Effect of dietary glutamine, glucose and/or sodium butyrate on piglet growth, intestinal environment, subsequent fattener performance, and meat quality

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ABSTRACT: The effect of feed supplementing with glutamine, glucose and/or sodium butyrate was estimated on 156 piglets. The after-effect of supplements on fattener performance, carcass traits, and meat quality was examined. Piglets were allocated to 5 groups and fed standard feed mixture alone (control – C) or supplemented with 10 g of L-glutamine, or 10 g of glucose, or 3 g of sodium butyrate per kg of diet or all of these compounds (groups GT, GC, SB, and GT+GC+SB, respectively). Six piglets from each group were slaughtered at 63 days of age, their intestines were prepared and their parts measured. Digesta from ileum and caecum was taken for analysis. Its acidity and volatile fatty acids content were evaluated. Morphological structure of duodenal and ileal epithelium was estimated. After 84 days of age 20 animals from each group were fed the standard mixture. After 100 days of fattening 8 pigs from each group were slaughtered, pH of meat was measured, and samples of the *longissimus muscle* were taken for analysis. Body weight gains of piglets fed diets supplemented with SB or all supplements were higher than those of controls. All supplements given together increased total intestinal weight and length. Epithelial villi in jejunum were the highest in piglets receiving all supplements. Their height in the duodenum ranged from 296 to 347 μm and in the jejunum they were higher: 336 – 424 μm. After 100 days of fattening body weight of all experimental animals was higher than that of control. There was no significant difference in carcass and meat quality. Sodium butyrate added to the diet improved piglet performance probably due to changes in intestine development and in intestinal epithelium structure. This positive effect was enhanced to some extent by the addition of glutamine or glucose.

Keywords: piglet feeding; piglet performance; intestinal structure; volatile fatty acids; carcass traits

INTRODUCTION

Several years ago, the European Union banned the use of antibiotic growth promoters in animal feeds thus some replacements have to be found, especially for young animal feeding (Anadon 2006). Weaned piglets are delicate animals with undeveloped gastrointestinal tract and immune system (Bailey et al. 2005), which makes weaning the most dangerous moment in piglet rearing. Growing animals have a high requirement for protein and energy, thus proper development and functioning of the gastrointestinal tract are crucial. After weaning, piglets have to adapt to the new stressful conditions which is associated with reduced feed consumption, temporary malnutrition, and growth retardation (Lalles et al. 2004). During this period, synthesis of protein increases in the

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intestine while decreasing in muscles (Seve et al. 1986), which protects the intestine against protein deficiency. Because intestinal epithelium cells are the main site of nutrient absorption, provision of easily digestible nutrients should improve the structure and function of intestinal epithelium (Thacker 1999). Another way of preventing intestinal atrophy can be to supplement piglet feed with substances which could be utilized by epithelium cells (enterocytes) as energy source.

Such substances may be, among others, glutamine and butyric acid (De Lange et al. 2010). According to Burrin and Stoll (2002) the gastrointestinal tract of the neonatal pig preferentially uses glutamine and glutamate as an energy source for growth and function. Also Lescano et al. (2013) found intestinal epithelium structure improved by glutamine and glutamate. Apart from improving the intestinal epithelium structure and piglet performance found by Zou et al. (2006), recent studies indicate that glutamine serves important regulatory functions in nutrient metabolism (Wu 2010) and is a regulator of gene expression (Wang et al. 2008). Though majority of research have shown the benefits of the supplement of glutamine in piglet diets, some authors found no such improvements (Domeneghini et al. 2004; Johnson et al. 2006).

Butyric acid is produced by bacterial fermentation of dietary fibre in distal parts of the digestive tract and is the main energy source for the epithelial cells of the large intestine (Chapman et al. 2005). On the other hand, given in the feed it improves digestive and absorptive capacities of the small intestine (Claus et al. 2007). In the experiment of Le Gall et al. (2009) the pre-weaning sodium butyrate supplementation was the most efficient to stimulate body growth and feed intake after weaning, though jejunal villus height in piglets receiving sodium butyrate was lowered. Ma et al. (2012) found butyrate improving antioxidative stress ability. Improved piglet performance due to butyrate supplement was found also by Lu et al. (2008) and in the experiment of Miller and Slade (2006) the positive effect of butyrate was enhanced by the addition of zinc oxide. Worse results were received by Biagi et al. (2007). In their experiment sodium butyrate did not improve piglet performance and in the experiment of Weber and Kerr (2008) butyrate lowered piglet body weight gains and feed utilization.

