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CAPRYLIC, CAPRIC AND/OR FUMARIC ACIDS AS ANTIBIOTIC REPLACEMENTS IN PIGLET FEED

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Abstract

The aim of the experiment was to compare the effect of dietary fumaric acid and fumaric acid mixed with caprylic or capric acid on performance and changes in the digestive system of piglets. The experiment was performed on 242 piglets (24 litters) from 7 to 84 days of age. Piglets were allocated to 4 groups with 6 litters per group. Group I (control) received standard feed mixture, group II received the same mixture supplemented with fumaric acid (15 g kg^{-1}). Groups III and IV were fed the same diets as group II but they additionally received 2 g kg^{-1} of caprylic (group III) or capric acid (group IV). Body weight gains and feed consumption were measured, and apparent digestibility of nutrients was estimated. Microbiological and histological analyses were also performed in the small intestine. Piglets receiving fumaric and caprylic acids had significantly higher body weight gains (276 g) than those receiving fumaric and capric acids (251 g) and the control piglets (234 g). Duodenal contents of experimental piglets had lower pH compared to that of control piglets, but this difference was significant only for the fumaric and caprylic acid group. Mixture of fumaric and caprylic acids improved apparent protein and fibre digestibility but there were no significant differences between experimental groups. These acids also reduced piglet mortality. All acids significantly decreased the amount of *Escherichia coli* in the small intestinal digesta but fumaric acid was the most active. Fumaric acid also reduced the *Clostridium* population but to a lesser extent than *E. coli*. All acids affected the structure of jejunal epithelium. Villi in the experimental groups were higher and crypts deeper than those in control animals. It can be stated that the mixture of fumaric and caprylic acids significantly improves performance of piglets. This improvement may result from changes in acidity in the upper part of the digestive tract and from structural changes of the small intestinal mucosa.

Key words: piglet feeding, fumaric acid, caprylic acid, capric acid

A few years ago the European Union banned antibiotics from farm animal feeds and thus some substitutes have to be found, especially for young animals (Anadón, 2006). Weaning piglets are sensitive animals with not fully developed gastrointes-

tinal tract and immune system (Bailey et al., 2005). Weaning is the most dangerous moment in which piglets are an easy target for microorganisms inducing gastrointestinal pathologies (Bikker et al. 2006). Different agents are proposed to prevent piglets' gastrointestinal disorders (Castillo et al., 2006). The principal of these are short chain fatty acids (SCFA) used for many years as antimicrobial acidifiers (Partanen and Mroz, 1999).

Medium chain fatty acids (MCFA) are another type of organic acids which could be considered as antibiotic substitutes; they have strong antibacterial activity against Gram-positive cocci (Bergsson et al., 2001) and *Escherichia coli* (Skrivanová 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 parameters of piglets as shown in the study in which piglets were fed whole *Cuphea* seeds, which is a natural source of MCFA (Dierick et al., 2003). Taking into consideration these partially similar and partially different (antimicrobial activity or improving the structure of intestinal epithelium) effects of SCFA and MCFA, it seemed interesting to determine the possible synergistic effect of both dietary fumaric (SCFA) and caprylic or capric acids (MCFA) on piglets' health and performance.

Material and methods

The Second Local Kraków Ethics Committee for Experiments with Animals gave its approval to all the experimental procedures relating to the use of animals.

Animals and diets

The experiment was performed on 242 piglets (24 litters) derived from Polish Landrace sows and a Duroc × Pietrain boar. Piglets received experimental mixtures *ad libitum* from 7 days of age to weaning. Rationed feeding was used from weaning (day 35) to the end of the experiment (day 84). The amount of feed was increased every 7th day by 200 g. Piglets were allocated to 4 groups with 6 litters each. Animals were kept in group pens, each litter in a separate pen. They were fed standard feed mixture (group C, control) or the same mixture supplemented with fumaric acid (15 g kg^{-1}) in group F. Groups F+C8 and F+C10 received the same mixture supplemented with the same amount of fumaric acid and 2 g kg^{-1} of caprylic or capric acid, respectively. Feed was given in loose form. All acids were supplied by Sigma-Aldrich. The composition of feeds is given in Table 1.

