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Article

Influence of protected organic acids on growth performance, fecal microbial composition, gas emission, and apparent total tract digestibility in growing pigs

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Abstract

This study was conducted to assess the effect of protected organic acids on growth performance, fecal microbial composition, gas emission, and apparent total tract digestibility (ATTD) in growing pigs. A total of 80 crossbred (Landrace × Yorkshire) × Duroc) growing pigs with average initial body weight (BW) of 22.66 ± 2.45 kg were allotted to one of two dietary treatments with 8 replications and 5 pigs (3 gilts and 2 barrows) per pen in a randomized complete block design in a 6-week study with basal diets (CON) and basal diets + 0.2% microencapsulated organic acids (MOA). A trend and significant effect on average daily gain (ADG) were observed during weeks 2 and 6 ($P < 0.05$), respectively. The gain–feed ratio (G:F) was increased ($P = 0.0032$) in the MOA group. ADG ($P = 0.0109$) and trend in G:F ($P = 0.1010$) were observed in the MOA group. However, no difference was observed in the BW and average daily feed intake of pigs. Fecal *Escherichia coli* counts showed reduction ($P = 0.0143$) at week 4. MOA supplementation had no influence on ATTD and fecal gas emission in growing pigs during the entire experiment ($P > 0.05$). The MOA supplementation to the basal diet had a positive effect on the growth performance and fecal microbial composition of growing pigs.

Key words: growing pigs, growth performance, apparent total tract digestibility (ATTD), organic acids

Résumé

Cette étude a été effectuée afin d'évaluer l'effet d'acides organiques protégés sur la performance de croissance, la composition microbienne fécale, les émissions de gaz, et la digestibilité apparente du tractus digestif complet (ATTD — « apparent total tract digestibility ») chez les porcs en croissance. Un total de 80 porcs croisés ([Landrace × Yorkshire] × Duroc) en croissance ayant un poids corporel (BW — « body weight ») initial moyen de 22,66 ± 2,45 kg ont été assignés à l'un de deux traitements alimentaires avec 8 réplicats et 5 porcs (3 cochettes et 2 castrats) par enclos dans un design expérimental aléatoire à blocs complets d'une étude de 6 semaines comme suivant : groupe CON (« control »; diète de base) et groupe MOA (« microencapsulated organic acids »; diète de base + 0,2 % d'acides organiques micro-encapsulés). Une tendance ainsi qu'un effet significatif sur le gain moyen quotidien (ADG — « average daily gain ») ont été observés au cours des semaines 2 et 6 ($P < 0,05$), respectivement. L'indice de consommation (G:F — « gain-feed ratio ») était augmenté ($P = 0,0032$) dans le groupe MOA. L'ADG ($P = 0,0109$) et une tendance en G:F ($P = 0,1010$) ont été observés dans le groupe MOA. Par contre, aucune différence n'a été observée sur le BW et la consommation moyenne quotidienne des porcs. Les comptes d'*Escherichia coli* fécaux ont montré une réduction ($P = 0,0143$) à la semaine 4. Les suppléments d'acides organiques micro-encapsulés n'ont pas eu d'effet sur l'ATTD ni les émissions fécales de gaz chez les porcs en croissance, et ce au cours de toute la période de l'expérience ($P > 0,05$). Les suppléments de MOA à la diète de base ont eu un effet positif sur la performance de croissance et la composition microbienne fécale des porcs en croissance. [Traduit par la Rédaction]

Mots-clés : porcs en croissance, performance de croissance, digestibilité apparente du tractus digestif complet (ATTD), acides organiques

Introduction

In animal farming, antibiotics are given for therapeutic purposes to treat infections, for preventive purposes before noticeable symptoms appear, and for non-curative purposes to promote growth and improve feed efficiency (Wegener 2003). The more antibiotics are misused or overused, the more likely the bacteria will become resistant to them (Romandini et al. 2021). This leads to antibiotic resistance. Therefore, given the antibiotic resistance and antibiotic residues in animal products, many countries, including the

