COMBINED EFFECT OF BUTYRATE AND *YUCCA SCHIDIGERA* EXTRACT ON THE GASTROINTESTINAL TRACT OF PIGS AROUND WEANING

KOMBINIRANO DJELOVANJE BITURATA I EKSTRAKTA *YUCCA SCHIDIGERI* NA GASTROINTESTINALNI SUSTAV SVINJA OKO ODBIJANJA

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SUMMARY

After the banning of antibiotics as growth promoters, animal production in the EU faced the problem of increased post-weaning disorders, and several solutions were proposed (acidifiers, probiotics, herb extracts, enzyme cocktails, etc.). Short chain fatty acids (SCFA) are the main end product of bacterial fermentation in the hind gut in monogastric animals and can be used as energy supply for the epithelial cells of gut mucosa. Thus the aim of the study was to test the usefulness of novel microencapsulated sodium butyrate and *Yucca Schidigera* extract (encourages friendly bacteria growth, reduces ammonia) preparation on pig performance and small intestine development in the critical weaning period. Microencapsulation helps to avoid stomach degradation and provides better distribution of the compounds along the small intestine. Suckling piglets, 14-day old, were divided into 2 groups, control and with butyrate addition. Control pigs received a standard feed supplemented with a microencapsulated acidifier (0.3%). Butyrate pigs received the same feed and acidifier, as controls, supplemented with butyrate/yucca preparation (0.3%). At days 28 (weaning), 35 and 56 of life, 6 pigs from each group were killed and the gastrointestinal tract was harvested and measured. The initial body weight (14 d) of pigs from the control group was higher than that of the experimental, but at day 56 it did not differ significantly (P=0.48). The relative stomach and pancreas weights in the experimental group showed a tendency toward higher values in all time points, and the small intestine relative weight in day 35 was higher as compared to the controls. The growth rate of the pigs at day 35 decreased significantly in the control (P=0.007), but did not in the experimental (P=0.058) group compared to day 31. Measurements of the small intestine sections revealed increased mucosa thickness in butyrate treated pigs. This was due to both villi and crypt enlargement. In conclusion, our results demonstrated the effectiveness sodium butyrate and *Yucca Shidigera* extract in the prevention of weaning problems in pigs, however further research on the novel preparation is needed.

Key words: feed additives, short chain fatty acids, small intestine development, pig

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INTRODUCTION

In intensive farming, the production animals are overloaded with stress resulting from high production demands and high animal concentration. This increases the possibility of organism malfunction, which in particular may concern the gastrointestinal system. Therefore introduction of new tools to protect the production animals is crucial. Pathogenic and conditionally pathogenic bacteria are hazardous factors in animal production. They can get into the alimentary tract together with feedstuffs and in the case of favorable conditions they can multiply and develop serious illnesses often invading the whole organism, and causing the animal death. The ban on the use of antibiotics as growth promoters in the EU countries in 2006 stimulated a great number of studies on the use of alternatives to antibiotics as the organic acid compositions, various plant extracts, enzyme cocktails as well as probiotics and other substances as the feed additives.

Short chain fatty acids (SCFA) are one of such alternatives. SCFA are the main end product of bacterial fermentation, and the main source of anions in the hind gut in animals and man (Reodriger, 1980, Lærke et al., 2007). The main SCFA are: acetic, propionic, butyric, lactic, formic, valeric, malic and capronic acid. In the study of Kotunia et al. (2004), on newborn piglets fed milk replacer formula supplemented with a sodium salt of butyric acid demonstrated positive effect on the development of the small intestine mucosa. Sodium butyrate exerts various effects in the organism but it is the main source of energy for the enterocytes and colonocytes showing more marked activity in the distal than in the proximal part of the colon (Bugat & Bentejac, 1993). Theoretically, sodium butyrate is able to cover 80% of the colonocytes and 5-10% of the whole organism energy needs (Cummings, 1995). Moreover, sodium butyrate has mitogenic and anti-inflamatory properties (Salminen et al., 1998).

