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Advantages of Sodium Butyrate in Weaned Piglet Diet

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ABSTRACT

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The aim was to examine production performance, carcass characteristics, and intestinal histomorphology of weaned piglets (28-to 54-day-old) fed diet with 3 or 5 g of sodium butyrate per kg of diet (group II and III). Groups II and III had higher final live weight and total weight gain. The feed to gain ratio was the best in group I. The highest carcass yield was of group III. Significant differences ($P < 0.05$) were observed in *Escherischia coli* counts in small intestine between control (I) and experimental groups (II and III), and in cecum between control (I) and experimental group II. There were significant differences ($P < 0.05$) between all groups for the intestine length, intestine weight, and both height and width of the ileal villus. The highest villus height/crypt depth ratio of jejunum occurred in group II piglets, while the highest villus height/crypt depth ratio of ileum was in piglets from group III. The significant correlations were determined between amount of sodium butyrate and final live weight, intestinal length, intestinal weight, jejunal and ileal crypt depth, and ileal villus widths.

Keywords: Carcass characteristics, Intestinal histomorphology, Intestine length, Intestine weight, Piglet performance

INTRODUCTION

Weaning is a critical period in the pig life cycle, caused by the fact that in a short period, piglets are subjected to many changes (social, nutritional and environmental). This period usually causes decreasing feed consumption with consequent atrophy of intestinal villus, reduction of digestive enzyme activity, growth rate reduction and increased inflammatory processes in the intestine, which lead to diarrhoea as the final result (Lallès *et al*., 2004; Roca *et al*., 2014; Raduloviæ *et al*., 2015; Šefer *et al*., 2015). During the last few decades, a common solution to this problem was the use of antibiotics as feed additives. After the prohibition of the use of antibiotics for growth stimulation in the European Union, from 2006, producers

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developed alternative solutions to prevent diarrhoea and growth check in this critical period. One alternative is organic acids, due to their positive effects on growth performance of all pig categories, including weaned piglets (Galfiand Bokori, 1990; Witte *et al.*, 2000; Piva *et al.*, 2002a; Mazzoni *et al.*, 2008). Short-chain fatty acids (SCFA), which are produced in the large intestine of mammals during microbial fermentation, are an important source of energy for animals (Cortyl, 2014). Large intestinal cells can use the produced SCFA, especially butyric acid, as a metabolism substrate (Jozefiak *et al.*, 2004). Also, it has been shown that butyrate, produced during bacterial fermentation, inhibits apoptosis in the mucosa, induces water and sodium absorption, intestinal cell proliferation and intestinal blood flow, and stimulates synthesis of gastrointestinal hormones (Wächtershäuser and Stein, 2000; Mentschel and Claus, 2003; Biagi *et al.*, 2007; Guilloteau *et al.*, 2010). Beside butyric acid produced during microbial fermentation, a subject of numerous studies has been the effect of butyric acid, ingested via feed, on the pig intestines' digestive and absorptive capacities. Instead of butyric acid, sodium butyrate is usually used as an additive, due to its powdery consistency, meaning it can easily be mixed in feed and used in lower amounts (1.0 g/kg) as compared to citric, acetic or propionic acids (4-20 g/kg). Kotunia *et al.* (2004) found that sodium butyrate has a positive effect on the development of small intestine in neonatal piglets fed artificial feed, while Claus *et al.* (2007) suggested that calcium butyrate improves digestive and absorptive capacity of the small intestine in piglets. Results of another study also showed that SCFA infusion, including sodium butyrate, in the ileum via silicone tubes, increases gastric emptying and intestinal motility in comparison with a similar solution of infused salts (Malbert *et al.*, 1994). Considering addition of sodium butyrate (more than 500 mg/kg of sodium butyrate) in pig diet changes intestine microflora composition, increases feed intake and production of serum cytokines (TNF-α, IL-6) and improves gastrointestinal health in weaned piglets, this leads to increase of the small intestine villus height and width, as well as the villus height/crypt depth ratio (Inan *et al.*, 2000; Lu *et al.*, 2008; Guilloteau *et al.*, 2010). The aim of the present study was to examine the use of sodium butyrate in weaned piglet diet and its influence on piglet production performance, carcass characteristics, and intestinal morphology and histology.

MATERIALS AND METHODS

Animal ethics

The experimental protocol was approved by the Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management and the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade.