European Union and South Korea, have banned the use of antibiotics in animal feed (Salim et al. 2013; Levy 2014). Antibiotic resistance poses a threat to global health and human development. This requires unprecedented global collective action across sectors and at all levels of society. Researchers and nutritionists are not too far behind to take action in finding the use of necessary and promising alternatives. One possibility is to use organic acids (OAs) as individual acids or as a blend of various acids to combat bacterial infections in livestock and these have been used in pig nutrition for decades and appear to offer many of the benefits of antibiotics (Dibner and Buttin 2002). OAs are weak acids that have been shown to have beneficial effects in animals and have antimicrobial activity (Dibner and Buttin 2002). Organic acids are alternative feed additive in animal production (Adil et al. 2011; Khan et al. 2012). In Europe, organic acids are usually included in the diets of monogastric animals as preservatives and acidifiers to replace antibiotics as growth promoters and prevent or control pathogens (Papatsiros et al. 2012). In addition, many studies on OAs in natural antibiotics have shown that they have similar beneficial effects as feed-containing antibiotics (Mathew 1991). A possible mechanism of action for organic acids includes lowering the pH of the digesta in the gastrointestinal tract (GIT) (Ravindran and Kornegay 1993), regulating the balance of microbial populations in the intestine, stimulating the secretion of digestive enzymes, and promoting the growth and restoration of intestinal morphology (Papatsiros et al. 2012).

Organic acids increase the digestibility of proteins and amino acids by increasing the breakdown of proteins in the stomach; in addition, they maintain the cellular integrity of the gut lining and improve the digestive process by maintaining normal gut flora (Sultan et al. 2015). It is reported that some of OAs are considered as a source of energy in the pig gut because they are the intermediary products of the tricarboxylic acid (Giesting and Easter 1985). Previously, Eckel et al. (1992) reported that feeding OAs to piglets was effective for growth performance. Similarly, Jongbloed et al. (2000) and Kiarie et al. (2018) found that organic acids significantly increased the growth performance and apparent total tract digestibility of dry matter (DM) in nursery pigs. Moreover, Nguyen et al. (2020) reported that organic acids help in lowering the pH of the digesta in the GIT. Previous studies indicated that OAs supplementation reduced the environmental problem by reducing the noxious gas emission (Upadhaya et al. 2014*a*, 2014*b*; Devi et al. 2016). However, these OAs have to be protected because the effectiveness of unprotected organic acids may be limited due to prompt absorption and metabolism in the duodenum, which limits the amount that reaches the lower gut (Cho et al. 2014; Upadhaya et al. 2014*a*; Lee et al. 2015). To overcome this limitation, matrix coating or encapsulation technologies have been developed, which allows controlling the microencapsulated organic acids (MOA) to reach the site of action (Hossain et al. 2018). Detailed research works on the concept of organic acids have emphasized that OAs can improve the growth efficiency of the pigs. Therefore, the objective of the current study was to assess the effect of protected organic acids on growth performance, fecal microbial composition, gas emission, and apparent total tract digestibility (ATTD) in growing pigs.

Materials and methods

The experiment was carried out in the Swine Research Unit of Dankook University, South Korea. All experimental procedures involving animals (approval no. DK-2-2030) used in this study were revised and approved by Institutional Animal Care and Use Committee of Dankook University. The protocol was evaluated and approved by Dankook University's Institutional Animal Care and Use Committee (approval no. DK-2-2030) before the experiment began. All animal procedures were carried out in accordance with the South Korean Council on Animal Care Guidelines.

Source of OA blend

The MOA mixture used in the experiment was a commercial product procured from Morning Bio Co., Ltd. (Cheonan, South Korea). The active ingredients were 17% fumaric acid, 13% citric acid, and 10% malic acid.