Animals were weighed individually at 1, 35, 56 and 84 days of age. Feed consumption was measured daily for each pen. Mean body weight gains and feed utilization were calculated from the results obtained.

Apparent digestibility of nutrients was estimated in 4 litters from each group (8–12 piglets on average) between 56 and 70 days of age using the indicator method with Cr_2O_3 as an indicator. The adaptation period lasted 10 days and the balance period was 5 days long.

Table 1. Composition and nutritive value of feed mixtures (g/kg)

Components	Control group	F	F+C8	F+C10
Wheat, ground	430	430	430	430
Triticale	100	100	100	100
Barley, ground	100	100	100	100
Soybean meal	250	250	250	250
Dried whey	50	50	50	50
Dried milk	30	30	30	30
Rapeseed oil	10	10	10	10
Salt (NaCl)	2.5	2.5	2.5	2.5
Limestone	8.0	8.0	8.0	8.0
Dicalcium phosphate	12.0	12.0	12.0	12.0
L-lysine	1.0	1.0	1.0	1.0
DL-methionine	1.5	1.5	1.5	1.5
Premix PP-prestarter*	5.0	5.0	5.0	5.0
Fumaric acid		15	15	15
Caprylic acid		2.0		1.0
Capric acid			2.0	1.0
Content of nutrients in 1 kg of mixture				
Metabolizable energy (MJ)**	12.68	12.94	13.03	12.93
Dry matter (g)	887.8	891.2	891.7	893.9
Crude protein (g)	200.0	200.0	203.0	203.0
Digestible protein (g)	145.8	151.6	155.3	155.2
Crude fat (g)	26.0	24.0	26.0	25.8
Crude fibre (g)	29.1	28.5	28.2	28.4
Crude ash (g)	52.7	53.6	55.2	52.2
N-free extractives (g)	580.0	585.1	579.3	584.5

*premix composition: vitamin A – 2700000 IU; D3 – 400000 IU; E – 8.0 g; K3 – 0.5g ; B_1 – 0.5 g; B_2 – 0.8 g; B_6 – 0.8 g; B_{12} – 0.008 g; pantothenic acid – 2.8 g; choline chloride – 70 g; folic acid – 0.2 g; nicotinic acid – 5.0; magnesium – 10 g; manganese – 12 g; iodine – 0.1 g; zinc – 30 g; iron – 20 g; copper – 32 g; cobalt – 0.06 g; selenium – 0.04 g; complete limestone to 1000 g.

** ME calculated using the equation of Hoffmann and Schiemann (1980).

Six piglets were randomly chosen from each group (i.e. one piglet from each litter) and slaughtered at 56 days of age. Their intestines were prepared and small intestinal (jejunal) digesta was collected for microbiological analysis.

Microbiological analyses

Microbiological tests were made in caecum and small intestinal digesta. The number of aerobic bacteria (especially *Escherichia coli*) and anaerobic bacteria (especially *Clostridium sp.*) was determined. The presence of yeasts and moulds was also estimated. The tests were made with the plate methods using agar medium by bioMerieux, according to Polish Standards (PN-ISO 7954, 1999; PN-EN ISO 4833/Ap1, 2005; PN-EN-ISO 7937, 2005; PN-ISO-15213, 2005; PN-ISO 7251, 2006).

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 under light microscope. Data acquisition was performed with 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 the standard PC computer.

Chemical analyses

Gross composition of feeds and faeces was analysed according to AOAC (1995). Chromium content of feed and faeces was determined after wet ashing with nitric acid and perchloric acid (AOAC, 2000).

Acidity of digesta in the stomach, small intestine and caecum was measured with a CP-411 pH-meter equipped with Metron 12-01 electrode.