Animal, housing, and trial

The study was conducted on 48 weaned piglets (50% male and 50% female), of the same origin, Yorkshire × Landrace crossbreed. Piglets were farrowed within a day, fed on sows' milk and from days 7 to 10 of life, started to feed on pre-starter

Table 1. Ingredients of the pig diets (per kg of the mixture)

Ingredients (%)	Starter diet			Pre-starter
	I	II	III	
Corn	45.56	45.45	45.37	42.02
Barley	18.0	18.0	18.0	15.82
Soybean meal	11.31	11.33	11.34	16.1
Soybean grits	4.5	4.5	4.5	5.3
AK 530 soy isolates	9.0	9.0	9.0	9.0
Potato protein	2.5	2.5	2.5	3.0
Whey 72%	2.5	2.5	2.5	2.5
Monocalcium phosphate	1.43	1.38	1.37	1.28
Cattle chalk	0.91	0.92	0.93	0.90
Cattle salt	0.50	0.33	0.20	0.5
Premix 1.5%*	1.5	1.5	1.5	1.5
Lysolecithin	0.05	0.05	0.05	0.05
Soybean oil	1.74	1.74	1.74	1.53
Mycotoxin adsorbent	0.2	0.2	0.2	0.2
Zinc oxide	0.3	0.3	0.3	0.3
Sodium butyrate	0.0	0.3	0.5	–

*Premix composition starter (per kg): Lysine 202.94 g; Methionine 72.65 g; Threonine 65.44 g; Tryptophan 20.00 g; St. Dig. Lysine 202.90 g; St. Dig. Methionine 72.65 g; St. Dig. Meth & Cyst 72.65 g; St. Dig. Threonine 65.44 g; St. Dig. Tryptophan 20.00 g; Calcium 137.16 g; Vit.A 800100 i.e; Vit.D 380030 i.e; Vit.E 10952.56 mg; Alpha-tocopherol 9966.80 mg; Vit.K3 306.83 mg; Vit.B1 153.53 mg; Vit.B2 306.83 mg; VitB6 233.33 mg; Vit.B12 1.54 mg; D-pantothenic acid 780.03 mg; Niacin 1533.47 mg; Choline chloride 16666.77 mg; Biotin 15.47 mg; Mn 3133.43 mg; Fe 15066.80 mg; Cu 11000.03 mg; Zn 8000.07 mg; I 15.47 mg; Cobalt-II-carbonate 33.37 mg; Se 26.83 mg; Phytase 33333.40FYT; Fungal xylanase (3.2.1.8) 13333.40 FXU

*Premix composition of pre-starter (per kg): Lysine 224.55 g; Methionine 83.85 g; Threonine 67.74 g; Tryptophan 22.00 g; St. Dig. Lysine 224.50 g; St. Dig. Methionine 83.85 g; St. Dig. Meth & Cyst 83.85 g; St. Dig. Threonine 67.74 g; St. Dig. Tryptophan 22.00 g; Calcium 139.18 g; Vit.A 800100 i.e; Vit.D 380030 i.e; Vit.E 10952.56 mg; Alpha-tocopherol 9966.80 mg; Vit.K3 306.83 mg; Vit.B1 153.53 mg; Vit.B2 306.83 mg; VitB6 233.33 mg; Vit.B12 1.54 mg; D-pantothenic acid 780.03 mg; Niacin 1533.47 mg; Choline chloride 16666.77 mg; Biotin 15.47 mg; Mn 3133.43 mg; Fe 15066.80 mg; Cu 11000.03 mg; Zn 8000.07 mg; I 15.47 mg; Cobalt-II-carbonate 33.37 mg; Se 26.83 mg; Phytase 33333.40FYT; Fungal xylanase (3.2.1.8) 13333.40 FXU

(Table 1). Before weaning, piglets were housed with sows in the same facility, with the same preconditions and the microclimate before entering the trial. All piglets were weaned on the 28th day.Weaned piglets were randomly allocated and housed in one of three weaning pens (dimensions 2×2.3 m) within same weaning facility, on concrete slatted floors, in groups of 16 animals per group, 4 piglets per pen. Weaned piglets were provided with *ad libitum* feed and water. Ventilation and light mode was regulated automatically, ventilation based on the temperature and humidity measured

in the premises, while light intensity was 100 lux and an illumination period of 16 hours per day. The weaning facility also had natural day-light. The trial was conducted over a 26-day period (when piglets were from 28 to 54 days old), during which animals were exposed to their respective experimental diets.