Experimental design, animals, and housing

In the 6-week trial, a total of 80 crossbred (Landrace × Yorkshire) × Duroc) growing pigs with average initial body weight (BW) of 22.66 ± 2.45 kg and sex were allotted to one of two dietary treatments with 8 replications and 5 pigs (3 gilts and 2 castrated barrows) per pen in a randomized complete block design. The dietary treatment included a basal diet based on corn-soybean meal and a basal diet supplemented with a 0.2% OA blends. The composition of the basal diets is presented in Table 1. The basal diets contained 3300 kcal of metabolized energy/kg and 15.50% crude protein and they were formulated to meet or exceed the nutritional requirements of swine (NRC 2012). All pigs were kept in an environmentally controlled room with slatted plastic floors. Each pen was equipped with a single-sided self-feeder and a nipple drinker, allowing ad libitum access to feed and water throughout the experiment.

Sampling and measurements

Individual BW of growing pigs was measured at the beginning and the end of weeks 2, 4, and 6. Feed consumption and residues were weighed and recorded on a pen basis to monitor average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

On day 36, 2 g/kg of chromic oxide (Cr_2O_3) as an indigestible marker was mixed in growing pigs' diets to calculate ATTD of DM, nitrogen, and gross energy (GE). At the end of week 6, fresh fecal samples were collected from 2 pigs per pen (1 gilt and 1 barrow) by rectal massage, placed on ice box transported to the laboratory, and stored at −20 °C until analyzed. All feed samples and fresh fecal samples were dried at 70 °C in the forced air oven for 72 h and then finely ground to pass through a 1 mm screen sieve. DM was analyzed following the methods outlined by the AOAC (2007). Chromium concentration was determined through UV absorption spectrophotometry (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan). The GE

Table 1. Composition of growing diet (as-fed basis).

Item	Basal diet
Ingredients (%)	
Corn	69.20
Soybean meal	14.23
DDGS	10
Tallow	2.87
DCP	1.35
Limestone	0.82
Salt	0.3
Methionine (99%)	0.07
Lysine (78%)	0.59
Threonine (99%)	0.1
Tryptophan (99%)	0.04
Mineral mix[a]	0.2
Vitamin mix[b]	0.2
Choline (25%)	0.03
Total	100
Calculated value	
CP (%)	15.50
ME (kcal/kg)	3300
Ca (%)	0.70
P (%)	0.60
TRP (%)	0.10
Lys. (%)	1.10
Met. (%)	0.30
Crude fat (%)	6.33

Note: DCP, dicalcium phosphate; DDGS, dried distillers grains; CP, crude protein; ME, metabolizable energy; TRP, tryptophan; Lys, lysine; Met, methionine.
[a]Provided per kg diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.
[b]Provided per kg of diet: vitamin A, 10 800 IU; vitamin D3, 4000 IU; vitamin E, 40 IU; vitamin K3, 4 mg; vitamin B1, 6 mg; vitamin B2, 12 mg; vitamin B6, 6 mg; vitamin B12, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; and D-calcium pantothenate, 25 mg.

was determined by measuring the heat of combustion in the samples, using a bomb calorimeter (Parr 6100; Parr Instrument Co., Moline, IL, USA). Nitrogen (protein) content was determined by using a Kjeltec 8600 analyzer (Foss Tecator AB, Hoeganaes, Sweden). The following formula was used to calculate the ATTD: N digestibility $= 1 - [(Nf \times Cd)/(Nd \times Cf)]$, where: Nf is the nutrient concentration in feces, Nd is the nutrient concentration in diet, Cd is the chromium concentration in diet, and Cf is the chromium concentration in feces.

During second, fourth, and sixth weeks of the experiment, the rectal massage technique was used to collect fresh fecal samples from two pigs per pen for fecal microbial count analysis. One gram of feces sample was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and mixed evenly. The total viable bacterial count in the fecal sample was found by plating a MacConkey agar plate (Difco Laboratories, Detroit, MI, USA) and Lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) with a 10-fold serial dilution to isolate both *Escherichia coli* and *Lactobacillus*, respectively. Lactobacilli medium III agar plates were kept in an incubator at 39 °C for 48 h under anaerobic conditions and MacConkey agar plates were kept in an incubator at 37 °C for 24 h. Immediately after removing the plate from the incubator, the number of *E. coli* and *Lactobacillus* colonies was counted. The microflora concentration was finally expressed as $\log_{10}$ CFU/g of feces.