Yucca Schidigera extract has been used in animal production as a factor encouraging friendly bacteria growth and reducing ammonia production in the gastrointestinal tract and fecal odors in animal excreta (Cheeke, 2000, Colina et al., 2001). These effects are mainly due to carbohydrate components and stilbenes that have urease-inhibiting properties (Oleszek et al., 1999, Cheeke, 2000). Yucca extract influences nitrogen metabolism trough the reduction of urea and ammonia in serum of cows (Hussain & Cheeke, 1995) rabbits (Hussain et al., 1996) and in rats (Killeen et al., 1998). In pigs increased growth rate and improved feed conversion efficiency (Cole & Tuck, 1995) as well as inhibition of Gram-positive bacteria and reductions in numbers of pigs born dead have been reported (Cline et al., 1996).

AIM

The aim of the present study was to determine the effect of protected sodium butyrate and *Yucca Schidigera* extract on small intestine structure and pig performance during the first 56 days of life.

MATERIAL AND METHODS

Experiments were carried out on Polish Large White x Pietrain breed pigs of both sexes from the 14^{th} to the 56^{th} day of life in standard farm conditions. Animals were divided into the control group and the experimental group and were fed on an appropriate diet for each developing period. Until weaning (28^{th} day) pigs were kept with the sows and received creep feed (M1) from the 14^{th} day of life. During the two weeks post-weaning pigs were fed the M2 diet, and from the 43^{rd} to the 56^{th} day of life pigs received the M3 diet (Table 1). Pigs from the control group (Control) received protected organic acids (fumaric, malic, citric), while pigs from the experimental group received the same acids and additionally sodium butyrate and *Yucca Schidigera* extract in the protected form in feed (Butyrate). Both feed additive preparation were administered at the level of 3 kg/ton feed from the 14^{th} to the 42^{nd} day of life and at the level of 2 kg/ton feed from the 43^{rd} to 56^{th} day of life. The composition of feed additive is displayed in Table 2.

From weaning to the 56^{th} day of life pigs were weighed weekly for body weight gain calculations. For studying the influence of sodium butyrate and *Yucca Shidigera* extract on the development of the gastrointestinal tract at the age of 28, 35 and 56 days six pigs from each group were killed by sodium pentobarbiturate overdose (20 mg/kg b.wt.). The entire gastrointestinal tract was isolated and

the weight of the stomach, small intestine, pancreas and liver was measured. Samples from the duodenum (10 cm distal to pylorus) and jejunum (50% of the length) were fixed in Bouin solution, dehydrated and embedded in ParaPlast Regular (P-3558, Sigma, Germany). Four µm slices were stained with haematoxylin and eosin and examined by a light microscope (Zeiss, Germany) fitted with image analysis system. Measurements of the villi length, crypt depth and tunica muscularis were done using Microimage v.4.0. Statistical analysis of the results was made using unpaired Student t-test and one-way analysis of variance followed by Tukey test (Statistica 6.0, StatSoft, Poland).

Table 1. Components of the feed mixtures (M1, M2i M3) applied in control and experimental pigs from the 14th to the 56th day of age

Tablica 1. Sastojci krmnih smjesa (M1, M2 i M3) primijenjenih u pokusnih i kontrolnih svinja u dobi od 14. i 56. dana

Components - Sastojci	M1 (14-28 day) (14-28 dan)	M2 (29-42 day) (29-42 dan)	M3 (43-56 day) (43-56 dan)
Calcium - Kalcij	1.0%	0.65 %	0.65 %
Phosphor - Fosfor	0.6%	0.50 %	0.50 %
Sodium - Natrij	0.1%	0.20 %	0.20 %
Lysine - Lizin	1.4%	1.25 %	1.15 %
Methionine - Metionin	1.0%	0.8 %	0.75 %
Threonine - Treonin	0.9%	0.8 %	0.75 %
Tryptophan - Triptofan	0.2%	0.2 %	0.20 %
Fat - Masnoća	10.3%	3.0 %	30.0 %
Protein - Bjelančevina	19.2%	16.5 %	16 %
Fiber - Vlakno	2.2%	4.0 %	4.0 %
Energy - Energija	15.4 MJ	13 MJ	13 MJ
Lactose - Laktoza	10.0%		
Ash - Pepeo	4.0%	7.0 %	6.0 %