Experimental diets

From the start (28-day-old piglets) until the end (54-day-old piglets) of the trial, each of the three groups of animals (16 animals per group) was fed one of three experimental diets. These comprised the same standard mixture for weaned piglets (starter diet), formulated to meet the maintenance and growth requirements of animals used in this study, but which differed in the addition of sodium butyrate. The diet for the experimental group I had no added sodium butyrate, the diet for experimental group IIcontained added 3 g of sodium butyrate per kg of the mixture, while the diet for experimental group III contained added 5 g of sodium butyrate per kg of the mixture (Table 1).

Chemical composition of the animal diets

Chemical analyses to determine crude protein, moisture, cellulose, fat, ash, calcium and phosphorus of the feed were conducted according to AOAC methods (AOAC, 1990).

Piglet production performance and carcass quality analyses

At the beginning and end of the study, live weights of weaned piglets were measured on technical scale with an accuracy of ± 10 g. The amount of feed ingested was recorded throughout the study. Feed consumption was measured daily for each pen and calculated for individual piglets. Measurements of feed was carried out on technical scale with and accuracy of ± 1 g. Based on the obtained data, the total and daily weight gain and feed to gain ratio was calculated. At the end of the study, animals were transported to the slaughterhouse, individually weighed, electrically stunned and immediately slaughtered. Subsequently, animals were processed using standard industrial techniques and hot carcass weight was recorded (carcass included heart, lungs, liver and kidneys). Carcass yield was calculated based on the live weight at slaughter and hot carcass weight and shown as a percentage.

Intestinal microflora

After slaughter, samples for bacteriological examination were taken directly from the intestine using a sterile syringe and 0.2 mL was transferred to 1.8 mL of reduced thioglycolate broth, and to saline solution. Further, serial dilutions were made down to 10^{-7}. Duplicate plates of selective media were inoculated with 0.5 mL of each dilution to determine the bacterial species defined by standard laboratory methods. Total viable count was determined using Plate count agar (PCA), total *Enterococcus* species and strains of *Escherichiacoli* were counted on UTI agar (Urogenital Tract Infections agar, HiMedia), while lactic acid bacteria counts were

determined on selective MRS agar supplemented with 20 mg/mL vancomycin (Sigma Aldrich) and 2 mg/mL cefotaxime (Sigma Aldrich). Microaerophilic atmospheres, used for the growth of lactobacilli on agar plates, were obtained using the GasPak CO_2 System (Becton Dickinson). After inoculation, UTI agar was incubated at 37°C for 24 to 48 hours, PCA at 30°C for 72 hours and MRS agar at 30°C for 3 to 5 days. Based on the appearance of colonies and cultural characteristics, subcultivation was performed on suitable substrates in order to obtain pure bacterial cultures. After cultivation, morphological and biochemical characterization of the isolated bacteria was conducted.

Morphological and histological analyses

After being emptied, the length and weight of individual intestinal segments (duodenum, jejunum, ileum, cecum, and colon) and the overall length and weight of the intestines were measured. The intestine length was measured using a steel ribbon with the accuracy of ± 0.5 cm, and intestines were weighed on technical scales with an accuracy of ± 1 g. For morphological and histological examination, samples of jejunum, ileum, and cecum were taken from 6 weaned piglets per group (18 animals in total). The intestine samples were taken immediately after slaughter and fixed in 10% formalin. After fixation and shaping, samples were dehydrated in increasing concentrations of ethyl alcohol, cleared with xylene, paraffin infiltrated and embedded in paraffin blocks. Sections 5 to 8 μm in thickness were cut and stained with Mayer's hematoxylin and eosin (HE) and with a combination of Periodic acid Schiff's stain and Alcian blue (PAS-AB; Yamabayashi, 1987; Smirnov *et al.*, 2005). Histological analysis was performed using a light microscope Olympus BX53 with the objective magnifications $\times 4$ and $\times 10$. Morphometric examinations were carried out using the Olympus cell Sens software (www.olympus-lifescience.com) and included the following measurements: the jejunal and ileal villus height and width, crypt depth, and the cecal crypt depth (Bergamo *et al.*, 2016).

Statistical analyses

Statistical analyses of the results were conducted using software GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego CA, USA, www.graphpad.com). One-way ANOVA with Tukey's post-hoc test was performed to assess the significance among experimental groups. Dependence of two parameters was expressed with Pearson's or Spearman's correlation coefficient (r) depending on the Shapiro-Wilk normality test. Values of $P < 0.05$ were considered significant.

RESULTS

Chemical composition of the animal diets

The chemical composition of the feed is shown in Table 2. Diets for all group of weaned piglets differed only in the amount of added sodium butyrate (0 g/kg, 3 g/kg and 5 g/kg).

