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ARTICLE

Effects of a matrix-coated organic acids and medium-chain fatty acids blend on performance, and in vitro fecal noxious gas emissions in growing pigs fed in-feed antibiotic-free diets[1]

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Abstract: This study evaluated the efficacy of a matrix-coated organic acids and medium-chain fatty acids blend (MCOFA) in growing pigs. Ninety six pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of 47.71 ± 3.73 kg were used in a 6 wk experiment. Pigs were allotted to diets containing 0 or 2 g kg^{-1} of MCOFA, and 0 or 2.5 g kg^{-1} of antibiotic growth promoters (AGP) according to a 2 × 2 factorial arrangement of treatments. Pigs fed diets supplemented with MCOFA had improved growth efficiency compared with those fed a diet without MCOFA ($P < 0.05$). Pigs receiving the diets supplemented with both AGP and MCOFA had higher apparent total tract digestibility of crude protein, dry matter, fat, and gross energy ($P < 0.05$). Pigs fed AGP × MCOFA diet had increased serum urea nitrogen ($P < 0.05$). Pigs fed diets supplemented with AGP had reduced fecal ammonia (NH_3) gas emissions compared with those fed without AGP ($P < 0.05$). Moreover, pigs fed diets supplemented with MCOFA had reduced fecal NH_3 and acetic acid gas emissions compared with those fed without MCOFA ($P < 0.05$). In conclusion, dietary supplementation with MCOFA improved performance in growing pigs.

Key words: ammonia, apparent total tract digestibility, growing pigs, matrix-coated organic acids blend, performance.

Résumé : Cette étude a évalué l'efficacité d'un mélange d'acides organiques et d'acides gras à moyennes chaînes recouverts de matrice (MCOFA — « matrix-coated organic acids and medium-chain fatty acids ») chez les porcs en croissance. Quatre-vingt-seize porcs [(Yorkshire × Landrace) × Duroc] de poids corporel (BW — « body weight ») moyen de 47,71 ± 3,73 kg ont été utilisés dans une expérience de 6 semaines. Les porcs ont été assignés aux diètes contenant 0 ou 2 g kg^{-1} de MCOFA, et 0 ou 2,5 g kg^{-1} de facteurs antibiotiques de croissance (AGP — « antibiotic growth promoters ») selon un arrangement factoriel 2 × 2 des traitements. Les porcs ayant reçu une diète avec supplément de MCOFA avaient une efficacité améliorée de croissance par rapport à ceux ayant reçu une diète sans MCOFA ($P < 0,05$). Les porcs ayant reçu les diètes avec les suppléments d'AGP et de MCOFA avaient une plus grande digestibilité apparente du tractus complet des protéines brutes, des matières sèches, de gras et d'énergie brute ($P < 0,05$). Les porcs ayant reçu la diète AGP × MCOFA avaient un taux d'azote d'uréique sanguin augmenté ($P < 0,05$). Les porcs ayant reçu la diète avec suppléments d'AGP avaient des émissions de gaz d'ammoniac (NH_3 — « ammonia ») fécal réduites par rapport à ceux ayant reçu une diète sans AGP ($P < 0,05$). De plus, les porcs ayant reçu la diète avec suppléments de MCOFA avaient des taux réduits de NH_3 fécal et d'émissions de gaz d'acide acétique par rapport à ceux ayant reçu la diète sans MCOFA ($P < 0,05$). En conclusion, les suppléments alimentaires de MCOFA améliorent la performance chez les porcs en croissance. [Traduit par la Rédaction]

Mots-clés : ammoniac, digestibilité apparente du tractus complet, porcs en croissance, mélange d'acides organiques recouverts de matrice, performance.

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Introduction

Traditionally, antibiotic growth promoters (AGP) have been used as growth promoters and to control gastrointestinal tract (GIT) pathogens in pigs and poultry (Pluske et al. 1997; Thacker 2013; Waititu et al. 2015). A major current interest in the livestock industry is to develop and apply feeding programs that do not include AGP, due to the emerging human health crisis of antibiotic resistance. To this end, an intensive amount of research has been focused on the development of a wide range of feed additives with potential to serve as alternatives to AGP (de Lange et al. 2010; Nyachoti et al. 2012; Heo et al. 2013). Among these alternatives, organic acids (OA) and medium-chain fatty acids (MCFA) with or without lipid microencapsulation has been proposed for the livestock feed industry (Luckstadt and Mellor 2011; Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015), which include fumaric acid, citric acid, malic acid, capric, caprylic acid, and a variety of composite OA. There is evidence to suggest that these products may improve dietary nutrient utilization to enhance body weight gain, and the efficiency of nutrient utilization in poultry and swine (Cho et al. 2014; Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015).