Lactose is one of the main constituents of sow milk. It is degraded by intestinal lactase, such that glucose and galactose are absorbed into the epithelium enterocytes. Glucose is a good energy source for epithelial cells (Mallet et al. 1986) though given at the level of 5% of feed may result in incidents of digestive disturbances (Bayley and Carlson 1970). According to Jin et al. (1998) lactose and sucrose supported a better growth than glucose (all given at the level of 5% of feed) during the first and second week postweaning but in the third week postweaning no difference was found. In the experiment of Vente-Spreeuwenberg et al. (2003) glucose and lactose did not affect growth performance and villus architecture. According to Posho et al. (1994) the capacity of enterocytes to utilize glucose increased threefold between birth and 2 days of age. Fleming et al. (1991) found that cells taken from the jejunum utilized exogenous substrates in decreasing order glutamine > glucose > butyrate.

The aim of this experiment was to investigate the effect of diets supplemented with glutamine (amino acid), glucose (monosaccharide) and/or sodium butyrate (short chain fatty acid) on piglet performance, acidity, and volatile fatty acids of intestinal content and intestinal villi height. The effects of these substances on fattener performance during the later period of the experiment, carcass traits, and meat quality were also estimated.

MATERIAL AND METHODS

All procedures used in this experiment were approved by the Second Local Krakow Ethics Committee.

Animals and diets. The experiment was performed on 156 piglets (15 litters) originating from Polish Landrace sows and a Polish Landrace boar. Piglets received experimental mixtures *ad libitum* from 7 days of age to weaning (35 days of age), whereas restricted feeding was used from weaning to the end of the experiment (84 days of age). The amount of feed was increased every 7 days by 200 g. Piglets were allocated to 5 groups with 3 litters in each group, kept in group pens (each litter in a separate pen), and fed a standard feed mixture (control – C) or the same mixture supplemented with 10 g of L-glutamine, or 10 g of glucose, or 3 g of sodium butyrate per kg of diet or all of these compounds (groups GT, GC, SB, and GT+GC+SB, respectively). All supplements were supplied by Sigma Aldrich (St. Louis, USA). The ingredients and composition of feed mixtures are given in Table 1.

Animals were weighed at 1, 35 (weaning), 56, and 84 days of age. Feed consumption was measured

Table 1. Composition of diets for piglets

	C	Diet + GT	Diet + GC	Diet + SB	Diet + GT+GC+SB
Component (g/kg)					
Barley, ground	200	200	200	200	200
Wheat, ground	353	338	338	350	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	45	45	40	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
Nutritional value and chemical composition					
Metabolizable energy (MJ)	12.9	12.8	12.8	12.9	12.7
DM (g)	884	885	874	884	878
Crude protein (g of DM)	173	170	174	173	172
Crude fat (g of DM)	35	36	36	35	38
Crude ash (g of DM)	56	58	52	56	58
Crude fibre (g of DM)	21	22	24	21	22

C = control diet, GT = glutamine, GC = glucose, SB = sodium butyrate, DM = dry matter

[1]premix (DSM Nutritional Products Ltd., Mszczonów, Poland) composition: vitamin: A 2 700 000 IU, D_3 400 000 IU, E 8.0 g, K_3 0.5 g, B_1 0.5 g, B_2 0.8 g, B_6 0.8 g, B_{12} 0.008 g, pantothenic acid 2.8 g, choline chloride 70 g, folic acid 0.2 g, nicotinic acid 5.0 g, magnesium 10 g, manganese 12 g, iodine 0.1 g, zinc 30 g, iron 20 g, copper 32 g, cobalt 0.06 g, selenium 0.04 g, complete limestone to 1000 g

daily for each pen and calculated for individual piglets. According to the results, mean body weight gains and feed utilization were calculated.