Table 2. Chemical composition of the animal diet

Parameters	Percent
Moisture	9.85
Ash	5.83
Crude protein	18.68
Crude fat	4.5
Crude fiber	3.64
Calcium	0,97
Phosphorus	0.64
NFE*	57.50

*Nitrogen Free Extract

Piglet production performance

Piglet production performances are presented in Table 3. The average total feed intake of group I piglets, as well as the average daily feed intake, was the highest while the lowest was in group II animals ($P<0.05$).

Table 3. Production performance and carcass characteristics of pigs fed diet with different levels of sodium butyrate (mean±SE; n=16)

Parameters	Diet		
	I	II	III
Total feed intake, kg	$12.22^{a}\pm0.26$	$11.54^{b}\pm0.12$	$11.72^{ab}\pm0.09$
Daily feed intake, kg	$0.47^{a}\pm0.01$	$0.44^{b}\pm0.01$	$0.45^{ab}\pm0.01$
Feed to gain ratio	$2.14^{a}\pm0.03$	$2.53^{b}\pm0.07$	$2.46^{b}\pm0.04$
Initial live weight, kg	6.55 ± 0.35	6.56 ± 0.33	6.57 ± 0.31
Final live weight, kg	$32.17^{a}\pm1.26$	$35.75^{b}\pm1.07$	$35.32^{b}\pm0.86$
Total weight gain, kg	$25.62^{a}\pm1.17$	$29.19^{b}\pm0.92$	$28.75^{b}\pm0.68$
Daily weight gain, kg	$0.48^{a}\pm0.02$	$0.54^{b}\pm0.02$	$0.53^{ab}\pm0.01$
Hot carcass weight, kg	$21.00^{a}\pm0.91$	$24.48^{b}\pm0.92$	$24.04^{b}\pm0.66$
Carcass yield, %	$65.10^{a}\pm0.77$	$67.99^{b}\pm0.53$	$68.35^{b}\pm0.93$

I- diet without sodium butyrate; II- diet with 3 g/kg mixture sodium butyrate; III- diet with addition of 5 g/kg mixture sodium butyrate; SE- standard error

^{ab}Means within row with different superscript significantly differ at $P<0.05$

The average animal live weights at the beginning of the study ranged from 6.55 ± 0.35 to 6.57 ± 1.25 kg, with no significant differences ($P>0.05$) among the groups. At the end of the study, weaned piglets from the groups fed a diet with added sodium butyrate (groups II and III) had significantly higher ($P<0.05$) average live weights as compared to group I piglets. Significantly higher ($P<0.05$) average total

weight gain occurred in both groups of piglets fed a diet with added sodium butyrate (group II and III) as compared with group I piglets fed diet without sodium butyrate.The best feed to gain ratio was in group I ($P<0.05$) as compared to other groups. Hot carcass weights were lower in group I piglets as compared to experimental groups II and III.

Intestinal microflora

Results of intestine microbiology are presented in Table 4. Significant differences ($P<0.05$) were observed in *E. coli* count in small intestine between control (I) and experimental groups (II and III), and in *E. coli* count in cecum between control (I) and experimental group II.

Table 4. Effect of sodium butyrate on intestinal microflora of weaned pigs (mean±SE; n=10)

Microorganisms	Diets		
	I	II	III
Small intestine			
TVC	5.56±1.00	5.52±0.88	5.22±0.62
Lactic acid count	5.93±0.47	6.08±0.75	6.28±0.77
Enterococcus spp.	4.01±0.45	3.85±0.73	3.79±0.53
Escherichia coli	5.21^{a}±0.66	4.92^{b}±0.65	4.42^{b}±0.50
Cecum			
TVC	6.64±0.79	6.57±0.72	6.42±0.83
Lactic acid count	6.14±0.93	6.57±0.75	6.71±0.77
Enterococcus spp.	4.28±0.79	4.01±0.65	3.99±0.68
Escherichia coli	5.92^{a}±0.61	5.79^{ab}±0.70	5.27^{b}±0.73

I- diet without sodium-butyrate; II- diet with 3 g sodium-butyrate/kg mixture; III- diet with addition of 5 g/kg mixture sodium-butyrate; SE- standard error

^{ab}Means within column with different superscript differ significantly at $P<0.05$

Morphological and histological analyses

The weight and length of the intestine are shown in Table 5. Piglets fed the control diet without sodium butyrate had, on average, intestinal sections (duodenum, jejunum, ileum, and cecum) that were significantly lighter ($P<0.01$) than group II and III piglets on the butyrate-supplemented diets. In contrast, the colons of group I control piglets were significantly heavier than those of piglets fed diets with added sodium butyrate. Overall, the average total weight of digestive tract of group I piglets was significantly lower ($P<0.01$) than the average weight of the digestive tract of the piglets fed sodium butyrate. Duodenum, jejunum, and ileum, as well as cecum and colon of piglets from the group I were significantly shorter ($P<0.01$) than the average length of these intestinal segments of piglets from groups II and III. Results of histological examination are shown in Figs. 1, 2, 3, 4 and Table 6.

