Influence of protected organic acid blends and diets with different nutrient densities on growth performance, nutrient digestibility and faecal noxious gas emission in growing pigs

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ABSTRACT: This study was conducted to evaluate the effects of dietary supplementation of protected organic acid blends including medium chain fatty acids and different nutrient density diets on growth performance, nutrient digestibility and faecal noxious gas content in growing pigs. A total of 80 crossed [(Landrace × Yorkshire) × Duroc] growing pigs with an initial body weight (BW) of 22.61 ± 2.32 kg were used in a six-week trial. Pigs were randomly allocated into one of four treatment groups in a 2 × 2 factorial arrangement with two levels of nutrient density (high and low) and protected organic acid (0% and 0.1%) according to their sex and BW (five replicates with two gilts and two barrows per pen). Pigs fed high nutrient density diets had increased ($P < 0.05$) average daily gain (ADG0 and gain: feed (G : F) than those fed low nutrient density diets. Likewise, pigs fed protected organic acid diets exhibited increased ($P < 0.05$) ADG compared with pigs fed no additional protected organic acids. An interactive effect (P = 0.03) between organic acid and nutrient density was observed on feed conversion by pigs. Dry matter (DM) digestibility tended to improve (P = 0.08) in pigs fed high nutrient density diets compared with low nutrient density diets. However, nitrogen (N) and energy (E) digestibility was not influenced by the nutrient density. Likewise, protected organic acid supplementation did not influence ($P > 0.05$) DM, N or E digestibility. Organic acid supplementation reduced ($P < 0.05$) H_2S content from faeces on Day 1, Day 3, Day 5 and Day 7 of incubation. Low nutrient density diets led to a reduction ($P < 0.05$) in H_2S gas content on Day 1 of incubation. No interactive effect on faecal noxious gas content was observed between nutrient density and organic acid. In conclusion, dietary supplementation of protected organic acids with a high nutrient diet improved growth performance and reduced H_2S acid emission.

Keywords: digestibility; growing pig; growth performance; microflora; protected organic acid

List of abbreviations

ADG = average daily gain, **ADFI** = average daily feed intake, **BW** = body weight, **DM** = dry matter, **E** = energy, **G:F** = feed efficiency, **H_2S** = hydrogen sulphide, **MCFA** = medium chain fatty acid, **N** = nitrogen

Organic acids are weak acids with at least one carboxylic group and a carbon chain with one to seven carbon atoms. Organic acids have been considered as potential substitutes for antibiotics. According to Canibe et al. (2001), organic acids can positively influence the microflora in the gastrointestinal tract, thus improving nutritional uptake and the health of pigs. Different organisms produce organic acids naturally and they are also produced by metabolism.

Previous reports indicated that organic acid supplements in pig diets had beneficial effects on swine performance (Mroz et al. 2006; Wang et al. 2009a), as well as in poultry (Wang et al. 2009b; Wang et al. 2010). Different combinations of organic acids blends and inorganic acids have been used increasingly in diets for weaning as well as growing-finishing pigs and sows due to their beneficial and synergistic effects.

The combination of organic acids and medium-chain fatty acids (MCFAs) has been reported to have beneficial effects on intestinal micro-ecology in piglets (Zentek et al. 2013). This was associated with a much higher antimicrobial strength of the acid in its undissociated form. With the development of coating technologies, protecting acids by matrix coating or encapsulation for targeted delivery to different gut segments has gained considerable attention. A study by Bosi et al. (1999) showed that protected organic acids are more effective in retarding absorption of dietary acids and allowing more effective delivery of the acids to the distal ileum, cecum, and colon of piglets. To our knowledge, most researchers that have evaluated the effects of organic acid blends have used concentrations of more than 0.2% and have utilised the same dietary nutrient concentration for all treatments.

Previous studies on probiotics indicated that their uptake was affected by the nutrient density of the diet (Meng et al. 2010). Thus, we hypothesised that there could be interaction between nutrient density and organic acid supplementation. Furthermore, studies on the administration of protected organic acid supplements to growing pigs are limited.

