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Effects of dietary caprylic and capric acids on piglet performance and mucosal epithelium structure of the ileum

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

[1]*National Research Institute of Animal Production, Department of Animal Nutrition and Feed Science 32-083 Balice, Poland*
[2]*Medical College Jagiellonian University, Department of Pathomorphology Grzegórzecka 16, 31-531 Kraków, Poland*

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ABSTRACT

Effects of a diet supplemented with caprylic and/or capric acid on piglet performance, apparent digestibility of nutrients, intestinal microflora and small intestine (ileum) structure were investigated. The experiment was performed on 252 piglets (24 litters) allocated to 4 experimental groups (6 litters each). The animals were fed with a standard feed mixture (control) or the same mixture supplemented with 2 g of caprylic or capric acid (groups C_8 and C_{10}, respectively) per 1 kg of feed. Group C_8+C_{10} received 1 g of caprylic and 1 g of capric acid. Apparent digestibility was estimated using Cr_2O_3 as an indicator, while microbiological analyses were performed using standard agar plates. The short-chain fatty acid (SCFA) content of the ileum and caecum digesta was analysed using Varian 340 analyzer. The piglets receiving caprylic or capric acids grew significantly ($P<0.01$) faster than the control ones (average daily gains during the whole experiment, i.e. between days 1 and 84 of age, were: 288, 269, 278 and 234 g, respectively). The best feed utilization (1.3 kg per kg) was found in animals receiving caprylic acid. The acids also lowered piglet mortality, while significantly increased protein digestibility ($P<0.01$) and, to a lesser degree ($P<0.05$), also fibre digestibility. There was no significant difference in acidity of the digesta between control and experimental groups. Capric acid increased the amount of aerobic bacteria as compared to the control group, but the amount of *Escherichia coli* remained unchanged. The population of *Clostridium perfringens* was reduced by both caprylic and capric acids ($P<0.01$). Acids had no effect on SCFA content of the ileum but lowered the acetic acid content of the caecum digesta. Capric acid had the strongest effect on villi, which were significantly higher (306 µm) than in the control group (233 µm). Differences

[3] Corresponding author: e-mail: e-mail: ehan@op.pl

in crypt depth were smaller but the crypts were also the deepest in piglets receiving capric acid. The results suggest that caprylic and capric acids added to the feed improve piglet performance, probably due to positive changes in the mucosal epithelium structure of the ileum.

KEY WORDS: medium-chain fatty acids, caprylic acid, capric acid, ileum epithelium structure, feed improvement, piglets

INTRODUCTION

Fatty acids, especially medium-chain fatty acids (MCFA), are substances which can be considered as antibiotic replacers. They have strong antibacterial activity against Gram-positive cocci (Bergsson et al., 2001) and *Escherichia coli* (Skřivanová et al., 2009). Moreover, they can improve post-weaning gut development (Tang et al., 1999). Such positive changes (e.g., greater villus height) may result in improved performance parameters of piglets, as shown in a study in which piglets were fed with whole *Cuphea* seeds, which is a natural source of MCFA (Dierick et al., 2003). These acids can also be a rapidly available energy source for young animals, due to their direct transport *via* portal blood to the liver, with no reestrification or chylomicron formation and preferential oxidation in the mitochondria (Odle, 1997).

The aim of this experiment was to investigate the effect of a diet supplemented with caprylic and capric acids on piglet performance, apparent digestibility of nutrients, intestinal microflora and structure of mucosal epithelium of the small intestine in piglets.

MATERIAL AND METHODS

The II Local Cracow Ethics Committee for Experiments on Animals approved all procedures used in this experiment.