Table 5. Weight and length of different intestine segments (mean±SE; n=10)

Parameters	Intestinal segment	Diets		
		I	II	III
Weight (g)	Duodenum[1]	$30.1^{a}\pm0.526$	$38.9^{b}\pm0.433$	$39.7^{b}\pm0.423$
	Jejunum[2]	$942.6^{a}\pm2.192$	$1165.0^{b}\pm1.965$	$1167.0^{b}\pm2.103$
	Ileum[3]	$36.1^{a}\pm0.823$	$40.3^{b}\pm0.746$	$39.7^{b}\pm0.559$
	Cecum[4]	$51.4^{a}\pm0.872$	$59.5^{b}\pm0.719$	$60.4^{b}\pm0.748$
	Colon[5]	$417.4^{a}\pm3.215$	$400.5^{b}\pm4.313$	$397.1^{b}\pm3.598$
	Total	$1478^{a}\pm4.568$	$1704^{b}\pm5.754$	$1704^{b}\pm5.352$
Length (cm)	Duodenum[1]	$30.1^{a}\pm0.690$	$26.9^{b}\pm0.526$	$26.5^{b}\pm0.637$
	Jejunum[2]	$1375^{a}\pm2.604$	$1424^{b}\pm4.738$	$1427^{b}\pm4.542$
	Ileum[3]	$25.0^{a}\pm0.760$	$29.6^{b}\pm0.872$	$30.2^{b}\pm0.940$
	Cecum[4]	$13.8^{a}\pm0.663$	$17.2^{b}\pm0.574$	$17.5^{b}\pm0.543$
	Colon[5]	$1726^{a}\pm6.18$	$1797^{b}\pm3.02$	$1800^{b}\pm3.98$
	Total	$3169^{a}\pm7.236$	$3295^{b}\pm6.426$	$3301^{b}\pm8.164$

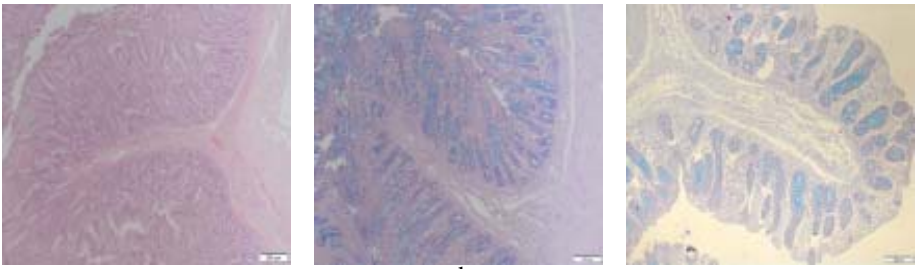
I- diet without sodium butyrate; II- diet with 3 g/kg mixture sodium butyratee; III- diet with 5 g/kg mixture sodium butyrate; SE- standard error; ^{a,b}means within row with different superscript differ significantly at $P<0.05$.

Table 6. Jejunal, ileal and cecal morphometric parameters (mean±SE; μm)

Intestinal segment	Parameters	Diets		
		I	II	III
Jejunum	Villus height	$581.6^{a}\pm7.976$	$605.4^{b}\pm13.85$	$608.6^{c}\pm9.689$
	Villus width	$72.83^{a}\pm0.970$	$68.65^{a}\pm1.504$	$84.02^{b}\pm1.465$
	Crypt depth	95.99 ± 1.879	73.46 ± 1.671	84.65 ± 1.842
Ileum	Villus height	$221.60^{a}\pm3.073$	$235.10^{b}\pm5.012$	$245.20^{c}\pm6.616$
	Villus width	$99.20^{a}\pm1.952$	$99.59^{ab}\pm2.845$	$104.90^{b}\pm1.773$
	Crypt depth	401.50 ± 11.220	409.80 ± 9.018	420.40 ± 8.158
Cecum	Crypt depth	$518.40^{a}\pm6.401$	$555.10^{b}\pm16.710$	$566.50^{b}\pm11.110$