At the end of weeks 2, 4, and 6 of the trial, fresh fecal samples were collected from randomly selected 2 pigs per treatment (1 gilt and 1 barrow per pen per treatment) to analyze fecal NH_3, H_2S, methyl mercaptan, CO_2, and acetic acid. Then, samples were packed in 2.6 L box with a small hole in the middle of one side that was sealed with adhesive plaster and filled with a total of 300 g of fecal samples. Samples were fermented for 24 h at room temperature (25 °C), and 100 mL samples were taken from the headspace from approximately 2.0 cm above the fecal sample. After that, the box was re-sealed with adhesive plaster to measure the fecal noxious content. The fecal samples were shaken manually for about 30 s before the measurement to disrupt any crust formation on the surface of the fecal sample and to homogenize the samples. Concentrations of NH_3, H_2S, methyl mercaptan, CO_2, and acetic acid were measured within the scopes of 5.0–100.0 ppm (No. 3La, detector tube; Gastec Corp., Kanagawa, Japan) and 2.0–20.0 ppm (4LK, detector tube; Gastec Corp.).

Statistical analysis

SAS's GLM technique was used to analyze all of the data as a completely randomized block design (version 9.2; SAS Institute, Cary, NC, USA). When significant differences between treatment means were found, they were separated using the *T* test. The pen was utilized as a testing unit. The standard errors of mean (SEM) were used to represent data variability, and values of $P < 0.05$ were considered statistically significant. Trends were defined as $P < 0.1$.

Results

Growth performance

The effects of MOA mixture supplementation on growth performance of the growing pigs are shown in Table 2. Dietary MOA supplementation showed trends or significant effects to improve the daily gain of growing pigs at weeks 2 ($P = 0.074$) and 6 ($P < 0.05$), respectively. Also, pigs fed a diet containing MOA supplement significantly increased the G:F ($P < 0.05$) compared with those fed CON. Over the entire trial, except ADG ($P = 0.019$) and G:F ($P = 0.101$), there were no differences observed in the BW and ADFI of pigs.

ATTD of nutrients

The ATTD results are summarized in Table 3. There were no significant differences observed in ATTD of DM, N, and GE between CON and MOA groups during the end of the trial ($P > 0.05$).

Fecal gas emission

The effects of MOA supplementation on the fecal gas emission of growing pigs are presented in Table 4. The dietary MOA supplementation had no influence on fecal gas emission in growing pigs during the entire experiment ($P > 0.05$).

Table 2. The effect of dietary microencapsulated organic acid mixture supplementation on the growth performance of growing pigs.

Items	CON	MOA	SEM	*P* value
	Basal diet	0.20%		
Body weight (kg)				
Initial	22.66	22.66	0.01	0.9962
Week 2	30.78	31.54	0.26	0.6066
Week 4	39.76	41.51	0.45	0.4740
Week 6	50.46	52.22	0.55	0.2634
Week 2				
ADG (g)	580	634	18	0.0742
ADFI (g)	1254	1318	39	0.2845
GF	0.466	0.483	0.017	0.5542
Week 4				
ADG (g)	690	712	16	0.1797
ADFI (g)	1555	1578	37	0.2316
GF	0.443	0.451	0.005	0.1554
Week 6				
ADG (g)	716	765	19	0.0414
ADFI (g)	1862	1908	36	0.3189
GF	0.385	0.401	0.004	0.0032
Overall				
ADG (g)	662	704	13	0.0109
ADFI (g)	1557	1601	23	0.1629
GF	0.425	0.439	0.005	0.1010

Note: Means in the same row with different superscripts differ ($P < 0.05$). kg, kilogram; ADG, average daily gain; ADFI, average daily feed intake; GF, gain-feed ratio; CON, basal diets; MOA, basal diets + 0.2% microencapsulated organic acids mixture; SEM, standard error of means.

Table 3. The effect of dietary microencapsulated organic acid mixture supplementation on the apparent total tract digestibility (ATTD) of nutrients of growing pigs.