Animals and diets

The experiment was performed on 252 piglets (24 litters) originating from Polish Landrace sows and Duroc × Pietrain boar. Piglets received experimental mixtures *ad libitum* from 7 days of age to weaning (35 days of age), whereas from weaning to the end of the experiment (84 days of age) restricted feeding was used. The amount of feed was increased every 7 days by 200 g. Piglets were allocated to 4 groups, with 6 litters in each group, kept in group pens (each litter in a separate pen) and fed with the standard feed mixture (control) or the same mixture supplemented with caprylic (octanoic) or capric (decanoic) acid or both of these acids (groups C_8, C_{10} and C_8+C_{10}, respectively). Both acids were supplied by Sigma Aldrich. Composition of feed mixtures is given in Table 1.

Table 1. Composition and nutritive value of feed mixtures

Item	Control	C_8	C_{10}	C_8+C_{10}
Components, g/kg				
wheat, ground	430	430	430	430
triticale	100	100	100	100
barley, ground	100	100	100	100
soyabean 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 [1]	5.0	5.0	5.0	5.0
caprylic acid	-	2.0	-	1.0
capric acid	-	-	2.0	1.0
Content of nutrients in 1 kg of mixture				
metabolizable energy[2], MJ	12.68	12.87	12.88	12.94
dry matter, g	887.8	889.7	888.5	889.6
crude protein, g	200.0	203.5	203.0	203.0
digestible protein, g	145.8	155.9	154.7	154.5
crude fat, g	26.0	26.6	26.0	26.3
crude fibre, g	29.1	28.2	27.0	28.0
crude ash, g	52.7	53.0	52.9	60.3
N-free extractives, g	580.0	578.4	579.6	572.0

[1] premix composition: IU: vit. A 2700000; vit. D_3 400000; g: vit. E 8.0; vit. K_3 0.5; vit. B_1 0.5; vit. B_2 0.8; vit. B_6 0.8; vit. B_{12} 0.008; pantothenic acid 2.8; choline chloride 70; folic acid 0.2; nicotinic acid 5.0; Mg 10; Mn 12; J 0.1; Zn 30; Fe 20; Cu 32; Co 0.06; S 0.04; complete limestone to 1000; [2] ME calculated using Hoffmann and Schiemann equation (1980)

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

Apparent digestibility of nutrients was estimated in 4 litters from each group (10-12 piglets per litter) between the 56 and 70 days of age, using Cr_2O_3 as indicator. The adaptation period lasted 10 days, whereas the balance period 5 days.

Six piglets from each group (i.e. one piglet from each litter) were slaughtered at 56 days of age, their intestines were prepared and digesta from the small intestine (ileum) was taken for microbiological analysis.

Microbiological analyses

Microbiological tests were made in the small intestine (ileum) digesta. Aerobic bacteria (especially *Escherichia coli*) and anaerobic ones (especially *Clostridium perfringens*) were counted. Presence of yeasts and moulds was also estimated. The tests were made with the plate method on an agar medium by bioMerieux, according to European Standards (ISO 4833:2003; EN ISO 16654:2001; ISO 15213:2003; EN ISO 7937:2004; ISO 7954:1987).

Histological analysis

Samples from the small intestine (ileum) 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 haematoxylin and eosin and embedded in paraffin. Villus height and crypt depth were evaluated under a light 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.

Chemical analyses

The composition of feeds and faeces was analysed according to AOAC (1995) methods, while chromium oxide content, after nitric acid and perchloric acid wet ash preparation (AOAC, 2000).

Acidity of the digesta in the stomach, various parts of the small intestine and caecum was measured with CP-411 pH-meter equipped with a Metron 12-01 electrode. Short-chain fatty acids (SCFA) in the ileum and caecum were separated on the CP-Wax-58 column (25 m, 0.53 mm, 1µ, carrier gas - helium, 6 ml/min), with a temperature programme in a column oven from 90ºC to 200ºC, using Varian 3400 gas chromatograph equipped with a Varian 8200 CX autosampler (2000 C), FID detector (2600 C) and Star Chromatography Workstation Software.

Statistical analysis

Analysis of variance, with comparisons of means by Duncan's multiple range test at $P<0.05$ and $P<0.01$, was performed using the Statistica 5.1 package.