Statistical analysis

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

Results

Mean body weights of piglets at the beginning of the experiment were 1.6–1.7 kg (Table 2). At weaning (day 35) piglets receiving fumaric acid or fumaric and caprylic acids were significantly larger ($P<0.01$) than control piglets. At the end of the experiment only piglets fed fumaric and caprylic acids had significantly better results than the control ones. Piglets fed fumaric acid or fumaric and caprylic acids also utilized feed more efficiently ($P<0.01$) than those of the two remaining groups. All acids caused a decrease in piglet mortality. Nine dead piglets were found in the control group during the whole experiment compared to 3, 5 and 3 in groups F, F+C8 and F+C10, respectively.

The mixture of fumaric and caprylic acids improved ($P<0.05$) apparent protein and fibre digestibility when compared to controls but there was no significant difference between experimental groups (Table 3).

Duodenal contents of experimental piglets had lower pH compared to the control piglets (Table 4) but this difference was significant ($P<0.05$) only for the fumaric and caprylic acid group. A similar phenomenon was found in jejunum but the difference was more apparent ($P<0.01$).

All acids significantly reduced the amount of *E. coli* in the small intestinal digesta but the activity of fumaric acid alone was also significantly ($P<0.05$) higher than that of the mixture of fumaric acid and MCFA (Table 5). Fumaric acid alone

and mixed with capric acid also lowered the amount of *Clostridium perfringens* but this difference was not significant. Acids had no distinct effect on fungi and moulds except for their significant increase (P<0.05) in piglets receiving MCFA when compared to fumaric acid alone.

Table 2. Rearing indices of piglets

	Control	F	F+C8	F+C10	SEM
No. of piglets born	66	63	56	57	
Average no. of piglets born per litter	11.0	10.5	9.3	9.5	
Average no. of piglets weaned per litter	10.2	10.1	9.3	9.0	
Average no. of piglets on day 84 per litter	9.5	10.0	8.5	9.0	
Dead piglets on day 35	5	2	0	3	
Dead piglets on days 35 to 84	4	1	5	0	
Body weight (kg) at days of age:					
1	1.67	1.73	1.65	1.62	0.02
35	7.38 A	8.31 B	8.46 B	8.00 AB	0.12
56	10.67 Aa	11.89 ABb	12.30 Bb	11.60 ABab	0.20
84	21.08 Aa	23.56 ABb	24.57 Bb	22.47 ABab	0.37
Average daily gain (g) in age periods (days):					
1 – 35	168 Aa	194 Ab	200 Bb	187 Ab	3.37
35 – 56	157	170	183	171	6.03
56 – 84	371 Aa	417ABa	438 Bb	388 ABa	5.10
35 – 84	279 A	311 AB	328 B	295 AB	7.97
1 – 84	234 Aa	263 ABb	276 Bc	251 Aab	4.35
Daily feed intake (g) in age periods (days):					
1 – 35	20	19	19	21	0.04
35 – 56	233	259	240	237	5.91
56 – 84	912	892	916	924	13.70
35 – 84	621	622	625	626	14.82
1 – 84	374	376	373	374	17.53
Feed conversion ratio (kg/kg) in age periods (days):					
1 –35	0.121 Cb	0.097 Ba	0.093 Aa	0.110 Bb	0.002
35 – 56	1.53 B	1.52 B	1.31 A	1.38 A	0.02
56 – 84	2.56 C	2.14 A	2.09 A	2.38 B	0.02
35 – 84	2.30 Cc	2.00 ABa	1.90 Aa	2.12 Bb	0.03
1 – 84	1.63 Cd	1.43 ABb	1.35 Aa	1.49 Bc	0.13

Mean values in the same row with different letters differ significantly A, B – P≤0.01; a, b, c – P≤0.05.

All acids had an effect on the structure of jejunal epithelium (Table 6). Villi in these groups were higher than those in control and in the case of groups F and F+C8 this difference was significant (P<0.05). Differences in crypt depth were not significant but they were also deeper in the jejunal epithelium of piglets receiving acids.

Table 3. Digestibility coefficients of nutrients (%)

	Control	F	F+C8	F+C10	SEM
Dry matter	81.6	82.9	83.5	82.3	0.32
Crude fat	72.9 a	75.8 ab	76.5 b	74.9 ab	0.56
Crude fat	32.3	34.2	35.6	35.8	1.45
Crude fibre	18.5 a	25.3 ab	27.0 b	21.9 ab	1.33
N-free extract	93.1	93.2	94.3	93.1	0.24

Mean values in the same row with different letters differ significantly a,b – P≤0.05.

