

Effect of different sodium butyrate levels in weaned pig diet on the antioxidant capacity of selected organs

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Abstract: The aim of this study was to evaluate the influence of different sodium butyrate levels on the antioxidant capacity of selected organs (liver and kidney) in pigs. The study was conducted on 48 weaned piglets (28 to 54 days old) fed one of three diets (group C had no added sodium butyrate, group E-I had 3 g and group E-II 5 g of sodium butyrate added per kg of diet). The guaiacol peroxidase and pyrogallol peroxidase activities in liver tissues of E-II pigs were significantly lower than those in E-I pigs, while the activities of these enzymes in kidney tissues were significantly lower than in control pigs for both butyrate levels. Glutathione peroxidase activity in liver tissues was not upregulated by either level of dietary sodium butyrate. Also, the contents of malondialdehyde, indicative of lipid peroxidation, were not significantly different among the pig dietary groups. No significant differences in the enzyme activities (catalase, superoxide dismutase, glutathione S-transferase or the oxidation product, reduced glutathione) of control pigs and of animals consuming 3 g or 5 g sodium butyrate per kg of diet was found. The results showed that oral administration of sodium butyrate had an impact only on guaiacol peroxidase and pyrogallol peroxidase enzyme activities.

Keywords: piglet diet, antioxidative enzymes, liver, kidney, lipid peroxidation.

Introduction

For young animals, weaning is a critical stage because of alterations in the gastrointestinal tract morphology and function, often challenged by post-weaning stresses including diarrhea, low feed intake, and body weight loss, and these stresses can adversely affect intestinal health and function (Song *et al.*, 2011). The organic acids have positive effects on growth performance of all pig categories, including weaned piglets (Galfi and Bokori, 1990; Witte *et al.*, 2000; Mazzoni *et al.*, 2008; Piva *et al.*, 2002). 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). Butyric acid is produced by bacterial fermentation of undigested carbohydrates in the intestine of human and animals and recent studies have shown effects on antioxidative activity (Mentschel and Claus, 2003; Biagi *et al.*, 2007; Guilloteau *et al.*, 2010).

Butyrate supplementation could improve antioxidative stress ability and piglet performance (Lu *et al.* 2008; Ma *et al.* 2012). Song *et al.* (2011) demonstrated that sodium butyrate can reduce diarrhea through a reduction in intestinal permeability and increasing the expression of mucosal tight junction proteins on the intestinal mucosa. Because of its functional properties and accessibility, sodium butyrate is widely used as a feed additive. Numerous studies have demonstrated that dietary inclusion of sodium butyrate did not disturb normal biochemical and physiological processes in animals (Inan *et al.*, 2000; McCracken and Lorenz, 2001; Kotunia *et al.*, 2004; Claus *et al.*, 2007; Lu *et al.*, 2008; Guilloteau *et al.*, 2010; Sunkara *et al.*, 2011).

The present study, therefore, investigated the effect of sodium butyrate addition to weaned piglet diet on antioxidative capacity of the feed mixture, and on antioxidant enzyme activities in liver and kidney tissues.

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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 x Landrace crossbreed. Sodium butyrate (commercial preparation of chemically protected sodium butyrate with 54% activity, Butirex C4, Novation, Spain) was included as a feed additive in sows' diet seven days before farrowing, and this continued during lactation until the day of insemination, and 30 days after insemination. The sodium butyrate preparation was used at the levels recommended by the manufacturer. The daily feed intake of lactating sows was 5 to 6 kg per day. Piglets were farrowed

within a day, fed on sows' milk and from days 7 to 10 of life, started to feed on pre-starter with 2 g added sodium butyrate (Butirex C4, Novation, Spain) per kg of feed. Before weaning, piglets were housed with sows in the same facility, with the same pre-conditions including microclimate, before entering the trial. Weaned, 28-day-old piglets were randomly allocated and housed in one of three weaning pens (dimensions 2x2.3 m) within the same weaning facility, on concrete slatted floors, in groups of 16 animals per pen (stocking density was 4 animals m⁻²). Weaned piglets were provided *ad libitum* with feed and water. The trial was conducted over a 26-day period (when piglets were from 28 days old to 54 days old), during which animals consumed 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