RESULTS

Mean body weight of newborn piglets was 1.65-1.77 kg (Table 2). At 35 days of age (weaning) the highest body weight was found in piglets receiving caprylic acid (C_8). Piglets fed with capric acid (C_{10}) or with both acids (C_8+C_{10}) grew also faster (P<0.01) than the control ones. At the end of the experiment these differences were still significant. Feed utilization was the best in C_8 group. The remaining two experimental groups did not differ significantly from the control group at P<0.01.

Table 2. Rearing indices of piglets

Item	Control	C_8	C_{10}	C_8+C_{10}	SEM
No. of piglets born	66	60	63	63	-
Average no. of piglets born per litter	11.0	10.0	10.5	10.5	-
Average no. of piglets weaned per litter	10.2	10.0	10.5	9.7	-
Average no. of piglets at 84 days per litter	9.5	9.8	10.0	9.7	-
No. of dead piglets at 35 days	5	0	0	5	-
No. of dead piglets at 35 to 84 days	4	1	3	0	-
Body weight at age, days, kg					
1	1.65	1.77	1.73	1.76	0.02
35	7.38^{Aa}	9.30^{Bc}	8.55^{Bb}	9.08^{Bbc}	0.13
56	10.68^{Aa}	12.96^{Bc}	11.91^{ABb}	12.87^{Bb}	0.20
84	21.08^{A}	25.73^{B}	24.04^{B}	24.89^{B}	0.34
Average daily gain, days, g					
1-35	169^{Aa}	221^{Bc}	200^{Bb}	214^{Bbc}	3.67
35-56	157	174	160	181	5.20
56-84	372^{A}	456^{B}	433^{B}	429^{B}	7.40
35-84	279^{Aa}	335^{Bb}	316^{ABb}	322^{Bb}	5.43
1-84	234^{A}	288^{B}	269^{B}	278^{B}	4.02
Feed conversion ratio, days, kg/kg					
1-35	0.115^{b}	0.073^{a}	0.096^{b}	0.116^{b}	0.01
35-56	1.58	1.34	1.50	1.42	0.06
56-84	2.58^{b}	2.01^{a}	2.19^{ab}	2.18^{ab}	0.09
35-84	2.32^{b}	1.86^{a}	2.02^{ab}	1.99^{ab}	0.07
1-84	1.63^{Bb}	1.30^{Aa}	1.43^{ABb}	1.39^{ABa}	0.04

mean values in the same row with different letters differ significantly: ^{a,b,c} - P≤0.05; ^{A,B} - P≤0.01

Acids lowered also piglet mortality. Nine dead piglets were recorded in the control group while 1, 3 and 5 such cases were found in experimental groups C_8, C_{10} and C_8+C_{10}, respectively (Table 2).

Acids significantly (P<0.01) increased apparent digestibility of crude protein (Table 3) and crude fibre (P<0.05). Digestibility of crude fat was also higher in the experimental groups but differences were not statistically significant.

There were no significant differences in the acidity of the digesta between the control and experimental groups (Table 4).

Table 3. Apparent digestibility of nutrients, % (based on 4 litters per group)

Item	Control	C_8	C_{10}	C_8+C_{10}	SEM
Dry matter	81.6	81.9	82.0	83.6	0.40
Crude protein	71.9[A]	76.6 [B]	76.2[B]	76.1[B]	0.46
Crude fat	32.3	37.4	38.1	42.6	1.75
Crude fibre	18.5[a]	31.5[b]	30.9[b]	28.6[b]	1.91
N-free extractives	93.1	92.0	92.2	93.7	0.36

mean values in the same row with different letters differ significantly: [a,b] - P≤0.05; [A,B] - P≤0.01

Table 4. Acidity of the digesta in the stomach and in various parts of intestines (based on 6 piglets per group)

Organ	Control	C_8	C_{10}	C_8+C_{10}	SEM
Stomach	2.71	2.17	2.36	2.57	0.16
Small intestine					
beginning (duodenum)	5.59	5.64	5.50	5.38	0.08
middle (jejunum)	5.89	5.61	5.46	5.54	0.11
end (ileum)	5.66	5.18	5.32	5.36	0.05
Caecum	5.53	5.40	5.38	5.58	0.07