Thus, the objectives of this study were to evaluate the effects of protected organic acid blends, including medium-chain fatty acids at lower concentrations than in previous studies, on growth performance. We also assessed nutrient digestibility and faecal gas emissions in growing pigs fed diets of different nutrient densities.

MATERIAL AND METHODS

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University.

Source of organic acids. The matrix-coated organic acid blends used in the experiment described here were provided by Morningbio Co., Ltd. (Cheonan, Korea). This protected organic acid blend consists of medium-chain fatty acids and composite organic acids. The active ingredients are 17% fumaric acid, 13% citric acid, 10% malic acid, and 1.2% medium chain fatty acid (capric and caprylic acid) and carrier (60%). The organic acid blends used in this experiment were protected by a matrix coating with a lipid base.

Experimental animals. In total, 80 cross-bred [(Landrace × Yorkshire) × Duroc] growing pigs with an initial BW of 22.61 ± 2.32 kg were used in a six-week trial. Pigs were allocated randomly into one of four treatments in a 2 × 2 factorial arrangement with two levels of nutrient density (high and low; Table 1) and protected organic acid (0% and 0.1%) according to sex and BW (five replicates with two gilts and two barrows per pen). All pigs were allowed *ad libitum* access to feed and water through a self-feeder and nipple drinker throughout the experimental period. The diets used in this experiment were formulated to meet or exceed NRC (2012). All pigs were housed in an environmentally controlled room. Room temperature and humidity were maintained at 25 °C and 60%, respectively.

Sampling and measurements. Body weight and feed intake were measured initially and at the end of Week 6 of the experimental period and feed consumption was recorded on a pen basis during the experiment to determine average daily gain (ADG), average daily feed intake (ADFI), and gain/feed ratio.

Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for seven days prior to faecal collection at the 6th week for calculation of dry matter (DM), nitrogen (N), and energy (E) digestibility. Faecal samples were collected at random from at least two pigs in each pen (one gilt and one barrow; 10 pigs per treatment). All feed and faecal samples were stored immediately at –20 °C until analysis. All samples were freeze-dried and finely ground, to allow passage pass through a 1-mm screen. DM, N, and E digestibility were determined in accordance with the methods established by the AOAC (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the apparent total tract digestibility (ATTD) of DM and N were calculated using indirect methods, as described by Williams et al. (1962). The digestibility was calculated using the following formula:

$$\text{Digestibility (\%)} = \{1 - [(N_f \times C_d)/(N_d \times C_f)]\} \times 100$$

where:

N_f = nutrient concentration in faeces (% DM)

N_d = nutrient concentration in diet (% DM)

C_f = chromium concentration in faeces (% DM)

C_d = chromium concentration in faeces (% DM)

Nitrogen was measured using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). The gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, USA).

At the end of the experiment, fresh excreta samples were collected from each replicate for the anal-

Table1. Composition of experimental diet for growing pigs (as-fed basis)[a]

Ingredients (%)	High nutrient		Low nutrient	
	OA^-	OA^+	OA^-	OA^+
Corn	56.75	56.65	62.48	62.38
Soybean meal	35.39	35.39	32.18	32.18
Tallow	5.63	5.63	3.12	3.12
Limestone	0.84	0.84	0.84	0.84
Salt	0.20	0.20	0.20	0.20
Tri-calcium phosphate	0.70	0.70	0.72	0.72
Lysine	0.14	0.14	0.11	0.11
Organic acid	–	0.10	–	0.10
Vitamin premix[b]	0.20	0.20	0.20	0.20
Mineral premix[c]	0.10	0.10	0.10	0.10
Calculated composition (g/kg)				
ME (MJ/kg)	14.65	14.65	14.24	14.24
CP (%)	20.0	20.0	19.0	19.0
Lysine (%)	1.3	1.3	1.2	1.2
Calcium (%)	0.65	0.65	0.65	0.65
Total phosphorous (%)	0.55	0.55	0.55	0.55
Analyzed composition (g/kg)				
GE (MJ/kg)	17.93	17.93	17.28	17.28
CP (%)	19.56	19.56	18.35	18.35
Calcium (%)	0.63	0.63	0.61	0.61
Total phosphorous (%)	0.53	0.53	0.51	0.51