Table 2. Composition of the feed additive preparation used in control and experimental diet

Tablica 2. Sastav pripravka dodanog hrani u kontrolnom i pokusnom obroku

	Control - Kontrolni	Experimental - Pokusni
Sodium butyrate (E-470) - Natrijev biturat	-	30%
Fumaric acid (E-297) - Fumarična kiselina	10%	10%
Malic acid (E-296) - Malična kiselina	5%	5%
Citric acid (E-330) - Citrična kiselina	5%	5%
Yucca Schidigeri extract Ekstrakt *Yucca Schidigeri*	-	2.5%
Triglycerides from vegetal origin Trigliceridi biljnog podrijetla	ad 100%	ad 100%

RESULTS

The mean body weight of pigs from the control group was significantly higher compared to that of the experimental at the 14th day of life (P=0.01). This difference was present at the 31st day of life (P=0.02), while from the 35th day of life the mean body weight of the experimental pigs showed a tendency towards lower value than that of controls. At the 56th day of life the body weight of pigs of both groups did not differ significantly (P=0.48) (Table 3). The dynamics of growth from the 35th to the 56th day of life did not show significant differences between the mean daily growth rate of pigs from the control and the experimental group (P=0.23). (Table 3). Feed conversion ratio (FRC) calculated from weaning (28th day) to the 56th day of life was better in the sodium butyrate and Yucca supplemented pigs (1.72 kg feed/kg b wt.) than in the control pigs (2.16 kg feed/kg b wt.). In the experimental pigs the relative weight of the stomach and pancreas showed a tendency towards higher values than in the controls at the 28th, 35th and 56th day of life (Table 4). The relative weight of the small intestine of the experimental pigs showed a tendency towards higher value at the 35th day of life compared to the controls (P=0.09), but at the 56th day of life it was significantly lower in the experimental pigs (P=0.01) compared to the controls (Table 4). The relative weight of the liver varied at different ages and showed significantly higher values sodium in butyrate and *Yucca Schidigera* fed pigs at the 35th day of life and significantly lower values at the 28th and 56th day of life compared to the controls (Table 4). The histometrical analysis of the structure of the duodenal wall did not differ between the control and the experimental group at the 56th day of age. In the middle jejunum the thickness of the mucosa, the length of villi and the depth of the crypts were significantly increased in sodium butyrate/yucca treated pigs compared to controls (Table 5).

Table 3. Body weight and mean daily growth rate of control and experimental pigs from the 14th to the 56th day of age. (Means ± SE)

Tablica 3. Tjelesna masa i prosječni dnevni prirast svinja pokusne i kontrolne skupine od 14. do 56. dana starosti (Prosjek SE)

Age (days) Dob (dana)	Body weight - Tjelesna masa (kg)		Mean daily growth rate (kg/d) Prosječni dnevni prirast (kg/d)	
	Control Kontrolna	Experimental Pokusna	Control Kontrolna	Experimental Pokusna
14 (n=24)	4.5±0.2[a]	3.9±0.1[b]		
28 (n=24)	7.2±0.2[a]	6.5±0.1[b]	0.19±0.01	0.19±0.02
31 (n=18)	8.0±0.3	7.2±0.1	0.29±0.04	0.29±0.04
35 (n=18)	8.7±0.3	8.0±0.2	0.17±0.02*	0.20±0.03
42 (n=12)	10.5±0.5	10.4±0.2	0.27±0.05	0.34±0.02
49 (n=12)	13.0±0.4	13.1±0.9	0.36±0.03	0.39±0.09
56 (n=12)	15.9±0.3	16.1±0.6	0.41±0.02	0.43±0.14

a, b – means in rows with different superscripts differ significantly at the respective pig's age, P<0.05

*- represents significant difference between the mean daily growth rate at the 35th and 31st day of age in control pigs, P<0.05

Table 4. Relative weight of the stomach, liver, pancreas and small intestine of control and experimental pigs. (Means n=6±SE)

Tablica 4. Relativna težina želuca, jetre, gušterače i tankog crijeva pokusnih i kontrolnih svinja

