Original article

Short- and medium-chain fatty acids as a feed supplement for weaning and nursery pigs

E. Hanczakowska[1], A. Szewczyk[2], M. Świątkiewicz[1], K. Okoń[3]

[1] National Research Institute of Animal Production, Department of Animal Nutrition and Feed Science, Krakowska 1, 32-083 Balice, Poland
[2] Graduate student at National Research Institute of Animal Production, Department of Animal Nutrition and Feed Science, Krakowska 1, 32-083 Balice, Poland
[3] Jagiellonian University, Medical College, Department of Pathomorphology, Grzegórzecka 16, 31-531 Krakow, Poland

Abstract

The effect of supplementing piglet diets with acidifiers containing the short-chain fatty acids – SCFA (propionic C_3 and formic) together with medium-chain fatty acids – MCFA (caprylic C_8 and capric C_{10}) on performance, nutrient apparent digestibility, intestinal microflora and small intestine structure was investigated. The study was performed on 326 piglets allocated to 5 experimental groups. They were fed a standard diet (Group I – control) or a standard diet supplemented with 0.5% propionic and formic acids (Group II – PF). Group III (PF + C_8), group IV (PF + C_{10}) and group V (PF + C_8 + C_{10}) received the same mixture as group II with a supplement of 0.2% of caprylic and/or capric acids, respectively. Apparent digestibility of nutrients and microbiological analyses were performed. The structure of jejunum mucosa was also examined. Piglets receiving capric acid (groups IV and V) had the highest body weight gains. Piglets receiving MCFA digested protein and fiber better ($P \leq 0.05$) than piglets receiving SCFA as acidifier. There was no difference in intestinal microflora except for *Clostridium perfringens*, the population of which was reduced by SCFA (group II). Villi of the mucosal epithelium were the highest ($P \leq 0.05$) in piglets receiving SCFA with capric acid (group IV). Under the conditions of this study a mixture of SCFA (propionic and formic) with capric acid significantly improves performance of piglets.

Key words: swine, propionic acid, formic acid, caprylic acid, capric acid, intestinal histology, intestinal microflora

Introduction

Weaning is the most dangerous time in a piglet's life. During this period, the intestinal tract and immune system of piglets are not fully developed (Bailey et al. 2005), which makes them easy targets for microorganisms which induce gastrointestinal pathologies (Castillo et al. 2006). They also have to adapt to the new stressful conditions which are associated with reduced feed consumption, temporary malnutrition

Correspondence to: E. Hanczakowska, e-mail : ewa.hanczakowska@izoo.krakow.pl

and growth retardation (Lalles et al. 2004). Antibiotic growth promoters were used to prevent these problems but they were banned by the European Union several years ago (Anadón, 2006).

Different agents are proposed to prevent piglet gastrointestinal disorders, among them short-chain fatty acids (SCFA), used for many years as antimicrobial acidifiers (Partanen and Mroz 1999). Gedek et al. (1992) found that the antimicrobial activity of fumaric acid is higher than that of hydrochloric acid or antibiotic tylosine. Medium-chain fatty acids (MCFA) are another type of organic acid which could be considered as antibiotic replacers as they have strong antibacterial activity against Gram-positive cocci (Bergsson et al. 2001) and *Escherichia coli* (Skřivanova et al. 2009). Apart from this antimicrobial activity they can also improve postweaning gut development (Tang et al. 1999). Such positive changes (greater villus height) may result in improved performance of piglets, as was found in our previous study (Hanczakowska et al. 2011a).

Research hypothesis: Short chain fatty acids have a positive effect on piglet performance. This effect can be enhanced by the addition of medium chain fatty acids.

The aim of this study was to determine possible synergistic effects enhancing the positive effect of both dietary SCFA (propionic C_3 and formic) mixed with MCFA (caprylic C_8 and capric C_{10}) on piglet health and performance, apparent digestibility of nutrients, intestinal microflora and structure of mucosal epithelium of the small intestine.