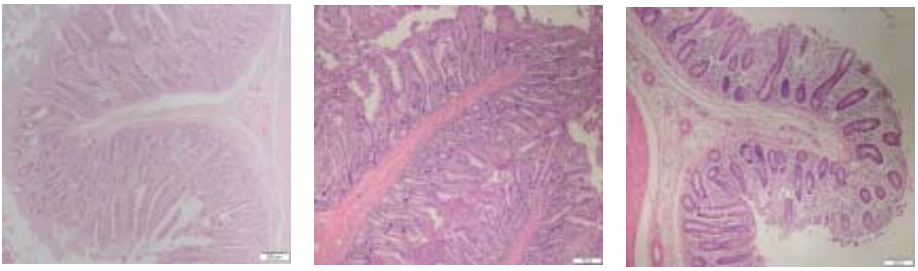
I- diet without added sodium-butyrate; II- diet with addition of 3 g sodium-butyrate per kg of the mixture; III- diet with addition of 5 g sodium-butyrate per kg of the mixture; SE - standard error; $^{a, b, c}$ means within row with different superscript differ significantly at $P<0.05$;

The villus heights of various parts of small intestine differed. Jejunal villus height and width were significantly different ($P<0.01$) in control (I) piglets and groups of piglets fed a diet with added sodium butyrate (II and III), while no difference was observed for crypt depth. Ileal villus heights were significantly lower in control (I) piglets than in piglets receiving the diets with added sodium butyrate (groups II and III piglets). Piglets from the group I had significantly wider ileal villus than control piglets ($P<0.05$), while ileal crypt depth was greater in piglets fed diets with added sodium butyrate, but differences were not significant. The cecal crypt depth of control piglets was lower as compared to that in both groups of piglets fed diets with added sodium butyrate. The both, jejunal and ileal villus height/crypt depth



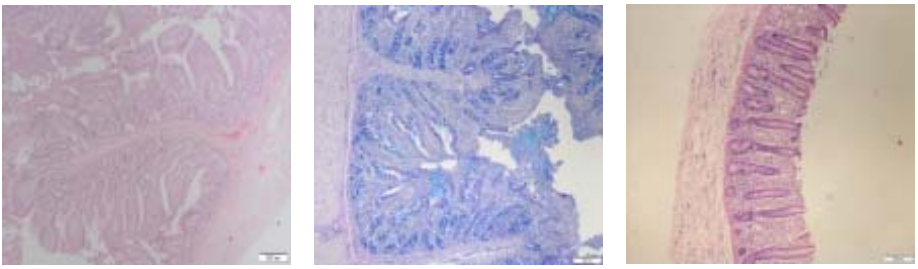
a b c

Fig. 1. Photomicrographs showing mucosa of the jejunum (a), ileum (b) and cecum (c) of group I pigs (PAS-AB, 200 μm)



a b c

Fig. 2. Photomicrographs showing mucosa of jejunum (a), ileum (b) and cecum (c) of group II pigs (PAS-AB, 200 μm)



a b c

Fig. 3. Photomicrographs showing mucosa of jejunum (a), ileum (b) and cecum (c) of group III pigs (PAS-AB, 200 μm)

ratio was the lowest ($P<0.05$) in the control group (I) as compared to both groups of piglets fed a diet with the addition of sodium butyrate (II and III; Fig. 4).

Correlations between piglet performance and results of morphological analyses

There were strong positive significant ($P<0.01$) correlations between added sodium butyrate, final live weight and intestinal length and weight, while correlations between different levels of sodium butyrate and morphometric parameters were weak, except jejunal villus height and cecal crypt depth where there was no or

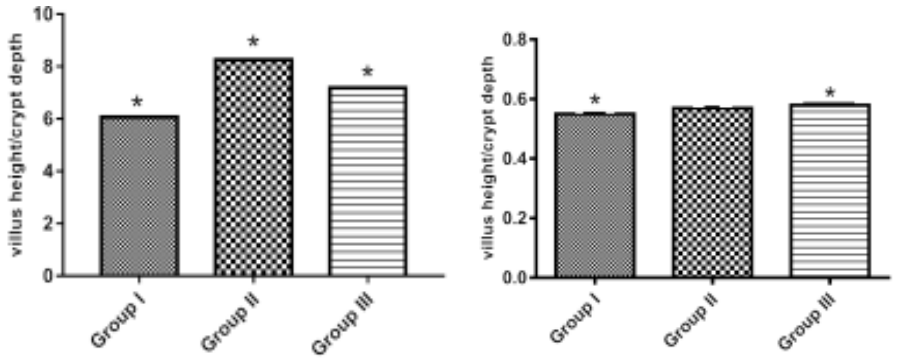


Fig. 4. Jejunal (a) and ileal (b) villus height/crypt depth ratio (*- significant difference $P<0.05$)

negligible correlation (Table 7).