Six piglets from each group (i.e. two piglets from each litter) were slaughtered at 63 days of age, the intestines were prepared, their content removed, and their individual parts were weighed and measured. Digesta from the small intestine (jejunum) and caecum was taken for analysis.

The remaining piglets were raised to 84 days of age and then 20 animals from each group were randomly chosen for further fattening. They were fed standard feed mixtures (Table 2): grower (to 60 kg of body weight) and finisher (from 60 kg to the end of the experiment). Fattening lasted 100 days, after which 8 animals from each group were slaughtered. Loin eye area, backfat thickness in point C, backfat thickness over the shoulder, loin, rump I, rump II, and over the rump III were measured. Samples of the *longissimus muscle*, obtained from the area of the last thoracic and the first lumbar vertebrae were collected for analysis.

Histological analysis. Samples from the piglet small intestine (duodenum and jejunum) were spread on polystyrene plates and fixed in 10% buffered formalin. The intestinal wall was precisely cut and four slices 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 Zeiss Axioskop light microscope (Zeiss GmbH, Jena, Germany) and by a CDD ZVS-47DE camera (Optronics Inc., Goleta, USA) connected by RGB line with a graphic card GraBIT PCI (Soft Imaging System GmbH, Münster, Germany) installed in a standard PC.

Chemical analyses. The composition of feeds was analyzed according to AOAC (2005) methods. Acidity of the digesta of stomach, ileum, caecum,

Table 2. Composition of diets for fatteners

	Grower	Finisher
Component (g/kg)		
Barley, ground	473	518.5
Wheat, ground	200	200
Soybean meal	150	60
Rapeseed press cake	100	150
Wheat bran	50	50
Salt	2.5	2.5
Calcium carbonate	10	10
Fodder phosphate	8	4
Premix PT1[1]/PT2[2]	5	5
L-Lysine	1.5	–
Nutritional value and chemical composition		
Metabolizable energy (MJ)	12.3	12.3
DM (g)	872	867
Crude protein (g of DM)	171	158
Crude fat (g of DM)	46	47
Crude fibre (g of DM)	36	36
Crude ash (g of DM)	51	58
L-Lysine (g of DM)	9.95	8.15
Methionine + cysteine (g of DM)	6.13	6.04
Threonine (g of DM)	6.48	6.16
Tryptophan (g of DM)	2.69	2.81
Ca (g of DM)	8.18	7.02
P (g of DM)	5.33	4.22

DM = dry matter

[1]PT-1 (grower; DSM Nutritional Products Ltd., Mszczonów, Poland): vitamins: A 1 600 000 IE, D_3 200 000 IE, E 6.0 g, K_3 0.3 g, B_1 0.2 g, B_2 0.6 g, B_6 0.3 g, B_{12} 0.002 g, calcium pantothenate 2.0 g, choline 40 g, folic acid 0.04 g, nicotinic acid 3.0 g, magnesium 8.0 g, manganese 10.0 g, iodine 0.06 g, zinc 14.0 g, iron 20.0 g, copper 4.0 g, cobalt 0.04 g, selenium 0.04 g, complete limestone to 1000 g

[2]PT-2 (finisher; DSM Nutritional Products Ltd., Mszczonów, Poland): vitamins: A 1 600 000 IE, D_3 200 000 IE, E 4.0 g, K_3 0.3 g, B_2 0.6 g, B_{12} 0.002 g, calcium pantothenate 1.6 g, choline 40 g, folic acid 2.0 g, magnesium 8.0 g, manganese 10.0 g, iodine 0.06 g, zinc 14.0 g, iron 10.0 g, copper 4.0 g, cobalt 0.04 g, selenium 0.04 g, complete limestone to 1000 g

and colon contents was measured with a CP-411 pH meter (Elmetron, Zabrze, Poland) equipped with a Metron 12-01 electrode (Metron, Torun, Poland). Volatile fatty acids (VFA) in the jejunum and caecum were separated on a 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 temperature program 90–200°C, using Varian 3400 gas chromatograph (Varian Associates Inc., Walnut Creek, USA) equipped with a Varian 8200 CX autosampler (200°C), FID detector (260°C), and Star Chromatography Workstation software (Version 4.5, 1996).