Materials and Methods

All procedures included in this study, relating to the use of live animals, were accepted by the II Local Ethics Committee for Experiments with Animals in Krakow, Poland.

Animals, housing and management

The study was performed on 326 weaning and nursery pigs derived from 30 Polish Landrace × Large White Polish sows mated with Duroc × Pietrain boars. All sows were in their 3rd or 4th parity. The trial started at 7 days of piglet age and lasted until 84 days of age. The piglets were weaned at 35 days of age. All animals were kept in groups in straw bedded pens (one litter per pen), and had free access to water. During the study all piglets were individually weighed at 1, 35, 56 and 84 days of age and the daily weight gains were calculated for every piglet. At 56 days of age six pig-

lets were randomly chosen from each group (i.e. one animal from each litter) and slaughtered. Their intestines were prepared and digesta from the small intestine (jejunum) was taken for microbiological analysis. At the end of the experiment (day 84) the remaining piglets were left for further rearing.

Between 56 and 70 days of age, randomly selected piglets from the growth study (10-12 animals from each group) were used for the digestibility trial. Apparent digestibility of nutrients was estimated using the indicator method with Cr_2O_3 (3.0 g/kg). The adaptation period lasted 10 days and the balance period 5 days. Feces from one litter were collected once a day and frozen at -20ºC. At the end of the collection period all daily feces were mixed together and an average sample was prepared for each litter.

Design of experiment

The piglets were allocated to 5 groups with different treatment. The design of the experiment was as follows: Group I (control) – standard feed mixture; Group II (PF) – standard mixture supplemented with 0.5% of 1:1 w/w ratio mixture of propionic and formic acids. Group III (PF + C_8), Group IV (PF + C_{10}) and Group V (PF + C_8 + C_{10}) received the same mixture as Group II but supplemented with 0.2% of caprylic (C_8) or 0.2% of capric (C_{10}) acid or 0.2% of 1:1 w/w ratio mixture of caprylic and capric acids (C_8 + C_{10}), respectively. All acids were supplied by Sigma Aldrich.

Diets and treatments

The experimental feed mixture was based on soybean, wheat, barley and milk products. It was fed *ad libitum* from the 7th day of age to weaning (day 35). Rationed feeding was used from weaning to the end of the experiment (day 84), according to the scheme: 35-42 days of age – 0.2 kg of feed per litter; 43-49 days of age – 0.4 kg; 50-56 days of age – 0.6 kg; 57-63 days of age – 0.8 kg; 64-70 days of age – 1.0 kg; 71-84 days of age – 1.25 kg. Diets contained 205 g of protein and 12.65 MJ EM per kg.

Microbiological analyses

Microbiological tests were made in cecum and small intestine digesta. The number of *Escherichia coli* and *Clostridium sp.* was determined. The presence of yeasts and molds was also estimated. The tests were carried out using plate methods with agar medium by

bioMerieux, according to European Standards (EN ISO 2001, 2004, ISO 1987).

Histological analysis

Samples from the small intestines were spread on polystyrene plates and fixed in 10% buffered formalin. The intestinal wall was precisely cut and four slides were prepared from each sample. They were stained with hematoxylin and eosin and embedded in paraffin. Villus height and crypt depth were evaluated using a light microscope. Data acquisition was performed with a Zeiss Axioscop microscope (Zeiss GmbH, Germany) and CDD ZVS-47DE camera (Optronics Inc., USA) connected by RGB line with a graphic card GraBIT PCI (Soft Imaging System GmbH, Germany) installed in a standard PC computer.

Chemical analyses

Gross composition of feeds and feces was analyzed according to AOAC (2005). Chromium content in feed and feces were determined after a nitric acid × perchloric acid wet ash preparation (AOAC, 2005). Apparent digestibility coefficients (ADC) were calculated using the following equation:

$$ADC\ (\%) = 100 - [100 \times (a/b) \times (c/d)]$$

where: a = chromium content in feed (%), b = chromium content in feces (%), c = nutrient content in feces (%), d = nutrient content in feed (%).

