamine activity but we have no explanation for this result.

All supplements significantly ($P<0.01$) lowered the content of SCFA in the jejunum chyme. Because these acids are produced by microbial fermentation in piglet intestines (Gancarcikova et al., 2009), this decrease could be due to the known antimicrobial activity of SB (Castillo et al., 2006). Also, a significantly higher content of isobutyric acid in the jejunum of the piglets in the negative control group may be evidence of higher microbial activity in the intestines of this group. According to Arkowitz et al. (1994) this acid is produced from amino acid valine by bacterial deamination. No such significant differences were found in the caecum chyme. The content of SCFA in the caecum was much higher than that of the jejunum. It is known that their amount increases from proximal to distal parts of the digestive tract. In addition, Nyachoti et al. (2006) found that acetic acid concentration increased from 0.907 mmol/l in the duodenum to 70.29 mmol/l in the ileum in early-weaned piglets.

A significantly lower content of acetic acid in the piglets receiving glucose, especially in their caecum, is hard to explain. The feed contained large amounts of cereals rich in starch which is hydrolysed to glucose in the digestive tract. Thus such a small supplement of this carbohydrate should not affect the environment in the distal parts of the intestines. Perhaps glucose causes some changes in the more proximal part of the digestive tract, which may affect the content of the large intestine. Gregory et al. (1987) found that a higher content of glucose in the piglet duodenum suppresses feed intake and that this influence is pre-absorptive. According to these authors the most likely mechanism is that duodenal infusion of glucose inhibits intake calorically or according to the duodenal osmotic concentration via gastric distension following duodenally-mediated slowing of gastric emptying. According to Imoto and Namioka (1978), changes in the carbohydrate feeding of pigs causes more changes in acetic acid than propionic and butyric acids content in the pig's large intestine, which was also found in the present experiment. Bayley and Carlson (1970) found that the glucose supplement did not reduce post-weaning check but appeared to increase the incidence of digestive disturbance. The authors suggest that it could be the result of low digestibility of fat (there was a higher amount of fat in the faeces than was ingested) and changes in the microflora of the lower part of the digestive tract. All these findings and results of this experiment suggest an active role of glucose in the intestines which requires further research.

Intestinal villi are the main site of nutrient absorption (Ray et al., 2002) and their better development could be the reason for higher nutrient utilisation (Mekbungwan et al., 2002) resulting in the better growth of piglets (Hanczakowska and Świątkiewicz, 2012). Wu et al. (1996) found better growth of intestinal villi in piglets fed with glutamine, and Lu et al. (2008) found the same using butyrate. No such effect of glutamine was found in the present experiment; villi height in the

duodenum and jejunum epithelium in piglets receiving glutamine was even lower than those in other groups. This could be due to the late completing of the experiment. According to Hou et al. (2006) the effect of glutamine is most distinct in the first two weeks after weaning but this experiment was completed when piglets were 60 days old. Despite the fact that glucose is a good energy source for epithelial cells (Mallet et al., 1986), it does not affect villus architecture (Vente-Spreeuwenberg et al., 2003). Also in this experiment neither glucose nor glutamine improved the villi height in the proximate part of the small intestine. According to Wilfart et al. (2007) the digestibility of nutrients in the duodenum is low and increases in the subsequent segments of the digestive tract. Thus the significantly highest villi found in the present experiment in the jejunum epithelium of piglets fed with all supplements could be the cause of the animals' higher body weight gains.

The large variation in the levels of amino acids in plasma – even between piglets which have been fed alike – was reported by Chavez and Bayley (1977). Also Keith et al. (1977) discovered significant differences in free amino acids level in pig blood depending on diet and time after eating. The levels of free amino acids found in this experiment were comparable, though mostly slightly lower than those found by Flynn et al. (2000). This difference could be due to the age of piglets tested. In our experiment they were slaughtered at the 60th day of life while in the experiment of Flynn et al. (2000) samples were obtained at 1, 3, 7, 14 and 21 days of age and the level of the majority of amino acids was reduced during this period. On the other hand Weiser et al. (1970) found that the total amount of free amino acids in pig blood grew during 6 months of an animal's life. Helland et al. (1986) in their experiment on the leucine metabolism in piglets found significantly increased postprandial protein synthesis in animals fed glucose-fortified diets. They found that glucose-fed pigs utilised three times as much leucine from the circulation as sucrose-fed pigs. There is also a possibility in this experiment, in the piglets receiving glucose, that a part of the amino acids from the bloodstream was used for protein synthesis.

Some increase of insulin content in the blood serum of piglets in all the groups receiving butyrate (i.e. compared to negative control) was in accordance with the results of Fang et al. (2014) who also obtained an apparent, though not significant ($P=0.058$) increase of this hormone level using a supplement of sodium butyrate. In this experiment the only group with a distinctly higher level of insulin than the negative control was that receiving butyrate and glutamine. It is known that glutamine enhances insulin secretion (Molfino et al., 2010). According to Maechler and Wollheim (1999) glutamate, which is derived from glutamine acts as an intracellular messenger that couples glucose metabolism to insulin secretion. The experiment of Brennan et al. (2003) also supports this hypothesis, but they suggest that the downstream metabolites of glutamate metabolism – e.g. glutathione – confer regulatory effects on insulin secretion. Thus, the significantly higher level of insulin in group (SB+GT) could be due to the synergistic effect of the butyrate and glutamine supplements.

The insulin-like growth factor 1 (IGF-1) is strictly connected with body protein turnover (Clemmons, 1992). Because the kind and amount of main protein sources (soybean meal and rapeseed press cake) were the same in all groups in the present experiment, significant differences in this hormone content in the blood must

have another reason. According to Takenaka et al. (2000) dietary essential amino acids restriction decreased IGF-1 production in rats, while Tomas et al. (1997) found a dose-related reduction in plasma amino acids after IGF-1 application in pigs. In the presented experiment IGF-1 concentration in the blood was significantly lower in the group with the lowest free amino acids level. This suggests an interdependence of glucose, IGF-1 and protein metabolism.

Conclusion

Summing up the results, it can be concluded that glutamine and glucose given together with sodium butyrate improve both the structure of the jejunum epithelium and average body weight gains. A supplement of glucose significantly lowers free amino acid content and IGF-1 level in piglet blood.

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