[a]High and low nutrient: high or low in energy, CP, and Lys. High or low diet was supplemented with 0 or 0.1%organic acid

[b]Provided per kg diet: 20 000 IU of vitamin A, 4000 IU of vitamin D3, 80 IU of vitamin E, 16 mg of vitamin K3, 4 mg of thiamine, 20 mg of riboflavin, 6 mg of pyridoxine, 0.08 mg of vitamin B12, 120 mg of niacin, 50 mg of Ca-pantothenate, 2 mg of folic acid, and 0.08 mg of biotin

[c]Provided per kg diet: 140 mg of Cu (as copper sulphate), 179 mg of Zn (as zinc oxide), 12.5 mg of Mn (as manganese oxide), 0.5 mg of I, 0.25 mg of Co (as $Co_2O_3 \times 7\ H_2O$), and 0.4 mg of Se (as $Na_2SeO_3 \times 5\ H_2O$)

ysis of noxious gas content according to the method described by Cho et al. (2008). In total, 300 g of excreta were incubated in 2.6 l sealed plastic boxes in duplicate. The samples were permitted to ferment for a period of 30 h at 32 °C. After the fermentation period, a Gas Detector instrument (GV-100S; Gastec Corp., Kanagawa, Japan) was used for gas detection. In these measurements, the plastic boxes were punctured, and headspace air was sampled ~2.0 cm above the samples at a rate of 100 ml/min. Levels of ammonia, H_2S, and total mercaptans (Gastec Detector Tube No. 3La, No. 4LL, and No.70L, respectively; Gastec Corp.) were measured at Day 1, Day 3, Day 5, and Day 7 of incubation.

Statistical analyses. Data were analysed as a completely randomised block design with 2 × 2 factorial arrangement using GLM procedures (SAS Institute, 1996). The main effect included dietary nutrient density and protected organic acid administration as well as any interaction between organic acids and dietary nutrient density. For all response criteria, the pen served as the experimental unit. The initial BW was used as a covariate for ADG and ADFI. Before carrying out statistical analysis of the microbial counts, logarithmic conversion of the data was performed. Variability in data was expressed as the standard error of the mean and P-values < 0.05 were considered to indicate statistical significance.

RESULTS

Growth performance and nutrient digestibility

Pigs fed the high nutrient density diets showed increased ($P < 0.05$) ADG and G : F versus those fed the low nutrient density diets. Likewise, pigs fed protected organic acid diets exhibited higher ADG than pigs fed no additional protected organic acids (Table 2). An interactive effect ($P = 0.03$) between

Table 2. Effect of organic acids supplementation with different nutrient density on growth performance and nutrient digestibility in growing pigs[1]

Items	High		Low		SEM	*P*-value		
	OA⁻	OA⁺	OA⁻	OA⁺		density	OA	density*OA
Growth performance								
ADG (g)	630	645	615	630	6.06	0.02	0.02	NS
ADFI (g)	1417	1465	1503	1449	25.00	NS	NS	0.06
G/F	0.445	0.440	0.409	0.435	0.007	0.01	0.10	0.03
Nutrient digestibility								
DM (%)	74.00	74.60	73.83	73.54	0.33	0.08	NS	NS
N (%)	72.05	72.02	71.52	71.24	0.50	NS	NS	NS
E (%)	71.32	71.82	71.31	71.27	0.37	NS	NS	NS

[1]High = high nutrient density diet minus (–) or plus (+) 0.1% protected organic acid. Low = low nutrient density diet minus (–) or plus (+) 0.1% protected organic acid

SEM = standard error of means, NS = non-significant, density*OA = interaction between density of diets and organic acid blends

organic acid and nutrient density was observed in feed conversion.