Analysis of meat. Meat acidity was measured with a pH-meter equipped with a Metron OSH 12-00 electrode 45 min after slaughter. Meat colour was estimated with a colourimeter CR-310 (Minolta, Osaka, Japan). Water holding capacity of meat was measured according to Grau and Hamm (1953).

Statistical analysis. Statistical analysis of treatment effects was performed using the 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 software package (Version 5.1, 1996).

RESULTS

All supplements had a beneficial effect on the health of piglets. There was no significant difference in piglet body weight gain before weaning. In the first period after weaning (i.e. at 35–56 days of age), experimental piglets grew faster than the control ones, but these differences were significant ($P < 0.01$) only for the group receiving all supplements. However, there was no significant difference between experimental groups (Table 3). During the whole rearing period (i.e. 1–84 days of age), piglets receiving glucose ($P < 0.05$) and those fed sodium butyrate and all supplements grew faster ($P < 0.01$) than control ones. At the end of the experiment the body weight of piglets from all groups receiving supplements (except glutamine) was significantly higher than that of controls. Piglets fed glutamine were also heavier than controls, but the difference was not significant. There was no significant difference in feed utilization.

Sodium butyrate numerically increased piglet total intestinal mass (Table 4) and in the case of jejunum this difference was significant ($P < 0.05$). Generally, the mass of each part of intestines of piglets receiving all supplements was higher than that in other groups. Similarly, the total intestine was the longest in the GT+GC+SB group, but this difference was significant ($P < 0.05$) only in comparison to the control group.

The villi height differed in the epithelium of various parts of the small intestine (Table 5). In duodenum the highest villi were found in piglets

Table 3. Results of piglets rearing

	C	Diet + GT	Diet + GC	Diet + SB	Diet + GT+GC+SB	SEM	*P*-value
Born piglets in treatment (*n*)	32	30	31	32	31	–	–
Dead and culled piglets (%)	12.5	6.6	3.2	6.2	3.2	–	–
Average body weight (kg)							
Day 1 of age	1.75	1.77	1.75	1.77	1.75	0.03	0.99
Day 35 of age	7.12	7.28	7.92	7.56	7.98	0.14	0.18
Day 56 of age	12.10^{a}	13.12^{ab}	13.64^{ab}	13.71^{ab}	14.96^{b}	0.28	0.02
Day 84 of age	25.68^{Aa}	27.69^{ABab}	29.11^{ABb}	30.33^{Bb}	32.01^{Cc}	0.53	0.002
Average BWG (g)							
Days 1–35 of age	158	162	182	170	183	3.93	0.15
Days 35–56 of age	237^{a}	278^{ab}	272^{ab}	293^{ab}	332^{b}	10.1	0.05
Days 56–84 of age	481^{Aa}	519^{ABab}	547^{ABab}	593^{Bbc}	608^{Bc}	13.3	0.01
Days 35–84 of age	377^{Aa}	416^{ABab}	430^{ABabc}	464^{Bbc}	490^{Bc}	9.95	0.003
Days 1–84 of age	287^{Aa}	312^{ABab}	328^{ABCbc}	344^{BCbc}	364^{Cc}	6.33	0.001
Feed intake (g)							
Days 1–35 of age	14	12	12	14	12	1.80	0.09
Days 35–56 of age	263	234	234	249	256	3.01	0.45
Days 56–84 of age	922	900	912	920	932	1.63	0.45
Days 35–84 of age	625	616	618	632	647	1.51	0.45
Days 1–84 of age	375	369	369	372	390	1.05	0.32
Average feed utilazation per 1 kg of BWG (kg/kg)							
Days 1–35 of age	0.09	0.08	0.07	0.08	0.06	0.01	0.26
Days 35–56 of age	1.12	0.84	0.86	0.85	0.77	0.77	0.73
Days 56–84 of age	1.90	1.73	1.65	1.55	1.53	0.06	0.34
Days 35–84 of age	1.65	1.48	1.43	1.36	1.32	0.06	0.47
Days 1–84 of age	1.30	1.18	1.12	1.08	1.07	0.04	0.50

C = control diet, GT = glutamine, GC = glucose, SB = sodium butyrate, BWG = body weight gain

$^{a–c}$mean values in the row with different letters differed at $P < 0.05$

$^{A–C}$mean values in the row with different letters differed at $P < 0.01$

receiving glutamine. In piglets receiving all supplements villi were lower than in the case of glutamine alone. In jejunum epithelium of piglets receiving glutamine villi were lower than in other groups and in relation to the group receiving all supplements this difference was significant ($P < 0.01$). There were also significant differences ($P < 0.05$) in crypt depth in duodenum and villi width in jejunum.