Capric acid increased the total amount of aerobic bacteria (Table 5) in the small intestine digesta, compared to the control group (P<0.05), but there was no significant difference in the amount of *Escherichia coli.* There was also no difference in total anaerobic bacteria, but the population of *Clostridium perfringens* was lowered by both caprylic and capric acids (P<0.01). Caprylic acid had no antifungal effect and capric acid even increased the amount of fungi and moulds in the digesta. In spite of these differences, when both acids were provided together, the amount of fungi was significantly lower (P<0.01).

Table 5. Microbial counts in the small intestine (ileum) digesta, $\log_{10}$CFU/(1 g chyme) (based on 6 piglets per group)

Microorganisms	Control	C_8	C_{10}	C_8+C_{10}	SEM
Aerobic bacteria	7.22[a]	7.47[ab]	7.98[b]	7.65[ab]	0.12
Escherichia coli	4.70	3.91	4.75	4.93	0.25
Anaerobic bacteria	6.61	5.95	6.85	6.85	0.23
Clostridium perfringers	3.68[B]	2.47[A]	2.51[A]	2.95[AB]	0.16
Candida albicans + *Candida* sp.	3.20[ab]	2.41[a]	3.82[b]	2.28[a]	0.22
Moulds	3.42[B]	3.55[B]	4.34[C]	2.96[A]	0.11
Fungi and moulds	3.69[B]	3.67[B]	4.72[C]	3.15[A]	0.13

mean values in the same row with different letters differ significantly: [a,b] - P≤0.05; [A,B,C] - P≤0.01

There was no significant difference in SCFA content of the small intestine except isobutyric and isovaleric acid, which were present in small amounts (Table 6). Both acids lowered the acetic acid content of the caecum digesta and in the case of caprylic acid this decrease was significant (P<0.05).

Table 6. Short-chain fatty acids (SCFA) content of piglets' ileum and caecum chyme, μmol/1 g chyme (based on 6 piglets per group)

VFA	Control	C_8	C_{10}	C_8+C_{10}	SEM
Ileum					
acetic	3.849	3.937	2.353	4.678	0.63
propionic	0.666	0.327	0.466	0.452	0.07
isobutyric	0.160^{ABb}	0.206^{Bb}	0.116^{ABab}	0.021^{Aa}	0.02
butyric	0.505	0.493	0.049	0.259	0.10
isovaleric	0.079^{a}	0.140^{ab}	0.035^{a}	0.218^{b}	0.02
valeric	0.047	0.106	0.047	0.103	0.18
total	5.306	5.208	3.066	5.731	1.02
Caecum					
acetic	77.153^{b}	52.052^{a}	58.01^{ab}	68.626^{ab}	3.87
propionic	40.875	35.294	36.403	35.206	1.41
isobutyric	0.423^{A}	0.333^{A}	0.524^{A}	1.204^{B}	0.10
butyric	22.763	20.032	21.584	16.285	1.40
isovaleric	0.420^{ab}	0.260^{a}	0.547^{b}	0.295^{a}	0.04
valeric	4.231	4.313	4.705	3.185	0.39
total	145.865	112.284	121.773	124.801	7.21

mean values in the same row with different letters differ significantly: ^{a,b} - P≤0.05; ^{A,B} - P≤0.01

Capric acid had a significant effect on the structure of the ileum epithelium (Table 7). Villi in this group were significantly (P<0.01) higher than in the control group. They were also higher, but not significantly, than in the remaining experimental groups. Differences in crypt depth were smaller. The villus height to crypt depth ratio was higher in the experimental groups than in the control, but these differences were not statistically significant.