Acidity of stomach, ileum, and cecum 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 ileum and cecum digesta were separated on a CP-Wax 58 column (Varian BV, Middelburg, the Netherlands) (25 m, 0.53 mm, 1 m, carrier gas – helium, 6 ml/min), with a column oven temperature program from 90 to 200ºC, using a Varian 3400 gas chromatograph (Varian Associates Inc., Walnut Creek, USA) equipped with a Varian 8200 CX autosampler (2000 C), FID detector (2600 C), and Star Chromatography Workstation Software.

Statistical analysis

Statistical analysis of treatment effects was conducted by analysis of variance with comparisons of means using Duncan's multiple range test at $P \leq 0.05$ and $P \leq 0.01$ levels of significance using the Statistica v 5.1 package.

Results

Mean body weight of piglets at the beginning of the study (7th day of age) were in the range of 2.62 to 2.79 kg (Table 1). At the end of the study piglets receiving acidifiers had significantly ($P \leq 0.05$) higher body mass than those of the control group. Differences between group II, obtaining SCFA alone, and groups obtaining SCFA with MCFA were not significant. There was also no significant difference in the number of culled piglets between particular groups. Groups with mixed acidifiers (III, IV and V) grew faster than the control and SCFA groups ($P \leq 0.05$) until weaning and also during the following three weeks. In the last period of the experiment (56-84 days of age) none of the acids used had any effect on piglet body weight gain. At the end of the experiment all piglets receiving MCFA had higher body weight than that of the control ($P \leq 0.05$). Differences in feed conversion were significant ($P \leq 0.05$) only in the period between 35 and 56 days of age and between control group I and group III (PF + C_8) receiving caprylic acid.

Piglets receiving MCFA digested protein significantly ($P \leq 0.05$) better than those from groups with SCFA alone or without any acidifier (Table 2). Digestibility of fat was significantly ($P \leq 0.05$) higher in piglets receiving capric acid (PF + C_{10}) and both MCFA (PF + C_8 + C_{10}). Digestibility of fiber was also significantly higher in all MCFA groups compared to control and SCFA groups.

Stomach contents of piglets from control and SCFA groups had a lower pH than the others (Table 3) and in the case of piglets receiving only propionic and formic (group II -PF) acids this difference was significant ($P \leq 0.01$). Some significant ($P \leq 0.05$) but irregular differences were also found in the jejunum contents. There was also no significant difference in the total content of volatile fatty acids in ileum or cecum contents between the control and experimental groups (Table 3). The total amount of these acids was much higher in the cecum than in the ileum. In the ileum the only significant difference ($P \leq 0.01$) was found for valeric and isovaleric acids and also for isobutyric acid ($P \leq 0.05$), the content of which were the highest in group II. Differences in cecum content, though significant in the case of butyric, isobutyric and valeric acids, were irregular.

Table 1. Rearing indices of piglets.