Significant ($P < 0.05$) differences were detected in acidity of digesta in proximal parts of the digestive tract (i.e. duodenum and jejunum) but not in its further parts (i.e. ileum and large intestine) (Table 6). There was no apparent dependence acidity from type of feed supplements.

The content of volatile fatty acids in caecum digesta was much higher than in jejunum (Table 7). In both these parts of the intestines acetic acid was the most abundant. In jejunum the content of butyric and isobutyric acids was higher ($P < 0.05$ and $P < 0.01$, respectively) in control group than in other groups. Total content of VFA in jejunum was lowered by supplements of glutamine ($P < 0.05$) and by all supplements ($P < 0.01$), which resulted from the lower level of acetic acid. There was no significant difference in total content of VFA in caecum.

At the end of the second part of the experiment (i.e. after 100 days of fattening) body weight of all test animals was higher than that of control ones but this difference was significant ($P < 0.01$) only in the case of the last two groups, i.e. those receiving butyric acid (Table 8). There was no significant difference in cold dressing, loin eye area, lightness of meat, and its water holding capacity. Fatteners earlier receiving glucose (alone or together with other supplements)

Table 4. Mass and length of piglet intestines

	C	Diet + GT	Diet + GC	Diet + SB	Diet + GT+GC+SB	SEM	*P*-value
Age at slaugther (days)	62.0	62.7	60.3	61.7	62.0	0.49	0.60
Live weight (kg)	16.3^{a}	17.5^{ab}	17.9^{ab}	17.2^{a}	19.5^{b}	0.35	0.04
Carcass weight (kg)	11.6	12.2	12.4	12.1	13.7	0.34	0.22
Intestinal mass (g)							
Duodeum	24^{a}	34^{ab}	28^{ab}	32^{ab}	38^{b}	1.45	0.01
Jejunum	747^{ABa}	773^{ABa}	706^{Aa}	926^{Bb}	928^{Bb}	27.1	0.01
Ileum	29	28	26	31	30	1.22	0.75
Caecum	42	49	42	48	53	2.14	0.41
Colon	334^{ab}	285^{a}	303^{a}	306^{a}	380^{b}	10.7	0.04
Total	1176^{ab}	1168^{ab}	1104^{a}	1343^{b}	1428^{bc}	35.6	0.01
Length of intestines (cm)							
Duodeum	24	22	21	21	23	0.79	0.42
Jejunum	1066^{a}	1115^{ab}	1074^{a}	1138^{ab}	1210^{b}	16.2	0.02
Ileum	18.5^{a}	19.0^{a}	19.0^{a}	24.7^{b}	20.7^{ab}	0.77	0.03
Caecum	11.5	12.7	11.8	13.8	13.1	0.46	0.52
Colon	246	224	246	232	252	5.22	0.41
Total	1366^{a}	1392^{ab}	1371^{ab}	1428^{ab}	1520^{b}	17.2	0.02

C = control diet, GT = glutamine, GC = glucose, SB = sodium butyrate
^{a,b}mean values in the row with different letters differed at $P < 0.05$
^{A,B}mean values in the row with different letters differed at $P < 0.01$

Table 5. Epithelium structure of piglet small intestine mucosa ($n = 6$)