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

Ingredient (%)	Diet		
	C	E-I	E-II
Corn	45.56	45.45	45.37
Barley	18.0	18.0	18.0
Soybean meal	11.31	11.33	11.34
Soybean grits	4.5	4.5	4.05
AK 530 soy isolate	9.0	9.0	9.0
Potato protein	2.5	2.5	2.5
Whey 72%	2.5	2.5	2.5
Monocalcium phosphate	1.43	1.38	1.37
Cattle chalk	0.91	0.92	0.93
Cattle salt	0.52	0.33	0.2
Premix 1.5%*	1.5	1.5	1.5
Lysolecithin	0.05	0.05	0.05
Soybean oil	1.74	1.74	1.74
Mycotoxin adsorbent	0.2	0.2	0.2
Zinc oxide	0.3	0.3	0.3
Sodium butyrate	0.0	0.3	0.5
Σ	100	100	100

Legend: *Premix composition (per kg): Lysine 202.94g; Methionine 72.65g; Threonine 65.44g; Tryptophan 20.00 g; St. Dig. Lysine 202.90g; St. Dig. Methionine 72.65g; St. Dig. Meth&Cyst 72.65g; St. Dig. Threonine 65.44g; St. Dig. Tryptophan 20.00 g; Calcium 137.16g; Vitamin (Vit). A 800100i.e; Vit. D 380030i.e; Vit. E 10952.56mg; Alpha tocopherol 9966.80mg; Vit. K3 306.83mg; Vit. B1 153.53mg; Vit. B2 306.83mg; Vit. B6 233.33mg; Vit. B12 1.54mg; D-pantotenic acid 780.03mg; Niacin 1533.47mg; Cholinchloride 16666.77mg; Biotin 15.47mg; Mn 3133.43mg; Fe 15066.80mg; Cu 11000.03mg; Zn 8000.07mg; I 15.47mg; Cobalt-II-carbonate 33.37mg; Se 26.83mg; Phytase 33333.40FYT; Fungal xylanase (3.2.1.8) 13333.40FXU

(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 C had no added sodium butyrate, the diet for experimental group E-I contained added 3 g of sodium butyrate per kg of mixture, while the diet for experimental group E-II contained added 5 g of sodium butyrate per kg of mixture (Table 1).

Chemical composition of the animal diets

Chemical analyses to determine protein, moisture, cellulose, fat, and ash of the feed were conducted according to AOAC methods (AOAC, 1990). Antioxidant capacity in diet is based on formation of the ABTS⁺ cation [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)], and its scavenging by antioxidant sample constituents measured by spectrophotometry (decay of green/blue chromophore absorbance is inversely associated with antioxidant sample content, while the control antioxidant is Trolox, a hydrophilic vitamin E analog) (Miller et al., 1993).

Biochemical analyses

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, liver and kidney weights were recorded and samples of the organs were taken. Homogenates of liver and kidney were used with phosphate buffers (pH=7.0) for further biochemical analysis. Activities of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD-1), glutathione peroxidase (GSHPx), guaiacol peroxidase (GPx), pyrogallol peroxidase (PPx), glutathione S-transferase (GST), reduced glutathione and lipid peroxidation were measured in selected tissues (liver and kidney). The CAT activity was assayed by the method of Aebi (1984). The SOD-1 activity was determined according to Kakkar et al. (1984). The GSH-Px activity was determined using the method of Paglia and Valentine (1967). The GPx activity was measured by following the H₂O₂ dependent oxidation of guaiacol at 470 nm (Agrawal and Laloraya, 1977). The activity of PPx was measured using pyrogallol as the substrate according to Chance and Maehly (1955). The formation of purpurogallin was followed at 430 nm. GST activity in samples was evaluated using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate, as previously described by Habig et al. (1974). Reduced

glutathione (GSH) was performed according to the method described by Sedlak and Lindsay (1968). Malondialdehyde (MDA) level was analyzed with 2-thiobarbituric acid using the method of Ohkawa et al. (1979).