Item	Group I control	Group II (PF)	Group III (PF+C_8)	Group IV (PF+C_{10})	Group V (PF+C_8+C_{10})	SEM
No. of piglets born	61	69	66	65	65	–
Average No of piglets born per litter	10.1	11.5	11.0	10.8	10.8	–
Average No of piglets weaned per litter	9.8	10.8	10.6	10.3	10.5	–
Average No of piglets at 84th day per litter	9.5	10.8	10.5	10.2	10.3	–
Dead piglets at 35 day, No	2	4	2	3	2	–
Dead piglets at 35 to 84, No	2	0	1	1	1	–
Body weight (kg) in days of age:						
7th	2.62	2.62	2.79	2.71	2.67	0.037
35th	7.45[a]	7.87[ab]	8.07[ab]	8.20[b]	8.12[ab]	0.106
56th	10.82[Aa]	11.36[ABab]	13.06[Cc]	12.59[Cbc]	12.93[Cc]	0.185
84th	21.85[a]	22.94[ab]	24.00[b]	24.54[b]	24.27[b]	0.311
Average daily gain (g) in periods of life:						
7th – 35th	168[Aa]	182[ABab]	189[ABb]	195[Bb]	190[ABb]	2.762
35th – 56th	160[Aa]	166[Aa]	238[Bb]	209[ABb]	229[Bb]	6.078
56th – 84th	393	413	391	427	405	6.983
35th – 84th	294[a]	308[ab]	325[ab]	334[b]	329[ab]	5.289
1st – 84th	250[a]	264[ab]	275[b]	284[b]	281[b]	3.906
Feed conversion ratio (kg/kg) in periods of life:						
7th – 35th	0.122	0.114	0.111	0.096	0.111	0.004
35th – 56th	1.59[b]	1.44[ab]	1.07[a]	1.20[ab]	1.16[ab]	0.068
56th – 84th	2.32	2.26	2.37	2.19	2.34	0.087
35th – 84th	2.15	2.06	1.96	1.91	2.00	0.074
7st – 84th	1.55	1.49	1.47	1.44	1.44	0.037

Explanations: Mean values in the same row with different letters differ significantly at P≤0.01 (A, B, C) or P≤0.05 (a, b, c).

Table 2. Apparent digestibility coefficients of nutrients, % (based on 4 litters per group).

Item	Group I control	Group II (PF)	Group III (PF+C_8)	Group IV (PF+C_{10})	Group V (PF+C_8+C_{10})	SEM
Dry matter	80.2	81.8	81.3	81.4	81.7	0.24
Crude protein	72.6[Aa]	73.3[Aa]	76.5[ABb]	78.5[Bb]	76.3[ABb]	0.59
Crude fat	30.6[a]	33.9[ab]	31.03[a]	39.9[b]	39.3[b]	1.23
Crude fiber	20.5[aa]	21.1[Aa]	35.3[Bc]	33.1[Bbc]	28.4[ABb]	1.58
N-free extract	90.7	91.3	90.9	90.8	91.4	0.30

Explanations: Mean values in the same row with different letters differ significantly at P≤0.01 (A, B) or P≤0.05 (a, b, c).

Significant differences were found only in the intestinal population of *Clostridium perfringens* (Table 4), which was significantly (P≤0.01) lowered by supplement of formic and propionic acids when compared with both groups fed caprylic or capric acids alone. No significant difference was observed in intestinal content of *Escherichia coli,* yeasts and molds.

The results of epithelium structure estimation are shown in Table 5. Villi of the mucosa epithelium of piglets receiving capric acid (PF + C_{10}) were higher than those from control and SCFA groups (P≤0.01) and also from the remaining groups receiving MCFA (P≤0.05). Crypts were deepest (P≤0.01) in the epithelium of piglets in the control group.

Discussion

Partanen et al. (2007) found a beneficial effect from a mixture of formic and propionic acids on piglet performance. However, according to Kabara et al. (1972) the effect of these acids is not as strong as that of MCFA; thus, mixing SCFA with MCFA should produce better results. In our earlier study (Hanczakowska et al. 2011b) with dicarboxylic fumaric acid we found such an improvement only in the case of caprylic, but not capric, acid. This could be due to better digestibility of protein, which in turn was the result of higher intestinal villi in this group. In the present experiment piglets receiving capric acid had slightly better body weight gains

Table 3. Acidity of digesta in the stomach and in various parts of intestines and volatile fatty acid (VFA) content of piglets' chyme, μmol/g chyme (based on 6 piglets per group).