	C	Diet + GT	Diet + GC	Diet + SB	Diet + GT+GC+SB	SEM	*P*-value
Duodenum							
Villus height (μm)	299	347	296	302	332	8.45	0.31
Villus width (μm)	160	150	157	151	156	2.37	0.73
Crypt depth (μm)	353^{ab}	340^{a}	382^{b}	389^{b}	355^{ab}	5.66	0.03
Villus height/crypt depth	0.85^{ab}	1.02^{b}	0.79^{a}	0.78^{a}	0.90^{ab}	0.03	0.02
Jejunum							
Villus height (μm)	394^{ABb}	336^{Aa}	367^{ABab}	398^{ABb}	424^{Bc}	9.06	0.02
Villus width (μm)	139^{ab}	134^{a}	146^{ab}	148^{b}	133^{a}	2.09	0.04
Crypt depth (μm)	306	303	269	291	297	5.25	0.21
Villus height/crypt depth	1.30^{ab}	1.11^{a}	1.37^{b}	1.38^{b}	1.45^{b}	0.03	0.03

C = control diet, GT = glutamine, GC = glucose, SB = sodium butyrate
^{a,b}mean values in the row with different letters differed at $P < 0.05$
^{A,B}mean values in the row with different letters differed at $P < 0.01$

had significantly ($P < 0.01$) thicker backfat (average of 5 measurements) than control animals.

DISCUSSION

The results show that supplements used in this experiment can, to a certain extent, be used as growth promoters. When sodium butyrate was fed, piglets grew faster than control ones. This is consistent with results of Lu et al. (2008), who found that piglet body weight gains grew proportionally to the butyrate doses. In the quoted experiment a relatively low dose of butyrate was used (0.5–1.0 g per kg of feed), which is why we decided to use its higher amount

Table 6. Acidity of chyme in different parts of digestive tract of piglets (n = 6)

	C	Diet + GT	Diet + GC	Diet + SB	Diet + GT+GC+SB	SEM	P-value
Stomach	3.59	2.99	2.88	3.51	3.60	0.14	0.30
Duodenum	6.43^{b}	5.79^{a}	6.47^{b}	5.93^{ab}	6.24^{ab}	0.09	0.03
Jejunum	6.77^{b}	6.31^{a}	6.28^{a}	6.54^{ab}	6.36^{a}	0.06	0.047
Ileum	6.54	6.18	6.55	6.21	6.54	0.08	0.32
Caecum	5.58	5.57	5.53	5.67	5.81	0.05	0.42
Colon	6.19	6.30	6.04	6.26	6.34	0.07	0.63

C = control diet, GT = glutamine, GC = glucose, SB = sodium butyrate

^{a,b}mean values in the row with different letters differed at $P < 0.05$

according to Biagi et al. (2007) and Mazzoni et al. (2008). Glutamine had no significant effect although an improvement of piglet performance after supplementing the soybean diet with glutamine was found by Zou et al. (2006). In their experiment, piglets receiving glutamine were also healthier: cases of diarrhoea were rare and less serious. Data in the literature concerning glucose are not so optimistic. Jin et al. (1998) reported that glucose did not give better results than other carbohydrate sources, such as lactose, sucrose or dried whey.

There are two possible explanations for the beneficial effect of the butyrate supplement. The first one is its ability to improve structure and function of intestine, especially the epithelium (Galfi and Bokori 1990; Miller and Slade 2006).

Perhaps the longer digestive tract, especially its proximal parts, enables better availability of nutrients. It is known that small intestine is the site where most nutrients are digested and absorbed (Rivest et al. 2000) and colon and caecum are less important (Rudolph et al. 1983). Thus sodium butyrate affecting intestine development may also improve nutrient absorption, especially in jejunum (Kotunia et al. 2004; Molino et al. 2012), and in consequence piglet performance.

Intestinal villi are the main site of nutrient absorption (Ray et al. 2002) and their proper devel-

Table 7. Short-chain fatty acids content of chyme in jejunum and caecum of piglets (μmol/g of wet weight) (n = 6)