Statistical analyses

Statistical analyses of the results were conducted using software GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego CA, USA, www.graphpad.com). All parameters for each group of weaned piglets are shown in tables as mean±standard deviation. One-way ANOVA with Tukey's post-hoc test was performed to assess the significance among experimental groups.

Results and Discussion

The chemical composition of the feed, i.e. protein, moisture, fat, ash, fiber, calcium, phosphorus and NFE content is shown in Table 2. Diets for all groups of weaned piglets differed only in the amount of added sodium butyrate (0 g kg⁻¹, 3 g kg⁻¹ or 5 g kg⁻¹).

Table 2. Chemical composition of the animal diet

Parameter	
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

The results of chemical analyses showed that the diets for all piglets were in accordance with technological and legislative norms (Official Gazette RS, 2010), and the nutrient content fully satisfied the needs of weaned piglets (NRC, 1998). The sodium butyrate preparation was used at the levels recommended by the manufacturer.

Among the available analytical techniques, the Trolox equivalent antioxidant capacity assay is the most frequently used for assessing antioxidant

Table 3. Antioxidant activity of different feed extracts, measured by applying an improved ABTS test (mixture)

Feed	Extract			
	70% acetone	70% methanol	70% ethanol	Puffer pH=4.5
C	0.566±0.012 ^{AB}	0.446±0.012 ^{AB}	0.421±0.093 ^a	0.247±0.008 ^{AA}
E-I	0.630±0.012 ^{AC}	0.506±0.037 ^{AC}	0.499±0.037	0.263±0.025 ^{AB}
E-II	0.653±0.003 ^{BC}	0.569±0.023 ^{BC}	0.542±0.034 ^a	0.296±0.010 ^{AB}

Legend: Values expressed as mean± SD;
ANOVA test with *post hoc* Tukey's test.

^{A,B} Means within column with same superscript significantly differ at $p<0.01$.

^a Means within column with same superscript significantly differ at $p<0.05$.

C – diet without added sodium butyrate; E-I – diet with 3 g sodium butyrate added per kg; E-II – diet with 5 g sodium butyrate added per kg.

properties of feed extract components. The antioxidant capacity of different feed mixture extracts (acetone, methanol, ethanol and puffer) are shown in Table 3. The total antioxidant capacity of acetone and methanol extracts from diet with 5 g of added sodium butyrate was significantly higher ($p<0.01$) compared to the other diets (control and E-I diets).

Significant differences were observed in antioxidant capacity of ethanol and puffer extracts between the experimental diets. The acetone, methanol, ethanol and puffer extracts of the diet with 5 g sodium butyrate added were fast and effective scavengers of the ABTS radical, so butyrate supplementation significantly improved the antioxidant properties of the

Table 4. Effect of sodium butyrate on antioxidant enzyme activities and lipid peroxidation in pigs' liver tissues

Parameter	Pig group		
	C	E-I	E-II
CAT (IU mg protein ⁻¹)	18.56±2.08	17.99±1.87	18.21±1.09
SOD-1 (IU mg protein ⁻¹)	10.77±2.48	10.42±1.67	8.47±2.03
GSHPx (IU mg protein ⁻¹)	30.96±4.22	32.51±3.32	32.08±1.86
GPx (IU mg protein ⁻¹)	6.41±0.17 ^A	6.83±0.24 ^{AB}	6.38±0.19 ^B
PPx (IU mg protein ⁻¹)	24.40±1.02	25.00±0.98 ^a	23.80±0.88 ^a
GST (IU mg protein ⁻¹)	190.87±16.88	181.89±12.00	191.11±14.25
Reduced glutathione (µmol GSH per mg protein)	20.11±2.87	19.44±2.07	21.36±2.00
Lipid peroxidation (nmol MDA per mg protein)	1.38±0.09	1.45±0.11	1.29±0.14

Legend: Values expressed as mean± SD.
ANOVA test with *post hoc* Tukey's test.

^{A,B} Means within row with same superscript significantly differ at $p<0.01$.

^a Means within row with same superscript significantly differ at $p<0.05$.

C – pigs consumed diet without added sodium butyrate; E-I – pigs consumed diet with 3 g sodium butyrate added per kg; E-II – pigs consumed diet with 5 g sodium butyrate added per kg.