Table 4. Acidity of digesta in the stomach and in different parts of the small intestine

pH	K	F	F+C8	F+C10	SEM
Stomach	2.72	2.48	2.68	2.60	0.103
Small intestine					
– beginning	5.59 b	5.00 ab	4.81 a	5.38 ab	0.095
– middle	5.89 Bb	5.39 ABa	5.20 Aa	5.54 ABab	0.080
– end	5.66	5.22	5.43	5.36	0.043
– caecum	5.53 b	5.12 a	5.33 ab	5.38 ab	0.060

Mean values in the same row with different letters differ significantly A, B – P≤0.01; a, b – P≤0.05.

Table 5. Microbiology of small intestinal digesta (Log10CFU/1g chyme)

Microorganisms	Control	F	F+C8	F+C10	SEM
Aerobic bacteria	7.216	6.593	7.414	6.891	0.321
Escherichia coli	4.707 Bb	2.254 Aa	4.319 Ab	4.127 Ab	0.310
Anaerobic bacteria	6.614	6.371	7.571	6.927	0.265
Clostridium perfringens	3.685	2.447	3.089	2.606	0.215
Candida albicans + Candida sp.	3.203 ab	2.816 a	3.559 b	3.561 b	0.122
Moulds	3.420 ab	3.229 a	3.536 ab	3.940 b	0.100
Fungi and moulds	3.689 ab	3.393 a	4.083 b	4.103 b	0.115

Mean values in the same row with different letters differ significantly A, B, C – P≤0.01; a, b – P≤0.05.

Table 6. Morphological characteristics of the small intestine

Jejunum morphology	Control	F	F+C8	F+C10	SEM
Villus height (μm)	233 a	301 b	314 b	287 ab	11.545
Villus width (μm)	116 a	122 ab	123 ab	127 b	2.667
Crypt depth (μm)	280	307	307	331	7.894
Villus height/Crypt depth	0.834 a	0.990 ab	1.033 b	0.870 ab	0.034

Mean values in the same row with different letters differ significantly A, B – P≤0.01; a, b – P≤0.05.

Discussion

According to Lawlor et al. (2006) fumaric acid improves performance of weaning piglets. Also medium chain fatty acids can improve piglet performance (Dierick et al., 2003). MCFA are usually considered as a readily available energy source

(Odle, 1999) but in such a situation their dose has to be high (Cera et al., 1989) and their odour limits feed consumption (Decuypere and Dierick, 2003). In this experiment the dose of MCFA was very low as they were considered an antimicrobial factor (Marounek et al., 2003). It seemed possible that mixing fumaric acid with MCFA would improve their effect in piglet feeding. The results, i.e. mean body weight gains and feed utilization suggest that this is true for caprylic but not capric acid. The reason for this improvement is not quite clear.

The increase in protein digestibility, which was the highest in piglets receiving fumaric and caprylic acids was probably too small to significantly improve piglet performance. Also Falkowski and Aherne (1984), who supplemented piglet feed with fumaric acid reported only a small increase in protein digestibility. In the experiment of Eidelsburger et al. (1992) fumaric acid improved performance of piglets only to a small degree and Giesting and Easter (1991) found no improvement in nutrient digestibility or piglet weight gain when fumaric acid was used as feed additive. Thus it can be assumed that the improvement achieved in the current experiment was mainly the effect of supplementing caprylic acid. We also found a significant improvement in piglet performance after supplementing feed with this acid in another experiment (unpublished).