Table 7. Correlations (r) between added sodium butyrate and pig production performance and results of histological analyses

Parameters	Shapiro-Wilk normality test (P value)	Sodium butyrate (Pearson's/ Spearman's correlation coefficient r)
Daily feed intake	<0.0001	0.500**
Final live weight	0.0785	0.732**
Daily weight gain	0.0514	0.273
Carcass yield	0.8059	0.319
Hot carcass weight	0.8873	0.226
Intestinal length	0.0010	0.729**
Intestinal weight	<0.0001	0.710**
Jejunal villus height	0.6992	0.005
Jejunal villus width	0.2080	0.358
Jejunal crypt depth	0.1528	-0.396*
Ileal villus height	0.8812	-0.263
Ileal villus width	0.1612	0.388*
Ileal crypt depth	0.3998	0.384*
Cecal crypt depth	0.1899	0.070

r=0.00-0.20 – none or negligible linear relationship; r=0.21-0.40 – weak linear relationship; r=0.41-0.70 – moderate linear relationship; r= 0.71-1.00 – strong linear relationship; **P<0.01; *P<0.05

DISCUSSION

The results of chemical analyses showed that the diets for all piglets were in accordance with technological and legislative norms (Slu•beni glasnik RS, 2010), and the nutrient content fully satisfied the needs of weaned piglets (NRC, 1998). The

sodium butyrate was added due to reduction of corn used in the diet, in order to reduce the influence on the composition and nutritional value of the diet. The sodium butyrate preparation was used in the amounts recommended by the manufacturer. The diets had the same chemical composition (beside sodium butyrate amount) in order to clearly indicate the influence of sodium butyrate on overall piglet production performances, intestine morphological properties, and microflora, which was the aim of the study.

We found that sodium butyrate caused decreases of the average daily feed intake, which is in agreement with the studies of Weber and Kerr (2008), whose experiment were conducted to determine the effects of dietary 0.05, 0.1, 0.2, or 0.4% sodium butyrate on growth performance in weanling pigs and study of Leeson (2005), who examined the efficacy of 0.2 and 0.4% butyric acid on performance and carcass characteristics of broiler chickens. On the other, Hanczakowska *et al*. (2014) and Piva *et al*. (2002b) showed no effect of sodium butyrate on average daily feed intake during 84 and 56 days of experiment, respectively. In contrast,the studies of Biagi *et al*. (2007) and Lu *et al*. (2008) showed higher average daily feed intakes of the pigs ingesting sodium butyrate. The variable response of added dietary sodium butyrate could be contributed to the different animal ages, feeding durations or diet types (Weber and Kerr, 2008).

Piglets fed with added sodium butyrate grew faster and these groups had higher final average live weights than control piglets. In accordance with the live weight of piglets at the end of the trial, hot carcass weights and carcass yields of piglets fed sodium butyrate diets were also higher. This likely was because butyrate supplementation lengthened the piglet digestive tract, especially the proximal part, the main site of digestion and absorption, which enabled better availability of nutrition. Sodium butyrate also improves the structure and function of the pig intestine (Hanczakowska *et al*., 2014), and these factors all together consequently led to the improvement of our pigs' growth performances. Kotunia *et al*. (2004) and Manzanilla *et al*. (2006) showed that addition of 0.3% sodium butyrate increased feed efficiency and weight gain of weaned pigs and neonatal piglets, while Hanczakowska *et al*. (2014) reported higher average live weight of pigs fed with added sodium butyrate, up to when pigs were 84 days old. Lu *et al*. (2008) detected differences in the effect of sodium butyrate levels, in their 30-days study of 21 days-old weaned piglets fed diets with added 0.5 and 1 g sodium butyrate per kg of feed. They found that final average live weight and average daily weight gain were significantly higher in animals fed diet with the greater amount of sodium butyrate than in other animals (which received less sodium butyrate and were control animals). Valverde Piedra *et al*. (2009) reported a higher average daily weight gain of pigs fed a diet with added sodium butyrate during 56 days, but the gain was non-significant.

The best feed to gain ratio in the present study was achieved by control group piglets, followed by group III, while the worst feed to gain ratio was seen in group

II piglets. This impairment caused by intake of sodium butyrate is in agreement with Biagi *et al*. (2007) and Lu *et al*. (2008), whose studies showed feed to gain ratio was worsened when piglets were fed diets with added sodium butyrate, but opposite to findings reported by Piva *et al*. (2002b).