	C	Diet + GT	Diet + GC	Diet + SB	Diet + GT+GC+SB	SEM	P-value
Jejunum							
Acetic	13.9^{ABbc}	8.5^{Aa}	15.6^{Bc}	10.8^{ABab}	6.8^{Aa}	0.93	0.002
Propionic	0.22^{b}	0.06^{a}	0.07^{a}	0.11^{ab}	0.06^{a}	0.02	0.03
Isobutyric	0.32^{B}	0.05^{A}	0.04^{A}	0.10^{A}	0.01^{A}	0.03	0.0005
Butyric	0.20^{Bb}	0.03^{Aa}	0.05^{Aa}	0.04^{Aa}	0.09^{ABa}	0.02	0.006
Isovaleric	0.01^{Aa}	0.03^{ABab}	0.03^{ABab}	0.12^{Bc}	0.09^{ABbc}	0.01	0.01
Valeric	0.02	0.09	0.08	0.12	0.02	0.02	0.52
Total acids	14.6^{BCbc}	8.8^{ABa}	15.8^{Cc}	11.3^{ABCab}	7.0^{Aa}	0.95	0.002
Caecum							
Acetic	79.6	83.5	77.8	73.5	81.9	2.52	0.78
Propionic	43.8^{b}	38.9^{ab}	33.5^{a}	32.7^{a}	38.4^{ab}	1.30	0.02
Isobutyric	0.93^{A}	1.23^{A}	1.23^{A}	1.29^{A}	2.66^{B}	0.15	0.00
Butyric	15.5	14.9	15.4	13.6	18.8	0.96	0.57
Isovaleric	0.53^{ab}	0.81^{b}	0.47^{a}	0.55^{ab}	0.59^{ab}	0.04	0.02
Valeric	2.58	2.59	2.82	2.61	3.29	0.27	0.92
Total acids	142.9	142.1	131.2	124.2	145.5	4.08	0.44

C = control diet, GT = glutamine, GC = glucose, SB = sodium butyrate

$^{a–c}$mean values in the row with different letters differed at $P < 0.05$

$^{A–C}$mean values in the row with different letters differed at $P < 0.01$

Table 8. Fattening results, carcass traits and meat quality after 100 days of pig fattening (n = 20; starting age= 85 days)

	C	Diet + GT	Diet + GC	Diet + SB	Diet + GT+GC+SB	SEM	P-value
Starting BW (kg)	26.5^{a}	27.2^{a}	28.6^{ab}	30.5^{ab}	31.7^{b}	0.51	0.049
BW on day 184 of age (kg)	94.2^{Aa}	95.7^{ABa}	98.8^{ABab}	103.1^{BCbc}	106.7^{Cc}	0.79	0.0003
ADG on days 85–184 of age (g/day)	677	686	702	725	749	7.81	0.156
ADG on days 1–184 of age (g/day)	504^{Aa}	513^{ABab}	530^{ABab}	553^{BCbc}	574^{Cc}	4.34	0.002
Cold dressing (%)	78.1	77.2	79.6	75.7	77.4	0.58	0.42
Backfat thickness (cm)							
Shoulder	2.51	2.56	2.80	2.62	3.00	0.07	0.18
Loin eye	1.16^{Aa}	1.63^{Bbc}	1.65^{ABbc}	1.33^{ABab}	1.78^{Bc}	0.06	0.002
Rump I	1.06^{Aa}	1.18^{ABa}	1.62^{Bb}	1.18^{ABa}	1.68^{Bb}	0.07	0.005
Rump II	0.66^{a}	0.74^{ab}	1.10^{b}	1.12^{b}	1.06^{b}	0.06	0.03
Rump III	1.05^{Aa}	1.13^{Aa}	1.92^{Bc}	1.33^{ABab}	1.75^{ABbc}	0.10	0.006
Backfat thickness, average of 5 measurements (cm)	1.29^{Aa}	1.45^{ABa}	1.82^{Bb}	1.52^{ABab}	1.85^{Bb}	0.06	0.006
Backfat thickness, average in point C (cm)	0.73	0.81	1.07	0.90	1.05	0.06	0.28
Loin eye area (cm^2)	50.1	53.1	53.3	50.6	51.4	1.12	0.85
pH after 24 h cooling	5.43^{ab}	5.28^{a}	5.41^{ab}	5.67^{b}	5.49^{ab}	0.04	0.03
Water holding capacity, loose water (%)	21.2	21.8	23.0	21.5	23.0	0.53	0.74
Meat colour							
Lightness L^*	54.1	52.2	52.7	52.7	53.3	0.34	0.45
Redness a^*	17.2	16.7	16.9	16.8	16.9	0.12	0.74
Yellowness b^*	3.2^{ABab}	4.0^{ABbc}	4.3^{Bc}	4.2^{Bc}	2.8^{Aa}	0.16	0.005