DM digestibility tended to improve ($P = 0.08$) in pigs fed high nutrient density diets compared with low nutrient density diets. However, N and E digestibility were apparently unaffected by nutrient density. Likewise, protected organic acid supplementation did not affect DM, N, or E digestibility ($P > 0.05$; Table 2).

Faecal noxious gas content

Organic acid supplementation reduced ($P < 0.05$) H_2S content from faeces on Day 1, Day 3, Day 5, and Day 7 of incubation. The low nutrient density diet led to reduced ($P < 0.05$) H_2S gas content on Day 1 of incubation. No interactive effect on faecal noxious gas emission was observed between nutrient density and organic acid inclusion (Table 3).

Table 3. Effect of organic acids supplementation with different nutrient density on faecal gas emission in growing pigs[1]

Items (ppm)	High		Low		SEM	*P*-value		
	OA⁻	OA⁺	OA⁻	OA⁺		density	OA	density*OA
Ammonia								
Day 1	17.1	15.3	15.2	16.7	1.9	NS	NS	NS
Day 3	19.7	17.1	17.8	19.4	1.9	NS	NS	NS
Day 5	22.3	18.7	20.4	21.6	1.9	NS	NS	NS
Day 7	25.7	23.1	23.8	25.6	1.9	NS	NS	NS
Total mercaptans								
Day 1	0.5	0.4	0.4	0.4	0.1	NS	NS	NS
Day 3	0.7	0.5	0.6	0.5	0.1	NS	NS	NS
Day 5	0.8	0.7	0.7	0.7	0.1	NS	NS	NS
Day 7	0.9	0.8	0.8	0.8	0.1	NS	NS	NS
Hydrogen sulfide								
Day 1	0.7	0.5	0.5	0.4	0.1	0.01	0.007	NS
Day 3	0.7	0.6	0.7	0.5	0.1	0.36	0.02	NS
Day 5	0.9	0.7	0.7	0.6	0.1	0.09	0.01	NS
Day 7	0.9	0.8	0.8	0.6	0.1	0.12	0.01	NS

[1]High = high nutrient density diet minus (–) or plus (+) 0.1% protected organic acid. Low = low nutrient density diet minus (–) or plus (+) 0.1% protected organic acid

SEM = standard error, NS = non-significant, density*OA = interaction between density of diets and organic acid blends

DISCUSSION

Effects of protected organic acids

The matrix-coated organic acid used in the current study is a blend of organic acids and MCFA. It contains fumaric acid, citric acid, malic acid, and medium-chain fatty acids. The supplementation of diet with organic acids has been reported to improve growth performance by reducing gastrointestinal pH leading to modification of the intestinal microflora (Burnell et al. 1988).

We observed that when pig feed was supplemented with 0.1% protected organic acid, average daily gain and feed efficiency improved versus pigs fed a diet without added organic acids. The improvement in ADG could be due to the antimicrobial activity of organic acids, which helps in the reduction of pathogenic microbial load, thereby reducing the metabolic demands of microbes and increasing the availability of dietary energy and nutrients to the host animals. In agreement with our findings, some researchers have shown positive effects with single or blends of dietary acidifiers. For example, Overland et al. (2008) reported that supplementation with a single acidifier such as formic acid or sorbic acid improved growth rate and feed efficiency. Likewise, Walsh et al. (2007) demonstrated that the addition of 0.4% organic acid blends, such as fumaric, lactic, propionic, citric, and benzoic acids, improved growth performance. In contrast, other reports indicate a lack of response or even negative effects with single acidifiers, such as fumaric, citric, or formic acid (Radecki et al. 1988; Manzanilla et al. 2004), or blends of acidifiers, such as formic acid, lactic acid, and volatile fatty acids (Lee et al. 2007). These apparently inconsistent results and variable responses may be due to several factors, such as the stage of growth, diet complexity, types of acids, inclusion level of acids, and the health status of the pigs.