Item	Group I control	Group II (PF)	Group III (PF+C_8)	Group IV (PF+C_{10})	Group V (PF+C_8+C_{10})	SEM
Stomach	1.65 AB	1.26[A]	2.06[B]	2.08[B]	2.15[B]	0.093
Small intestine						
Duodenum	5.76	5.29	5.37	5.32	5.58	0.075
Jejunum	5.87[b]	5.69[ab]	5.57[ab]	5.45[a]	5.88[b]	0.076
Ileum	5.58	5.46	5.37	5.40	5.29	0.039
Caecum	5.50	5.46	5.58	5.41	5.48	0.052
Volatile fatty acid content of ileum and caecum chyme:						
Ileum						
acetic	4.590	5.930	3.690	4.690	4.060	0.699
propionic	0.805	0.671	0.254	0.708	0.233	0.589
isobutyric	0.033	0.298	0.062	0.066	0.068	0.045
butyric	0.320	0.172	0.335	0.305	0.348	0.043
isovaleric	0.037[A]	0.351[B]	0.089[A]	0.084[A]	0.091[a]	0.031
valeric	0.017[A]	0.199[B]	0.036[A]	0.027[A]	0.032[A]	0.02
Total	5.804	7.620	4.466	5.881	4.830	1.183
Caecum						
acetic	53.233	68.747	53.085	41.818	57.880	4.135
propionic	38.432	37.31	40.547	35.117	31.989	1.689
isobutyric	0.472[ab]	1.161[b]	0.675[ab]	0.681[ab]	0.442[a]	0.104
butyric	20.332[ab]	14.755[ab]	22.497[b]	21.814[b]	12.837[a]	1.345
isovaleric	0.222	0.576	0.329	0.509	0.252	0.061
valeric	3.217[ABa]	3.555[ABab]	5.314[Bb]	3.037[ABa]	1.639[Aa]	0.353
Total	115.909	126.104	122.447	102.476	105.041	6.103

Explanations: Mean values in the same row with different letters differ significantly at P≤0.01 (A, B) or P≤0.05 (a, b).

Table 4. Microbial counts in small intestine (ileum) digesta, $\log_{10}$CFU/(1 g chyme) (based on 6 piglets per group)

Item	Group I control	Group II (PF)	Group III (PF+C_8)	Group IV (PF+C_{10})	Group V (PF+C_8+C_{10})	SEM
Escherichia coli	5.68	5.48	5.44	5.49	5.69	0.40
Clostridium perfringens	2.14[ABb]	0.41[Aa]	2.84[Bb]	2.75[Bb]	1.89[ABb]	0.25
Candida albicans + *Candida* sp	0.80	1.05	1.54	0.93	0.74	0.21
Moulds	2.57	2.01	1.73	1.84	2.60	0.18
Fungi and moulds	2.13	2.03	2.19	1.93	2.65	0.19

Explanations: Mean values in the same row with different letters differ significantly at P≤0.01 (A, B) or P≤0.05 (a, b).

Table 5. Mucosal epithelium structure of the ileum (based on 6 piglets per group).

Ileum morphology	Group I control	Group II (PF)	Group III (PF+C_8)	Group IV (PF+C_{10})	Group V (PF+C_8+C_{10})	SEM
No	271	257	157	297	150	–
Villus height, μm	255[Aa]	264[Aa]	272[ABb]	292[Bc]	272[ABb]	2.453
Villus width, μm	116[Bb]	122[BCb]	127[Cc]	129[Cc]	109[Aa]	0.913
No	161	168	107	85	92	–
Crypt depth, μm	287[Aa]	391[Cc]	328[Bb]	293[Aa]	305[ABa]	3.520
Villus height/Crypt depth	0.888	0.675	0.829	0.996	0.892	–

Explanations: Mean values in the same row with different letters differ significantly at P≤0.01 (A, B) or P≤0.05 (a, b).

than those fed caprylic acid. This could be due to the slightly better protein and significantly better fat digestibility of caprilic acid, which, in turn, could result from higher intestinal villi.