C = control diet, GT = glutamine, GC = glucose, SB = sodium butyrate, ADG = average daily gain, BW = body weight

$^{a–c}$mean values in the row with different letters differed at $P < 0.05$

$^{A–C}$mean values in the row with different letters differed at $P < 0.01$

opment could be the reason for better nutrient utilization (Mekbungwan et al. 2002) resulting in better growth of piglets (Hanczakowska et al. 2011). This mechanism was found earlier for glutamine (Wu et al. 1996) and for butyrate (Lu et al. 2008). Glucose is a good energy source for epithelial cells (Mallet et al. 1986), but it does not affect villus architecture or growth performance (Vente-Spreeuwenberg et al. 2003). Digestibility of most nutrients increases along the gastrointestinal tract from duodenum onwards (Wilfart et al. 2007). Thus the highest villi found in this experiment in the jejunum epithelium of piglets fed sodium butyrate could be another reason for higher body weight gains in animals.

The second way to improve piglet performance is changing intestinal microflora and reducing the population of pathogenic bacteria by butyrate (Castillo et al. 2006). No microbiological analyses were performed in this experiment but better growth of piglets from experimental groups could be due to changes in their intestinal microflora. This argument can be confirmed by significant differences in volatile fatty acids content in jejunum.

It is known that the amount of volatile fatty acids in the digestive tract increases from its proximal to distant parts. Also Nyachoti et al. (2006) found that acetic acid concentration increased from 0.907 mmol/l in duodenum to 70.29 mmol/l in ileum in early weaned piglets. Significantly lower content of acetic acid in jejunum digesta of piglets of both groups receiving glutamine could be due to antibacterial activity of this compound. Acetic acid is the product of microbial fermentation of glucose also in pig small intestine (Nafikov and Beitz 2007). Glutamine can change the metabolism and the number of bacteria in piglet intestine (Gianotti et al. 2012; Dai et al. 2013). Also according to Wang et al. (2008)

glutamine enhances expression of the gene improving antibacterial activity in the piglet small intestine. Bacteria were not estimated in this experiment but it can be assumed that their lower activity resulted in lower level of acetic acid in piglet intestine. Lower amount of this acid in piglet blood after glutamine addition was found by Xiao et al. (2012).

There were no significant differences in acetic acid content in caecum of all groups and in propionic acid between experimental groups. This could be due to the fact that all supplements being readily soluble were probably absorbed in small intestine. High content of isobutyric acid in caecum digesta of piglets receiving all supplements may indicate degradation of protein because this acid is produced from the amino acid valine by bacterial deamination (Arkowitz et al. 1994). This result is hard to explain. Biagi et al. (2007) found a higher concentration of isobutyric acids in caecum (but not in jejunum or ileum) of piglets receiving dietary butyric acid. In this experiment neither butyric acid alone nor any other supplement raised isobutyric acid concentration. Unfortunately we cannot explain this phenomenon on the basis of the results obtained.

In the second part of the experiment, the initial weight of animals was not levelled due to the different body weight gains of piglets at the end of the first part of the experiment. These differences remained until the end of the experiment also due to higher body weight gains of pigs from experimental groups. It is known that growth retardation at the beginning of fattening is later compensated (Fabian et al. 2002), but it is usually found in the case of undernutrition which has not occurred in this experiment. Feeding pigs during 100 days with the same feed was probably the reason for very similar results of carcass estimation and meat quality.

The greater backfat thickness of pigs fed glucose could be due to more developed fatty tissue in piglets receiving this carbohydrate, which may persist also in older animals (Shankar et al. 2010).

CONCLUSION

In conclusion, sodium butyrate added to the diet for piglets improved their performance probably due to changes in the intestine development and in the intestinal epithelium structure. This positive effect was enhanced to some extent by the addition of glutamine or glucose. These differences persisted during later fattening despite feeding the same diets.

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