Age (days) Dob (dana)	28		35		56	
	Control Kontrolna	Experimental Pokusna	Control Kontrolna	Experimental Pokusna	Control Kontrolna	Experimental Pokusna
Stomach weight (%) Težina želuca (%)	0.54±0.02	0.70±0.06	0.76±0.04	0.88±0.07	0.82±0.02	0.86±0.09
Liver weight (%) Težina jetre (%)	3.00±0.09[a]	2.76±0.08[b]	2.71±0.12[a]	3.32±0.13[b]	3.08±0.06[a]	2.80±0.15[b]
Pancreas weight (%) Težina gušterače (%)	0.17±0.01	0.21±0.03	0.18±0.02	0.20±0.02	0.20±0.01	0.21±0.01
Small intestine weight (%) Težina tankog crijeva (%)	4.61±0.21	5.22±0.58	4.81±0.19	5.57±0.41	5.32±0.12[a]	4.92±0.44[b]
Small intestine length (cm/kg) Duljina tankog crijeva (cm/kg)	126.1±4.8	123.4±3.1	109.1±2.2[a]	121.5±3.8[b]	70.0±1.9	70.5±7.0

a, b – means in rows with different superscripts differ significantly at the respective pig's age, P<0.05

Table 5. Mucosa thickness, villi lenght, crypt depth and muscularis of the duodenum and jejunum of control and experimental pigs at the 56th day of age. (Mean n=6±SE)

Tablica 5. Debljina sluznice (mukoze), duljina dlačica (villi), dubina udubljenja (crypt) i muskulatura jejunuma kontrolnih i pokusnih svinja 56. dana starosti

	Mucosa thickness Debljina sluznice (µm)	Villi length Duljina dlačica (µm)	Crypt depth Dubina udubina (µm)	Muscularis Muskulatura (µm)
Duodenum				
Control Kontrolna	842.0±21.6	539.1±41.9	311.4±20.3	231.4±14.1
Experimental Pokusna	830.9±48.0	523.1±68.0	310.3±18.6	221.5±3.9
Jejunum				
Control Kontrolna	714.5±26.0[a]	506.7±29.7[a]	224.6±16.0[a]	277.7±38.0
Experimental Pokusna	842.0±47.9[b]	597.9±47.5[b]	243.6±6.4[b]	226.1±37.3

a, b – means in columns with different superscripts differ significantly at the respective intestine segment, P<0.05

DISCUSION

The process of weaning is complex and concerns not only changes connected with the kind of ingested food, but it is also connected with the environmental and psychical changes, that influence the function of the digestive tract. During this time a decrease in feed intake and body weight gain or even body weight decrease occur. Histological and biochemical changes in the structure and functioning of the small intestine decrease the digestion and absorption capacity of gastrointestinal tract leading to serious economic losses. After the ban of antibiotics in feed in the EU various alternatives have been proposed to overcome postweaning problems in pig production. Besides acidifiers, the use of factors promoting the development of the gastrointestinal tract in growing pigs is probably one of the most promising strategies (Valverde Piedra et al 2006).

In our study we have used a microencapsulated blend of organic acid, SCFA and *Yucca Schidigera* extract in order to achieve the target and minimize side effects. The substances protected by the lipid matrix are slowly released all along the gastrointestinal tract preventing abrupt changes in the pH in the upper gastrointestinal tract which may take place when unprotected acids are used.