It is known that MCFA are valuable energy sources especially for weaning piglets as their absorption is less dependent on both enzymatic and emulsifying agents (Chiang et al. 1990). On the other hand they have to be fed in relatively high amounts to be a source of energy, i.e. 8% as in the study of Cera et al. (1989). In this study a lower amount of MCFA was used (0.2%), which shows that the significant improvement in performance of piglets, especially before 56 days of age, must be a result of other factors. In any case, limited nutrition was used to supply piglets with the same amount of supplements.

Marounek et al. (2003) observed strong antimicrobial activity of caprylic acid and, to a lesser extent capric acid against *Escherichia coli*. No lowering effect of MCFA on the population of these bacteria was found in the present study. This could be due to the differences in *E. coli* strains as there is a strain-dependent variability in the susceptibility of *E. coli* to MCFA (Skřivanova and Marounek 2007). In the study of Marounek et al. (2003) caprylic acid had antimicrobial activity against *E. coli* at pH about 6.0 or higher. In this study pH was lower than 6.0 in all parts of the digestive tract. A significant reduction in the amount of *Clostridium perfringens* was found in group II. These bacteria are the most important cause of clostridial enteric disease in domestic animals (Songer and Uzal, 2005) but no improvement of piglet health or performance was found in this group, though such an improvement was found earlier by Partanen et al. (2007). Of the two types of *C. perfringens* infections, the type C infection is characterized by frequently hemorrhagic, often fatal, necrotic enteritis in young pigs (Songer and Meer, 1996). Because no such cases were found in this study, it can be assumed that *C. perfringens* type A was found in these animals; it is a member of the normal flora of the pig intestine (Nabuurs et al. 1983). Thus changes in intestinal microflora were perhaps not the reason for differences in piglet performance between 7 and 56 days of age.

In the present study the mixture of propionic and formic acids lowered the pH of chyme in the stomach but this difference was not significant. In the small intestine and cecum differences were small. This could be due to the buffering capacity of feed which is high especially in feeds with high protein content (Lević et al. 2005), and such feeds are used in piglet feeding. Similar results were obtained by Bolduan et al. (1988). The significantly weaker acidic reaction of stomach contents in piglets fed with MCFA is hard

to explain, although Zentek et al. (2012) found increased production of ammonia, which could increase the pH of digesta in piglets fed with MCFA not in the stomach, but in the small intestine.

Neither SCFA nor MCFA had an effect on volatile fatty acid (VFA) content in the ileum except for isobutyric, isovaleric and valeric acids, but their content was low. These acids originate from decomposition of proteins, from valine, leucine and proline, respectively (Arkowitz et al. 1994, Mackie et al. 1998) and their higher content suggests higher protein fermentation. In this study only numbers of *Escherichia* and *Clostridium* were estimated and results did not confirm this view, but changes in other kinds of bacteria, e.g. *Lactobacillus* (Castillo et al. 2006) cannot be excluded. Similar results were obtained earlier by Gabert et al. (1995).

Results of studies on supplementing pig diets with propionic or formic acids are not consistent. It seems that they are active primarily in the stomach; Risley et al. (1992) found no appreciable effect of organic acids on stomach, ileum or cecum pH, VFA profile or microflora population. Perhaps the gut ecosystem has a substantial capacity to resist changes (Jensen 1998).

The large intestine in monogastric animals is a main site of microbial hydrolysis of undigested feed, mainly fiber, hence the large content of VFA in the cecum. In pigs, this VFA production is intensified by a rapid rate of digesta passage. In spite of this higher VFA content, the pH of digesta in the cecum and ileum did not differ, as also reported by Argenzio and Southworth (1975).

Piglets' intestinal epithelial cells serve, among others, digestive and absorptive functions. They also serve as a barrier against antigens and bacteria and maintain proper viscosity of the luminal contents (Pacha 2000). The epithelial cells near the villous tip have the greatest digestive and absorptive capacity, and hence villous height gives an indication of the functional capacity of enterocytes (Hampson 1986). In the present study villi in the ileum of piglets receiving SCFA with caprylic or capric acids were slightly higher than those of control animals. These differences could be one of the reasons for higher nutrient absorption and digestibility, especially in the case of piglets receiving SCFA and capric acid.