In accordance to our study, where significantly higher number of *E. coli* ($P<0.05$) were observed in small intestine of control (I) group as compared to experimental groups (II and III), and in cecum of control group (I) as compared to experimental group III, the study of Lin *et al*. (2020) showed that compared with control, groups of weaned piglets fed diet supplemented with 300 or 450 mg sodium butyrate/kg feed in form of coated sodium butyrate increased the ratio of *Lactobacilli* and *E. coli* in the jejunum and colon ($P<0.05$). The antibacterial effect of sodium butyrate was dose-dependent. Lactic acid bacteria counts were higher in the piglets which received sodium butyrate, while *E. coli* and *Enterococcus* counts in those piglets were lower compared with control piglets. These results are in agreement with those reported by Galfiand Bokori (1990), Wielen *et al*. (2000) and Lu *et al*. (2008). Addition of sodium butyrate did not influence pH changes of intestinal contents (data not shown), which is why the antibacterial effect of sodium butyrate is not attributed to acidification, but rather, is due to its biological action. Sodium butyrate might help pig intestines resist invasion from opportunistic bacteria, including direct inhibition of bacterial growth and interference with adhesion to host tissue (Lu *et al*., 2008).

Beneficial effects of sodium butyrate could be due to increasing length and weight of pigs'digestive tracts and improving the structure of the intestinal mucosae, which leads to better absorption through the intestinal wall and improved growth performance. In the weaning period, piglet small and large intestines increase three times faster than the body weight increases (Piva *et al*., 2001), and so these sodium butyrate-induced changes could be of special importance at this time. In addition to the level of sodium butyrate added to diet, different studies showed that pig age or duration of feeding with added sodium butyrate also can influence pig production performance, so response to dietary sodium butyrate is variable (Piva *et al*., 2002b; Biagi *et al*., 2007; Weber and Kerr, 2008; Valverde Pierde *et al*., 2009).

The results of present study shows that sodium butyrate fed to healthy growing piglets greatly influenced small intestinal histomorphology and function. By increasing the length of small intestinal villi, sodium butyrate enlarged the absorptive surface, favorably influencing the transport processes (Galfiand Bokori, 1990). Development of intestine (increase of weight and length) of piglets fed diet with added sodium butyrate, shown in the present study, is in accordance with results of Hanczakowska *et al*. (2014), who also has shown increasing total intestinal weight and length. A longer digestive tract increases the absorptive surface, nutrition absorption and utilization, and consequently, influences animal growth.

Significant development of jejunal and ileal villus height and width were found in piglets receiving sodium butyrate than in control piglets, which could be another reason for a higher body weight gain of these piglets.This is in agreement with Hanczakowska *et al*. (2014),who found higher jejunum villus in pigs fed added sodium butyrate alone or in a mix with glutamine and glucose. Kotunia *et al*. (2004) also found that sodium butyrate increases the development of the small intestine mucosae in neonatal piglets fed artificial food, causes proliferation of intestinal cells, and stimulates blood circulation and intestinal synthesis of gastrointestinal hormones, while Biagi *et al*. (2007) found no effect of feeding of pigs with added sodium butyrate on intestinal morphology. Different effects of sodium butyrate on intestinal morphology can be attributed to the different absorption of sodium butyrate. This can occur rapidly through stomach tissue, meaning no butyrate arrives intact in the lower digestive tract, or it can be absorbed in the small intestine, if it is chemically protected, as was the case for the sodium butyrate used in our study, and have bigger influence on intestinal histomorphology (Manzanilla *et al*., 2006; Claus *et al*., 2007; Weber and Kerr, 2008). As a result of enzymes and bile, effects on feed components' digestibility increases along the gastrointestinal tract and is more available in distal parts of the intestine, which is in agreement with our results showing increases of jejunal and ileal villus heights (Hanczakowska *et al*., 2014).

In determining the relationship between the addition of sodium butyrate and piglet production performance and morphological and histological analyses of individual intestine segments, the present data showed the strongest, most significant correlations for final live weight and intestinal length and, weight. Pluske *et al*. (1996), in their study, showed that villus height is positively correlated with added butyrate in piglet diet.

CONCLUSION

Based on the results of the present study, it can be concluded that through development changes to intestinal epithelial structures, the addition of both examined doses (3 and 5 g per kg of feed mixuture) of sodium butyrate to the diet showed a positive influence on the pig production performance and could be suitable for use as a growth promoter.

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