Feeding pigs from the 14th to the 56th day of life on sodium butyrate and yucca extract supplemented diet showed a positive effect in their growth. Despite the fact that the beginning body weight of pigs from this group was substantially lower than that of control pigs, at the 56 day of life their body weight was the same as that of controls. Concomitant to that the butyrate and yucca supplemented pigs demonstrated better daily body weight gain. However, at the 35th day of life there was a decrease in the growth rate of booth groups, but this is connected with weaning and the change of feed. It is noteworthy that the changes in the growth rate of pigs of the experimental group showed a tendency towards lower values while, in the pigs of the control group they were significant as compared to the values from the 31st day of life. The studies of Galfi and Bokori (1990) showed that in pigs fed a diet supplemented with 0.17% unprotected sodium butyrate from 7 to 102 kg. the average daily body gain increased by 23% and the feed conversion ratio was optimized by 11.8% due to specific feed utilization. In our studies FCR was 20.3% higher in the experimental pigs, suggesting that this is due to the effect of both sodium butyrate and yucca extract. One may suggest that these effects are the results of more dynamic development of the gastrointestinal tract and adaptation to post-weaning challenge. The increased relative small intestine weight and the increased mucosa thickness in the jejunum in the sodium butyrate and yucca extract treated pigs support this statement. Moreover, the studies of Galfi and Bokori (1990) demonstrated increased microvilli length and crypt depth in the ileum of fattened pigs. Studies of Kotunia et al. (2004) on piglets fed a milk formula supplemented with sodium butyrate showed increased mucosa thickness, villi length and crypt depth in the distal jejunum and ileum in comparison to controls.

In conclusion, enrichment of protected organic acids with sodium butyrate and *Yucca Schidigeri* extract increases feed conversion ratio and to some extent the mean daily body weight gain after weaning. Moreover, it protects against the excessive body weight loss after weaning. Higher values of the relative small intestine weight and increased mucosa thickness due to both villi and crypt enlargement in the pigs receiving sodium butyrate demonstrated stimulation of the development of the intestinal mucosa leading to better feed conversion.

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SAŽETAK

Nakon zabrane antibiotika kao promotora rasta, proizvodnja životinja u EU suočila se sa sve više poremećaja nakon odbijanja pa je predloženo nekoliko rješenja (acidifikatori, probiotici, biljni ekstrakti, kokteli enzima, itd.). Kratkolančane masne kiseline glavni su krajnji proizvod bakterijske fermentacije u stražnjem crijevu u monogastričnih životinja pa se mogu upotrijebiti za snabdijevanje energijom epitelnih stanica crijevne sluznice /mukoze. Stoga je cilj ovog rada bio testirati korisnost novog natrijevog biturata u mikrokapsuli i pripravka ekstrakta *Yucca Schidigera* (potiče rast dobroćudnih bakterija, smanjuje amonijak) na performansu svinja i razvoj tankog crijeva u kritičnom razdoblju odbijanja. Mikrokapsule pomažu da se izbjegne oštećenje želuca i pruža bolju razdiobu spojeva duž tankog crijeva. Praščići na sisi, u dobi od 14 dana, bili su podijeljeni u dvije skupine, kontrolnu i skupinu s dodavanjem biturata. Kontrolni praščići dobivali su standardnu hranu s dodatkom acidifikatora/zakiseljivača u mikrokapsuli (0.03%). Praščići na biturati ma dobivali su istu hranu i acidifikator/zakiseljivač s dodatkom biturata/yucca pripravka (0.03%). U dobi od 28 (odbijanje), 35 i 56 dana žrtvovano je 6 svinja iz svake skupine

te je pregledan i izmjeren gastrointestinalni sustav. Početna tjelesna masa (14. dan) svinja iz kontrolne skupine bila je veća od mase svinja pokusne skupine ali 56. dan nije se značajno razlikovala (P=0.48). Relativne mase želuca i gušterače u pokusnoj skupini pokazale su tendenciju viših vrijednosti u svim razdobljima, a relativna masa tankog crijeva 35. dana bila je viša u usporedbi s kontrolnom skupinom. Stopa rasta svinja 35. dana znatno se smanjila u kontrolnoj skupini (P=0.007) ali ne u konrolnoj skupini (P=0.058) u usporedbi s 31. danom. Mjere tankog crijeva pokazale su povećanu debljinu sluznice u svinja tretiranih biturato m. To se pripisuje povećanju dlačica i udubina (villi i crypt). U zaključku, naši su rezultati pokazali djelotvornost natrijevog biturata i ekstrakta *Yucca Schidigeri* u prevenciji problema odbijanja u svinja, međutim, potrebna su dalja istraživanja novih pripravaka.

Ključne riječi: dodaci hrani, kratkolančane masne kiseline, razvoj tankog crijeva, svinja