CAT – catalase; SOD-1 – superoxide dismutase; GSHPx – glutathione peroxidase; GPx – guaiacol peroxidase; PPx – pyrogallol peroxidase; GST – glutathione S-transferase; GSH – reduced glutathione; MDA – malondialdehyde.

feed. Animal feeds contain a range of different compounds that possess antioxidant activities, including vitamin E (consisting of eight compounds tocopherols and four tocotrienols), carotenoids (more than 600 compounds), flavonoids (more than 8000 compounds), ascorbic acid and some other compounds that contribute to anti-oxidant/pro-oxidant balance in animals and that have positive effects on major physiological functions (Surai, 2007).

Effects of sodium butyrate on the activities of endogenous antioxidant enzymes and lipid peroxidation in the liver tissue of pigs are shown in Table 4. The inclusion of sodium butyrate induced significant change in the activity of the measured enzymes, GPx and PPx. Significant decreases in GPx and PPx activities were observed in E-II pigs' livers (Table 4; $p < 0.05$; $p < 0.01$). There were no significant differences in the other measured enzyme activities (CAT, SOD-1, GSHPx, GST and reduced glutathione) between the control pigs and animals treated with 3 g or 5 g sodium butyrate per kg of feed mixture ($p > 0.05$). A decrease in SOD after weaning in our study indicated that oxidative stress was present in weaning pigs, which caused increased free-radical generation. The MDA level increased somewhat in livers of pigs consuming 3 g sodium butyrate per kg of the feed compared with in livers of control and E-II pigs, but not significantly.

The mechanism of butyrate effects on inflammatory and oxidative status were presented by Canani et al. (2011). Butyrate has anti-inflammatory effects, primarily via inhibition of nuclear factor κ B (NF- κ B) activation, which can result from the inhibition of histone deacetylase. NF- κ B regulates many cellular genes involved in early immune inflammatory responses, including IL-1b, TNF- α , IL-2, IL-6, IL-8, and IL-12. Butyrate can act on immune cells through specific G-protein-coupled receptors (GPRs) for SCFAs, i.e., GPR41 (or FFA3) and GPR43 (or FFA2), which are both expressed on immune cells, including polymorphonuclear cells, suggesting that butyrate might be involved in the activation of leucocytes. The possible immune-modulatory functions of SCFAs are highlighted by a recent study on GPR43 $-/-$ mice in which they exhibit aggravated inflammation, related to increased production of inflammatory mediators and increased immune cell recruitment.

Data related to factors influencing the activity of antioxidant enzymes in pig tissues are limited. Antioxidant enzyme activities differ between different tissue types (Pradhan et al., 2000; Hernández et al., 2002). Variations in the activity of these enzymes between animals of a single species and different genetic types could lead to differences

in oxidative stability of the tissues (Hernández et al., 2004). Antioxidant enzymes are indispensable key factors against oxidative stress induced by xenobiotic factors in animals' defense systems (Hwang et al., 1993). The antioxidant enzyme defense system consists of CAT, SOD and GSH-Px. SOD converts radicals ($\text{HO}_2^-/\text{O}_2^-$) to the less toxic H_2O_2 , while CAT and GSH-Px detoxify H_2O_2 into O_2 and H_2O (Ahmad et al., 2012). After treatment with 5 g kg^{-1} sodium butyrate, GSH, one of the non-enzymatic antioxidant components, increased in our pig liver tissue, whereas MDA, a source of free radical mediated lipid peroxidation injury, decreased compared with the control (Table 4). Butyrate had no effect on other enzymes (including CAT and SOD-1). These results, therefore, suggest that the mechanism by which butyrate exhibits its effects may not be fully due to antioxidant stress (Song et al., 2011). The alteration in antioxidant indices by sodium butyrate, including in the amounts of MDA and GSH detected, suggest an improvement in the level of oxidative stress in the liver cells, which could result in improved healing. Previously, studies suggested that sodium butyrate improves the intestinal tight junction and depresses permeability by improving antioxidant ability. According to Sunkara et al. (2011), butyrate strongly induces synthesis of endogenous HDPs (Host Defense Peptides) and their expressions in different cell and tissue types including HD11 macrophages, primary monocytes, bone marrow cells, jejunum and cecal explants as well as in crop, cecum, and cecal tonsils of chickens, thus inhibiting the harmful proinflammatory response. The present study demonstrated that treatment with sodium butyrate did not enhance overall antioxidative ability in pig livers.