Organic acids are weak acids with at least one carboxylic group, and a carbon chain with one to seven carbon atoms. The OA and their salts can have a preventive effect on postweaning diarrhea in piglets (Partanen and Mroz 1999; Franco et al. 2005; Halas et al. 2010; Zentek et al. 2013). Moreover, OA and MCFA have numerous therapeutic effects including antibacterial, antimicrobial, and bacteriostatic properties in their undissociated form, which may improve intestinal microecology, and thereby increase the energy and nutrients digestibility and improve performance in pigs and poultry (Dierick et al. 2002; Halas et al. 2010). However, the effectiveness of unprotected OA may be limited due to prompt absorption and metabolism in the duodenum which limits the amount that reaches the lower gut and therefore, the ability to modulate intestinal flora and the immune system (Cho et al. 2014; Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015). To overcome this limitation, matrix coating or encapsulation techniques to protect OA for targeted delivery to different gut segments have been developed.

The matrix-coated organic acids and medium-chain fatty acids blend (MCOFA) have been reported to maintain optimum pH in the intestinal tract (Upadhaya et al. 2014*a*, 2014*b*) and improve nutrient digestibility (Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015). Moreover, it has been reported that AGP can increase growth rate, improve feed utilization, and maintain gut health of pigs (Thacker 2013; Hossain et al. 2016). It has been demonstrated that OA along with AGP supplementation can increase average daily feed intake (ADFI) and reduce feed conversion ratio of broiler chickens compared with AGP-free diet (Bagal et al. 2016). Thus, it is possible that the performance of pigs might be affected by the diets containing in-feed AGP during supplementation with MCOFA. Many studies have examined the performance-enhancing properties of OA in poultry or pigs, however, to the best of our knowledge, studies on the possible effects of MCOFA as an alternative to AGP in growing pigs has not been reported. Thus, the present study tested the hypothesis that MCOFA supplementation would improve the performance of growing pigs in-feed antibiotic-free feeding programs. Thus, the objectives of the current study were to evaluate the impact of a matrix-coated OA and MCFA blend on the growth performance, energy and nutrient digestibility, fecal consistency score, fecal pH, hematological profiles, and in vitro fecal noxious gas emissions in comparison with AGP in growing pigs, and examine the interaction of dietary MCOFA with AGP in the diet.

Materials and Methods

The experimental protocol (F13-002/1/2/3) was reviewed, and approved by the University of Manitoba Animal Care Committee, and pigs were handled in accordance with the guidelines described by the Canadian Council on Animal Care (2009).

Source of protected organic acids blend

The matrix-coated OA used in the current experiment was provided by a commercial company (Morningbio Co., Ltd., Cheonan, South Korea) and it consisted of composite OA and MCFA. The active ingredients were 170 g kg^{-1} fumaric acid, 130 g kg^{-1} citric acid, 10 g kg^{-1} malic acid, 12 g kg^{-1} MCFA (i.e., 6 g kg^{-1} capric and 6 g kg^{-1} caprylic acid), and carrier.

Experimental design, animals, diets, and management

A total of 96 grower pigs [(Yorkshire × Landrace) female × Duroc male; Genesus, Oakville, MB, Canada] with an average body weight (BW) of 47.71 ± 3.73 kg (mean ± SD) were used in a 6 wk experiment. Pigs were randomly allotted to one of four experimental diets according to initial BW (IBW) in a randomized complete block design. There were 12 replicate pens per treatment with two pigs per pen. There were four dietary treatments with two levels of MCOFA (0 or 2 g kg^{-1}) and two levels of AGP (aureomycin; 0 or 2.5 g kg^{-1}). The aureomycin supplement, Aureo S-P 250 G (Zoetis Canada Inc., Kirkland, QC, Canada), contains 44 g chlortetracycline, 44 g sulfamethazine, and 22 g penicillin kg^{-1}. All supplements were top-dressed to the basal diet. All diets were formulated to meet or exceed NRC (2012) recommendation for growing pigs. The composition and analyzed nutrient contents of the experimental diets are given in Tables 1 and 2, respectively. Pigs were obtained from the University of Manitoba's Glenlea Swine Research Unit, and housed in metallic pens (1.47 m × 1.14 m) with raised plastic-covered expanded metal floors in an environmentally controlled room with ambient temperature

Table 1. Composition of the basal diet (as-fed basis).[a]

Items	Content
Corn (g kg^{-1})	680.0
Soybean meal, 440 g CP kg^{-1}	268.4
Canola oil (g kg^{-1})	12.50
Limestone (g kg^{-1})	10.50
Dicalcium phosphate (g kg^{-1})	9.6
Salt (g kg^{-1})	5.0
L-Lys HCl (g kg^{-1})	0.8
DL-Met (g kg^{-1})	0.1
L-Thr (g kg^{-1})	0.1
Vitamin-mineral premix[b] (g kg^{-1})	10.0
TiO_2 (g kg^{-1})	3.0
Calculated values	
ME (kcal kg^{-1})	329.2
CP (g kg^{-1})	180.0
NDF (g kg^{-1})	101.0
ADF (g kg^{-1})	44.0
SID Lys (g kg^{-1})	8.7
SID Met (g kg^{-1})	2.6
SID Met+ Cys (g kg^{-1})	5.2
SID Thr (g kg^{-1})	5.7
SID Trp (g kg^{-1})	1.8
Ca (g kg^{-1})	8.0
Available P (g kg^{-1})	3.0