Items (%)	CON	MOA	SEM	*P* value
	Basal diet	0.20%		
Week 6				
Dry matter	75.36	74.91	0.59	0.6391
Nitrogen	72.63	72.34	0.62	0.8101
Gross energy	73.99	73.45	0.65	0.5374

Note: CON, basal diets; MOA, basal diets + 0.2% microencapsulated organic acids mixture; SEM, standard error of means.

Table 4. The effect of dietary microencapsulated organic acid mixture supplementation on gas emission in growing pigs.

Items (ppm)	CON	MOA	SEM	*P* value
	Basal diet	0.20%		
Week 2				
NH_3	1.1	0.6	0.3	0.1947
H_2S	2.1	1.9	0.6	0.5671
Methyl mercaptans	1.8	1.7	0.3	0.5728
CO_2	325	400	150	0.7375
Acetic acid	1.0	0.9	0.3	0.8693
Week 4				
NH_3	1.0	0.7	0.2	0.4963
H_2S	3.0	2.1	0.6	0.3461
Methyl mercaptans	1.0	0.9	0.4	0.8904
CO_2	575	425	155	0.4703
Acetic acid	1.5	1.1	0.4	0.1757
Week 6				
NH_3	1.2	0.8	0.2	0.1239
H_2S	2.4	2.1	0.6	0.8051
Methyl mercaptans	1.6	1.3	0.4	0.4845
CO_2	525	450	184	0.7595
Acetic acid	1.9	1.5	0.4	0.4939

Note: ppm, parts per million; NH_3, ammonia; H_2S, hydrogen sulfide; CO_2, carbon dioxide; CON, basal diets; MOA, basal diets + 0.2% microencapsulated organic acids mixture; SEM, standard error of means.

Table 5. The effect of dietary microencapsulated organic acid mixture supplementation on fecal microbial composition in growing pigs.

Items (log_{10} cfu/g)	CON	MOA	SEM	*P* value
	Basal diet	0.20%		
Week 2				
Escherichia coli	6.47	6.32	0.65	0.1223
Lactobacillus	9.52	9.73	0.06	0.1132
Week 4				
Escherichia coli	6.59	6.35	0.07	0.0143
Lactobacillus	9.70	9.84	0.05	0.1405
Week 6				
Escherichia coli	6.51	6.62	0.06	0.1274
Lactobacillus	9.77	9.80	0.06	0.6040

Note: Means in the same row with different superscripts differ ($P < 0.05$). cfu/g, colony forming units per gram; CON, basal diets; MOA, basal diets + 0.2% microencapsulated organic acids mixture; SEM, standard error of means.

Fecal microbial composition

Fecal microbial composition test results are represented in Table 5. Compared with the CON diet, pigs fed a diet supplemented with MOA significantly reduced ($P = 0.0143$) *E. coli* counts at week 4. However, the *Lactobacillus* population ($P > 0.05$) remained unaffected throughout the experiment.

Discussion

Growth performance

Previous researchers demonstrated that feeding a protected blend of OAs led to improved growth performance of piglets and growing–finishing pigs (Cho et al. 2014; Upadhaya et al. 2014*a*, 2014*b*; Lei et al. 2017; Xu et al. 2018). In this study, we observed that when growing pigs were fed a diet supplemented with an MOA mixture, the ADG and G:F ratio improved compared with the diet without MOA supplementation. This is consistent with Walsh et al. (2007), who found better results in G:F when 0.4% OAs were included in piglets' diets. Similarly, Kuang et al. (2015) found that weaning pigs fed the diet supplemented with an OA blend showed improved ADG. This is also in agreement with Upadhaya et al. (2018) who found an increase in ADG when 0.1% and 0.2% of the OA mixture was supplemented to the diets of weaning

pigs. The possible reasons for improvements are the mechanism of action for organic acids that includes lowering the pH of the digesta in the GIT (Ravindran and Kornegay 1993), which might have helped in regulating the balance of microbial populations and promoting the growth and restoration of intestinal morphology (Papatsiros et al. 2012).