It is possible that better digestibility of protein, especially in piglets receiving fumaric and caprylic acids was due to pH being lowered by these acids in the upper part of the digestive tract. Regarding reduced secretion of the pancreas in young piglets (Lindemann et al., 1986), lower pH could enable longer activity of pepsin originating from the stomach. According to Piva et al. (2002) higher gastric pH leads to ineffective proteolysis as a result of limited pepsin activity. Fumaric acid is known as an active antimicrobial factor (Gedek et al. 1992; Blank et al., 2003), which was found also in the current experiment. On the other hand, Marounek et al. (2003) observed strong antimicrobial activity of caprylic acid and, to a lesser extent, of capric acid against *Escherichia coli*. But in the current experiment none of these acids enhanced the effect of fumaric acid. 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 (Skřivanová and Marounek, 2007). In the current experiment MCFA were not active also in the case of other microorganisms. The few dead piglets had no symptoms of illness and thus they were not subjected to postmortem examination.

Piglets' intestinal epithelial cells serve, among others, digestive and absorptive functions. They also act as a barrier against antigens and bacteria and maintain proper viscosity of the luminal contents (Pacha, 2000). The epithelial cells near the villus tip have the greatest digestive and absorptive capacity and hence villus height gives an indication of functional capacity of enterocytes (Hampson, 1986). In the current experiment villi in the experimental groups, especially those receiving fumaric and caprylic acids were higher than villi in control animals, and villus height to crypt depth ratio was also higher in the experimental groups, which could be one of the reasons for higher protein digestibility and better performance of piglets.

Summing up the results of the present experiment, it is concluded that mixture of fumaric and caprylic acid significantly improves performance of weaning piglets.

This improvement can be due to the changes in acidity in the upper part of the digestive tract and structural changes of the small intestinal mucosa.

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Kwasy kaprylowy, kaprynowy i /lub fumarowy jako zamienniki antybiotyków w paszy dla prosiąt

STRESZCZENIE

Celem doświadczenia było porównanie wpływu dodatku kwasu fumarowego lub mieszaniny kwasu fumarowego z kwasem kaprylowym lub kaprynowym do paszy dla prosiąt na wskaźniki produkcyjne i zmiany w przewodzie pokarmowym. Doświadczenie przeprowadzono na 242 prosiętach (24 miotach) od 7. do 84. dnia życia. Prosięta przydzielono do 4 grup po 6 miotów w każdej. Grupa I (kontrolna) otrzymywała standardową mieszankę paszową, grupa II otrzymywała tę samą mieszankę z dodatkiem kwasu fumarowego (15 g kg^{-1}). Grupy III i IV były karmione jak grupa II ale otrzymywały dodatkowo 2g kg^{-1} kwasu kaprylowego (grupa III) lub kaprynowego (grupa IV).

Mierzono przyrosty prosiąt i spożycie paszy. Określano również strawność pozorną składników pokarmowych. Przeprowadzono też analizy mikrobiologiczne i histologiczne w jelicie cienkim.

Prosięta otrzymujące kwasy fumarowy i kaprylowy miały przyrosty (276 g) istotnie wyższe niż otrzymujące kwasy fumarowy i kaprynowy (251 g) i kontrolne (234 g). Treść dwunastnicy prosiąt doświadczanych miała niższe pH niż kontrolnych, ale różnice były istotne tylko w przypadku grupy otrzymującej kwas fumarowy i kaprylowy. Mieszanina kwasu fumarowego i kaprylowego poprawiła pozorną strawność białka i włókna, ale nie było istotnych różnic pomiędzy grupami doświadczalnymi. Kwasy zmniejszyły też śmiertelność prosiąt. Wszystkie kwasy obniżyły istotnie ilość *Escherichia coli* w treści jelita cienkiego, ale najaktywniejszy był kwas fumarowy. Kwas fumarowy zredukował też populację *Clostridium*, ale w mniejszym stopniu niż *E. coli*. Kwasy zmieniły strukturę nabłonka jelita

cienkiego. Kosmki w grupach doświadczalnych były wyższe, a krypty głębsze niż u prosiąt kontrolnych.

Można stwierdzić, że mieszanina kwasu fumarowego i kaprylowego istotnie poprawia wskaźniki produkcyjne prosiąt. Poprawa ta może być wynikiem zmian kwasowości w wyższych partiach przewodu pokarmowego, a także zmian w strukturze ich nabłonka.