[a]Pigs were allotted diets supplemented with or without 2 g kg^{-1} of matrix-coated organic acids and medium-chain fatty acids blend (MCOFA) and 2.5 g kg^{-1} of antibiotic growth promoters, respectively. CP, crude protein; Lys, lysine; HCl, hydrogen chloride; Met, methionine; Cys, cysteine; Thr, threonine; Trp, tryptophan; TiO_2, titanium oxide; ME, metabolizable energy; ADF, acid detergent fiber; NDF, neutral detergent fiber; SID, standardized ileal digestible; Ca, calcium; P, phosphorus.

[b]Provided the following nutrients (per kg of air-dry diet): retinyl palmitate, 2000 IU; cholecalciferol, 200 IU; vitamin α-tocopherol acetate, 40 IU; menadione sodium bisulfite, 2 mg; thiamine, 1.5 mg; riboflavin, 7 mg; pyridoxine, 2.5 mg; cobalamin, 20 μg; calcium pantothenate, 14 mg; folic acid, 1 mg; niacin, 21 mg; biotin, 70 μg; choline, 350 mg. Minerals: Cu, 10 mg (as copper sulphate); iodine, 0.4 mg (as potassium iodine); iron, 120 mg (as ferrous sulphate); Mn, 10 mg (as manganous oxide); Se, 0.3 mg (as sodium selenite); Zn, 110 mg (as zinc oxide).

set at 22 °C in the T.K. Cheung Centre for Animal Science Research. Pigs had ad libitum access to feed and water from drinking nipples throughout the study.

Laboratory analysis

Fecal samples were dried at 60 °C for 72 h, and along with feed samples, were finely ground to pass through a 1 mm screen in a Thomas–Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). All samples were analyzed for dry matter (DM, method 934.01; AOAC 2000), ash (method 942.05; AOAC 2000), nitrogen (N, method 968.06; AOAC 2000) by an N analyzer (model NS-2000, Leco Corporation, St. Joseph, MI, USA), and gross energy (GE) by an oxygen bomb calorimeter

Table 2. Analyzed chemical composition of the experimental diets, as-fed.

	−AGP		+AGP	
Items	−MCOFA	+MCOFA	−MCOFA	+MCOFA
GE (kcal kg^{-1})	3880.8	3859.2	3913.3	3824.2
DM (g kg^{-1})	867.6	870.7	868.4	866.8
CP (g kg^{-1})	163.4	175.9	170.3	166.7
Crude fat (g kg^{-1})	44.2	42.2	42.6	41.0
Ca (g kg^{-1})	7.6	8.6	8.4	8.8
P (g kg^{-1})	6.2	6.6	6.2	6.2
Ash (g kg^{-1})	49.6	50.5	50.3	53.3

Note: Pigs were allotted diets supplemented with or without 2 g kg^{-1} of matrix-coated organic acids and medium-chain fatty acids blend (MCOFA) and 2.5 g kg^{-1} of antibiotic growth promoters (AGP), respectively. GE, gross energy; DM, dry matter; CP, crude protein; Ca, calcium; P, phosphorus.

(Parr Instrument Co., Moline, IL, USA). Crude fat was determined using hexane as the solvent according to the AOAC (method 920.39; AOAC 2000). Dietary total calcium (Ca, method 984.01; AOAC 1995) and phosphorus (P, method 965.17; AOAC 1995) were analyzed using a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA). Titanium was determined according to the method described by Lomer et al. (2000) with some modifications in the duration of digestion time and temperature settings. Briefly, samples were ashed at 550 °C for 12 h followed by digestion in sulfuric acid for 2 h at 350 °C, and then diluted with deionized water before quantification using an Inductively Coupled Plasma Mass Spectrometry (Varian Inc., Palo Alto, CA, USA).

Sampling and measurements

Individual pig BW and feed refusals were recorded on day 0 and day 42 to calculate average daily gain (ADG), ADFI, and growth efficiency (G:F). To determine the apparent total tract digestibility (ATTD) for DM, N, GE, crude fat, Ca, P, and ash, pigs were fed diets containing titanium dioxide (3 g kg^{-1} of diets) as an indigestible marker for 5 d followed by fecal grab sampling from 12 randomly selected pigs per treatment via rectal palpation on day 42. Samples for digestibility assessment were collected in plastic bags, and immediately frozen (−20 °C). All feed and fecal samples were then analyzed for DM, N, GE, crude fat, Ca, P, and ash as described before. The occurrence and severity of diarrhea was monitored, and assessed during the whole trial using a fecal consistency scoring system (0 = normal, 1 = soft feces, 2 = moderate diarrhea, and 3 = severe diarrhea; Marquardt et al. 1999). Fecal subsamples were used for pH determination using a pH meter (AB15 plus; Fisher Scientific, Toronto, ON, Canada).