In contrast, Zentek et al. (2013) also reported that 0.416% fumaric acid or 0.328% lactic acid in the feed had no influence on the growth performance of weaned piglets. In addition, Manzanilla et al. (2004) observed that there are no effects with individual OAs, such as citric, formic, or fumaric acids on early weaned pigs. The inconsistent results among different studies could be due to age differences of animals, the types of OAs used, and OA dosage used.

ATTD of nutrients

Lowering the pH in the upper region of the GIT may improve the digestibility of nutrients. OAs were commonly used as an acidifying agent in livestock feed and are considered a promising alternative to antibiotics as they can improve the digestibility of nutrients (Nguyen et al. 2020). In the present study, we noticed that inclusion of MOA to the diet had no significant effect on the digestibility of DM, N, and GE, which is consistent with results of Upadhaya et al. (2016) and Muniyappan et al. (2021) who observed similar results when OA supplementation was introduced to the diets of pigs. However, some studies have reported a positive effect on ATTD when organic acid was used in pig diets (Upadhaya et al. 2014*a*; Kuang et al. 2015; Hossain et al. 2018). ATTD has not improved maybe due to the lack of influence of MOA on *Lactobacillus* counts in the current study as it helps in the breakdown of feeds and facilitates absorption.

Fecal gas emission

Intensive pig farming is responsible for significant air pollutant emissions (Costantini et al. 2020). The significant air pollutants from pig farming include NH_3, H_2S, and total mercaptans (Lesschen et al. 2011). Therefore, it is important to find some useful methods to reduce the noxious gas emission either by proper management or by dietary modification. Many studies explained that supplementing OAs to pigs' diets significantly reduced noxious gases (Eriksen et al. 2010; Upadhaya et al. 2014*a*, 2014*b*; Devi et al. 2016; Hossain et al. 2018). However, in the current study, the supplementation of 0.20% MOA in the diet of growing pigs did not influence the noxious gas emission of NH_3, H_2S, methyl mercaptans, CO_2, and acetic acid. The obtained findings are in agreement with Nguyen et al. (2018) who did not find any influence on NH_3, H_2S, and acetic acid in finishing pigs. Also, Upadhaya et al. (2018) pointed out that the dietary supplement of OA mixture had no effect on NH_3, H_2S, and total mercaptans in weaning pigs. The reduced fecal pH inhibits the invasion and proliferation of pathogenic bacteria in the GIT, which further limits the production of toxic bacterial metabolites and ammonia (Kil et al. 2011; Upadhaya et al. 2014*a*). Therefore, in this study, the pH of the fecal might have not been reduced by the supplementation of MOA in growing pigs, which led to the insignificant effects in gas emission.

Fecal microbial composition

The GIT is the interface at which digestion, secretion, and absorption take place (Ramani et al. 2021). The gastrointestinal microbiota plays a crucial role in the host's gut-associated immune system. In addition, the intestinal microbiota affects physiological development, health, and productivity (Upadhaya et al. 2021), leading to the hypothesis that the use of feed additives such as organic acids can be useful to control the microbial community. The recent results agree with the published evidence that indicate that the dietary supplementation of OAs had reduced *E. coli* and increased *Lactobacillus* counts in weaned piglets and weaning pigs (Long et al. 2018; Upadhaya et al. 2018; Yang et al. 2019). However, other researchers have shown no significant difference in fecal microbial composition (*E. coli* and *Lactobacillus)* by the addition of OAs. (Oh et al. 2018; Lee et al. 2021) and Cho et al. 2018 found no significant difference in *Lactobacillus* population in weanling pigs. Inconsistency in results may be related to the age of the animals, the composition of the diet, or the amount and type of OAs mixed.

Conclusion

Dietary supplementation of the protected blend of OAs at 0.20% level improved growth performance and shifted fecal microbial composition by reducing *E. coli* population. However, no significant difference was observed in other parameters of ATTD and fecal gas emission.

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Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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O.M.: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. S.M.: Writing – original draft, Writing – review & editing. I.H.K.: Conceptualization, Data curation, Resources, Supervision, Validation, Writing – review & editing.

Competing interests

No potential conflict of interest relevant to this article was reported.

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