Feed additives, such as organic acids and some phytogenic substances, have the potential to improve total tract digestibility of DM, N, and E because of improved pre-caecal digestion, leading to reduced influx of bacterially fermented substrates into the hindgut. This may reduce the total quantity of faecal protein and hence increase the digestibility of nitrogen and also of DM and E, as an indirect consequence. However, in the present study, nitrogen and energy digestibility were apparently not improved by organic acid supplementation, consistent with the findings of Kil et al. (2006) and Radecki et al. (1988), who reported that DM and CP were not improved by the addition of organic acids such as formic, fumaric, or lactic acid. In contrast, other researchers have observed favourable effects on nutrient digestibility with organic acid supplementation (Franco et al. 2005). The variation in results could be due to the age of the animals, composition of diet, and amount of organic acid supplemented (Ravindran and Kornegay 1993). The dosage of organic acids in the current study might have been too low to observe a significant impact on nitrogen and energy digestibility.

We found that pigs fed the protected organic acids exhibited a reduced emission of noxious gases such as H_2S, compared to the pigs fed diets without extra organic acids. The reduction in these odorous gases could possibly be due to a reduction of the pathogenic bacterial population in the gastrointestinal tract or due to enhancement of beneficial microbial activity, leading to changes in end products of microbial fermentation and a shift in the ecosystem towards a more anabolic status. Our preliminary experiments showed that the 0.1% organic acid blends numerically increased the lactobacillus population and reduced the *E. coli* population in growing pigs and significantly increased lactobacillus numbers in finishing pigs.

Effects of nutrient density

In this study, a portion of corn was substituted by soybean meal and tallow, which increased CP and fat. The increase in energy and nutrient density improved the ADG and G : F and decreased the average daily feed intake (ADFI), consistent with previous studies by Meng et al. (2010) and Yan et al. (2010). The impact of increased energy density was found to be associated with feed intake, indicating that the pigs were able to increase energy intake when the high nutrient density diet was provided. The high density diet tended to improve DM digestibility. However, neither N nor E digestibility was influenced by the nutrient density of the diet. As mentioned, the high energy diet was coupled with a higher content of soybean meal. Soybeans contain large amounts of non-starch carbohydrates known to be anti-nutritional, because they adversely affect digestibility and the absorption of nutrients. Thus, replacing highly digestible starch from corn with non-starch polysaccharide from soybean meal might have resulted in an increased influx of bacterial fermented substrates into the hindgut, leading to the lack of improvement in digestibility.

The levels of faecal noxious gases such as H_2S were higher in the high nutrient density diet, in agreement with our previous report, (Yan and Kim 2013), in which we found that a high nutrient density diet increased the concentration of ammonia and H_2S gas. However, ammonia and total mercaptan emissions were unaffected by nutrient density in the current study. A possible reason for the high faecal noxious gas content in the high nutrient density diet could be that corn in the high nutrient diet was replaced with soybean meal. Soybean meal contains non-starch carbohydrate, which is not easily digested by pigs. This leads to an increase in the viscosity of digesta, thus modifying gut transit time and altering the morphological and physiological functions of the gut. This, in turn, influences the microbial population, which might have elicited an increase in the influx of fermentable substrate into the hindgut system, leading to the formation of more faecal matter with more noxious gases.

Interactive effects between organic acids and nutrient density

An interactive effect ($P = 0.03$) between organic acid and nutrient density was observed in feed conversion by the pigs. There appeared to be some interaction in feed intake also. This may be because of the optimisation of beneficial microflora, due to the inclusion of protected organic acids. Moreover, the microflora could then improve the conversion of feed to body mass. However, no interaction was observed in nutrient digestibility in response to organic acid supplementation with the high nutrient density diet. No comparison could be made with other studies because there are only a small number of reports on factors that may affect the response to organic acids. However, diet was considered by Si et al. (2006) to be the main factor that influenced the antimicrobial effect of plant extracts *in vitro*.

In conclusion, we suggest that energy and nutrient density influence the effects of organic acids in the growth performance of pigs.

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