These piglets also had high body weight gains before 56 days of age, i.e. the day when samples of intestine were collected. Similar results were obtained in previous studies on the effects of medium-chain fatty acids alone or mixed with fumaric acid on nursery pig performance (Hanczakowska et al. 2011a,b).

Conclusion

Mixture of short-chain fatty acids (propionic and formic) with capric acid significantly improves performance of weaning and nursery pigs. An acidifier mixture of SCFA with capric acid improves the digestibility of nutrients, probably due to structural changes in the small intestine mucosa.

References

Anadón A (**2006**) The EU ban of antibiotics as feed additives: alternatives and consumer safety. J Vet Pharm Therap 29: 41- 44.

AOAC (**2005**) Official Methods of Analysis, 18th ed., AOAC International, Gaithersburg, USA.

Argenzio RA, Southworth M (**1975**) Sites of organic acid production and absorption in gastrointestinal of the pig. American Journal of Physiology 228: 454-460.

Arkowitz RA, Dhe-Paganon S, Abeles RH (**1994**) The fate of the carboxyl oxygens during D-proline reduction by clostridial proline reductase. Arch Biochem Biophys 311: 457-459.

Bailey M, Haverson K, Inman C, Harris C, Jones P, Corfield G, Miller B, Stokes C (**2005**) The development of the mucosal immune system pre- and post-weaning: balancing regulatory and effector function. Proc Nutr Soc 64: 451-457.

Bergsson G, Arnfinnsson J, Steingrimsson O, Thormar H (**2001**) Killing of Gram-positive cocci by fatty acids and monoglycerides. APMIS 109: 670-678.

Bolduan VG, Jung H, Schneider R, Block J, Klenke B (**1988**) Influence of propionic and formic acid on piglets. J Anim Physiol Anim Nutr 59: 72-78.

Castillo M, Martin-Orúe SM, Roca M, Manzanilla EG, Badiola I, Perez JF, Gasa J (**2006**) The response of gastrointestinal microbiota to avilamycin, butyrate and plant extracts in early-weaned pigs. J Anim Sci 84: 2725-2734.

Cera KR, Mahan DC, Reinhart GA (**1989**) Postweaning swine performance and serum profile responses to supplemental medium-chain free fatty acids and tallow. J Anim Sci 67: 2048-2055.

Chiang SH, Pettigrew JE, Clarke SD, Cornelius SG (**1990**) Limits medium-chain and long-chain triacylglycerol utilization by neonatal piglets. J Anim Sci 68: 1632-1638.

EN ISO 7937 (**2004**) Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of *Clostridium perfringens.* Colony-count technique. European Standard, 3rd ed., BSI, London, UK.

EN ISO 16654 (**2001**) Microbiology of food and animalfeeding stuffs – Horizontal method for the detection of *Escherichia coli* 0157. European Standard, 1st ed., BSI, London, UK.

Gabert VM, Sauer WC, Schmitz M, Arens F, Mosenthin R (**1995**) The effect of formic acid and buffering capacity on the ileal digestibilities of amino acids and bacterial populations and metabolites in the small intestine of weanling pigs fed semipurified fish meal diets. Can J Anim Sci 75: 615-623.

Gedek B, Roth FX, Kirchgessner M, Wiechler S, Bott A,

Eidelsburger U (**1992**) Influence of fumaric acid, hydrochloric acid, sodium formate, tylosin and toyocerin on the microflora in different segments of the gastrointestinal tract. J Anim Physiol Anim Nutr 68: 209-217.

Hampson DJ (**1986**) Alterations in piglet small intestinal structure at weaning. Res Vet Sci 40: 32-40.