Table 7. Mucosal epithelium structure of the ileum (based on 6 piglets per group)

Ileum morphology	Control	C_8	C_{10}	C_8+C_{10}	SEM
Villus height, μm	233^{A}	267^{AB}	306^{B}	268^{AB}	7.86
Villus width, μm	116	114	120	116	2.25
Crypt depth, μm	280^{a}	304^{ab}	338^{b}	280^{a}	0.02
Villus height/crypt depth	0.835	0.959	0.908	0.971	0.03

mean values in the same row with different letters differ significantly: ^{a,b} - P≤0.05; ^{A,B} - P≤0.01

DISCUSSION

Medium-chain fatty acids (MCFA) can improve piglet performance. As they are less dependent on both enzymatic and emulsifying agents, they may be utilized effectively by weanling piglets. On the other hand, such an improvement found by Cera et al. (1989) and Dierick et al. (2003) was not significant and the amount of MCFA used (8% in the experiment of Cera et al., 1989) was much higher than the amount used in the present experiment (0.2%). Thus a significant (P<0.01) improvement of piglet body weight gains found in this experiment is not due to utilizing MCFA as an energy source. In our earlier experiment (Hanczakowska et al., 2011) piglets receiving a mixture of caprylic and fumaric acids grew significantly better than those receiving a mixture of capric and fumaric acid or fumaric acid alone. This suggests that caprylic acid has a better effect on piglet growth, especially before 56 days of age, than capric acid.

Escherichia coli infections can be the most common reason of piglet diseases (Laine et al., 2004). Although Marounek et al. (2003) observed a strong antimicrobial activity of caprylic acid and, to a lesser extent, capric acid against *E. coli*, no lowering effect of MCFA on the population of this bacterium was found in the current experiment. It could be due to differences in *E. coli* strains, as there is a strain-dependent variability in the susceptibility of *E. coli* to MCFA (Skřivanová and Marounek, 2007). However, a significant decrease was observed in the amount of *Clostridium perfringens*, which is the most important cause of clostridial enteric disease in domestic animals (Songer and Uzal, 2005). From the two types of *C. perfringens* infections, type C infection is characterized by frequently haemorrhagic, often fatal, necrotic enteritis in young piglets (Songer and Meer, 1996). No such cases were found in this experiment. Thus it can be assumed that *C. perfringens* type A was present in these animals. It is a member of the normal flora of the pig intestine, but it can also become a cause of enteric disease (Nabuurs et al., 1983). The decline of the population of this microorganism could be the cause of improvement in health, lower mortality and better performance of piglets in the current experiment. The decrease in mortality of young rabbits receiving the feed containing caprylic acid was found also by Skřivanová and Marounek (2002).

MCFAs had no effect on pH along the intestinal tract. This was probably due to their low doses and quick absorption from its proximal part (Odle, 1997). In monogastric animals the large intestine is a main place of decomposing of nutrients undigested in upper parts of intestines by bacteria that produce short-chain fatty acids (Yasuda et al., 2007). Thus the lower amount of SCFA found in the caecum of piglets receiving MCFA could be due to the higher digestibility of nutrients, especially fibre.

Intestinal epithelial cells serve, among others, digestive and absorptive functions. They serve also as a barrier against antigens and bacteria and keep a 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 the functional capacity of enterocytes (Hampson, 1986). In the current experiment, villi in the experimental groups were higher than in the control animals. This could be one of the reasons for the higher protein digestibility and piglets' better performance. Weaning induces morphological changes of the intestinal mucosa, i.e. reduction of villus height due to a loss of enterocytes, resulting in impairment of nutrient absorption (Scholten et al., 1999). These unfavourable changes may be responsible for slightly worse results obtained by experimental piglets after weaning. Only at 35-56 days of age, differences between experimental and control animals were not statistically significant.

CONCLUSIONS

Results of the present experiment indicate that medium-chain fatty acids, caprylic and capric, improve piglet performance and protein digestibility. Caprylic acid has the most beneficial effect on piglet mortality and weight gain. The improvement caused by both acids can be due to structural changes of the small intestine mucosa and changes in the bacterial population.

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