Table 5 presents the activity of the measured antioxidant enzymes and MDA levels in pig kidney tissue. Compared to the control group, treatment with sodium butyrate significantly decreased PPx activity and GPx activity in the kidney tissues of pigs ($p < 0.01$). No significant alterations in other antioxidant enzymes (CAT, SOD-1, GSHPx, GST and reduced glutathione) between the control pigs' kidneys and those of animals with sodium butyrate added to their diets were observed.

Some in vitro studies indicated that butyrate could increase the activity of antioxidant enzymes. The activity of antioxidant enzymes in non-malignant human colon cells significantly increased after exposure to a butyrate environment (Jahns et al., 2015). Namely, butyrate could contribute to chemoprotection in colon cells by reducing the growth of tumor cells, committing them to more rapidly go into

Table 5. Effect of sodium butyrate on antioxidant enzyme activities and lipid peroxidation in pigs' kidney tissues

Parameter	Pig group		
	C	E-I	E-II
CAT (IU mg protein ⁻¹)	22.46±2.85	23.69±4.01	21.87±2.11
SOD 1 (IU mg protein ⁻¹)	21.20±1.46	22.18±3.50	23.59±4.40
GSHPx (IU mg protein ⁻¹)	29.33±3.05	30.22±2.54	30.10±2.00
GPx (IU mg protein ⁻¹)	2.18±0.08 ^{AB}	1.96±0.09 ^A	1.95±0.12 ^B
PPx (IU mg protein ⁻¹)	64.32±1.75 ^A	62.02±2.20 ^B	57.18±1.89 ^{AB}
GST (IU mg protein ⁻¹)	97.31±7.87	94.14±9.25	95.46±6.07
Reduced glutathione (µmol GSH per mg protein)	15.75±1.52	14.89±1.30	15.07±1.02
Lipid peroxidation (nmol MDA per mg protein)	1.92±0.09	2.05±0.14	1.95±0.15

Legend: Values expressed as mean± SD.

ANOVA test with the *post hoc* Tukey's test.

^{A,B} Means within row with the same superscript significantly differ at $p < 0.01$.

C – pigs consumed diet without added sodium butyrate; E-I – pigs consumed diet with 3 g sodium butyrate added per kg; E-II – pigs consumed diet with 5 g sodium butyrate added per kg.

CAT – catalase; SOD-1 – superoxide dismutase; GSHPx – glutathione peroxidase; GPx – guaiacol peroxidase; PPx – pyrogallol peroxidase; GST – glutathione S-transferase; GSH – reduced glutathione; MDA – malondialdehyde.

apoptosis, serving as a survival factor for normal, non-transformed colon cells, enhancing mucin synthesis and via the mechanism of favorably altering patterns of drug metabolism (Scharlau *et al.*, 2009). The antioxidant properties of feed additives which contain a complex mixture of antioxidants (including ascorbate, carotenoids, vitamin E and other phenolics such as the flavonoids) can also act within the digestive tract and improve overall gut functions (Halliwell *et al.*, 2000). Apart from the positive effect on antioxidant feed capacity, the changes in liver and kidney antioxidant enzyme activity, observed in this study, indicated that the sodium butyrate generally did not improve the antioxidant properties in the animal tissues, which is not in agreement with above-mentioned studies.

Conclusion

Results from the present study showed that oral intake of sodium butyrate had no effect on lipid peroxidation or antioxidative enzymes activity of pigs' kidney and liver tissues, with the exception of GPx and PPx. For these, addition of 3 g sodium butyrate per kg of pig diet generally had better influence than the addition of 5 g per kg. The lower-level sodium butyrate supplementation increased GPx enzyme activity in liver, but reduced it in kidney tissues. The addition of 5 g sodium butyrate had negative effects on PPx activities in both tissues. Sodium butyrate did have a positive effect on the antioxidant capacity of the feed.

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