Hanczakowska E, Szewczyk A, Okoń K (**2011a**) Effects of dietary caprylic and capric acids on piglet performance and mucosal epithelium structure of the ileum. J Anim Feed Sci 20: 556-565.

Hanczakowska E, Szewczyk A, Okoń K (**2011b**) Caprylic, Capric and/or fumaric acids as antibiotic replacements in piglet feed. Ann Anim Sci 11: 115-124.

PN-EN ISO 7954: 1999-Microbiology-general guidance for enumeration of yeasts and moulds-Colony Mount technique at 25ºC (ISO 7954:1987).

Jensen BB (**1998**) The impact of feed additives on the microbial ecology of the gut in young pigs. J Anim Feed Sci 7: 45-64.

Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP (**1972**) Fatty acids and derivatives as antimicrobial agents. Antimicrob Agents Chemother 2: 23-28.

Lallés JP, Boudry G, Favier C, Le Floc'h N, Luron I, Montagne L, Oswald IP, Pie S, Piel C, Séve B (**2004**) Gut function and dysfunction in young pigs: physiology. Anim Res 53: 301-316.

Lević J, Prodanović O, Sredanović S (**2005**) Understanding the buffering capacity in feedstuffs. Biotechnol Anim Husb 21: 309-313.

Mackie RI, Stroot PG, Varel VH (**1998**) Biochemical identification and biological origin of key odor components in livestock waste. J Anim Sci 76: 1331-1342.

Marounek M, Skřivanová E, Rada V (**2003**) Susceptibility of Escherichia coli to C2-C18 fatty acids. Folia Microbiologica 48: 731-735.

Nabuurs MJ, Haagsma J, Van de Molen EJ, Van der Heijden PJ (**1983**) Diarrhea in one to three week-old piglets associated with Clostridium perfringens type A. Ann Rech Vet. 14: 408-411.

Pacha J (**2000**) Development of intestinal transport function in mammals. Physiol Rev 80: 1633-1667.

Partanen KH, Mroz Z (**1999**) Organic acids for performance enhancement in pig diets. Nutr Res Rev 12: 117-145.

Partanen KH, Siljander-Rasi H, Pentikainen J, Pelkonen S, Fossi M (**2007**) Effects of weaning age and formic acid-based feed additives on pigs from weaning to slaughter. Arch Anim Nutr 61: 336-356.

Risley CR, Kornegay ET, Lindemann MD, Wood CM, Eigel WN (**1992**) Effect of feeding organic acids on selected intestinal content measurements at varying times postweaning in pigs. J Anim Sci 70: 196-206.

Skřivanová E, Marounek M (**2007**) Influence of pH on antimicrobial activity of organic acids against rabbit enteropathogenic strain of Escherichia coli. Folia Microbiologica 52: 70-72.

Skřivanová E, Molatová Z, Skřivanová V, Marounek M (**2009**) Inhibitory activity of rabbit milk and medium-chain fatty acids against enteropathogenic Escherichia coli O128. Vet Microbiol 135: 358-362.

Songer JG, Meer RR (**1996**) Genotyping of Clostridium perfringens by polymerase chain reaction is a useful adjunct to diagnosis of clostridial enteric disease in animals. Anaerobe 2: 197-203.

Songer JG, Uzal FA (**2005**) Clostridial enteric infections in pigs. J Vet Diagn Invest 17: 528-536.

Tang M, Laarveld B, Van Kessel AG, Hamilton DL, Estrada A, Patience JF (**1999**) Effect of segregated early weaning on postweaning small intestinal development in pigs. J Anim Sci 77: 3191-3200.

Zentek J, Buchheit-Renko S, Männer K, Pieper R, Vahjen W (**2012**) Intestinal concentrations of free and encapsulated dietary medium-chain fatty acids and effects on gastric microbial ecology and bacterial metabolic products in the digestive tract of piglets. Arch Anim Nutr 66: 14-26.