For the blood profiles, one pig from each pen was randomly selected, and blood samples (10 mL per pig) were collected via jugular vena cava puncture from the same

pig on day 0 and day 42. Half of the sample was transferred into either ethylenediaminetetraacetic acid tubes or a 5 mL nonheparinized Vacutainer tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Blood samples were incubated at room temperature for 1 h before being centrifuged at 3000*g* for 10 min at 4 °C to obtain serum. Serum samples were stored at −80 °C until required for analysis. Whole blood samples were sent to the Manitoba Veterinary Diagnostic Services Laboratory (Winnipeg, MB, Canada) for routine hematological analysis using an automated analyzer (Orthoclinical Johnson & Johnson VITROS 250 Chemistry System; Diamond Diagnostics, Holliston, MA, USA), whereas serum urea nitrogen (SUN) was measured using an automatic biochemistry blood analyzer (HITACHI 747; Hitachi, Tokyo, Japan) by calorimetric methods.

For the analysis of fecal ammonia (NH_3), hydrogen sulfide (H_2S), and acetic acid, 300 g of fresh feces were randomly collected from 12 pigs per treatment by rectal palpation on the last day of the experiment according to the method described by Hossain et al. (2015). The total freshly sampled feces were then stored in 2.6 L plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster. The samples were allowed to ferment for 5 d at room temperature (25 °C). After the fermentation, a gas sampling pump (Model GV-100; Gastec Corp., Ayase, Japan) was utilized for gas detection (Gastec detector tube No. 3La for NH_3, No. 4LK for H_2S, and 81L for acetic acid; Gastec Corp.). Before measurement the fecal samples were manually shaken for approximately 30 s to disrupt any crust formation, and to homogenize the samples, and then 100 mL of the headspace air was sampled from approximately 2.0 cm above the sample.

Calculations and statistical analysis

The ATTD was calculated using the following formula: digestibility (%) = $\{1 - [(Nf \times Td)/(Nd \times Tf)]\} \times 100$, where, Nf is nutrient concentration in feces (% DM), Nd is nutrient concentration in diet (% DM), Td is titanium concentration in diet (% DM), and Tf is titanium concentration in feces (% DM).

All data were statistically analyzed using the MIXED procedure of SAS/STAT® version 9.2 (SAS Institute Inc., Cary, NC, USA) for a randomized complete block design with a 2 × 2 factorial arrangement. The model included diet as a fixed effect, where pig and period were included as random effects. Analysis of variance revealed that the effect of experimental period and pig were not significant, and thus, the effects of period and pig were removed from the model. The pig was considered as the experimental unit. Data on growth performance, nutrient digestibility, fecal consistency score, fecal pH, and fecal noxious gas emissions were based on a pen basis, whereas data on blood profiles were based on individual pigs. The data were tested for the treatment effects of dietary AGP, MCOFA, and their interaction. When a significant interaction was observed, the means of each treatment were compared using Fisher's protected least significant difference. Variability in the data was expressed as the pooled standard error of mean (SEM). Statistical significance is considered at $P \leq 0.05$.

Results

There were significant AGP × MCOFA interactions for G:F, but not for ADG and ADFI (Table 3). Pigs fed AGP along with MCOFA supplemented diets had improved G:F compared with those fed control diet ($P < 0.05$). Pigs receiving MCOFA diet had improved G:F compared with those fed MCOFA-free diet ($P < 0.05$). Moreover, pigs fed diet supplemented with AGP had improved G:F compared with those fed without AGP diet ($P < 0.05$). No significant differences in IBW and final BW were found among treatment groups ($P > 0.05$). Effects of MCOFA and AGP on ATTD of energy and nutrients are presented in Table 4. There were AGP × MCOFA interactions for ATTD of crude protein (CP), DM, fat, and GE ($P < 0.05$). Pigs fed AGP along with MCOFA supplemented diets had higher ATTD of CP, DM, fat and GE than those fed control diet ($P < 0.05$). Pigs fed the diet supplemented with AGP had higher ATTD of CP, DM, fat, GE, and ash than those fed the diet without AGP ($P < 0.05$). Moreover, pigs fed the diet supplemented with MCOFA had higher ATTD of CP, DM, fat, and GE ($P < 0.05$) than those fed the diet without MCOFA. No significant ($P > 0.10$) difference in diarrhea scores of growing pigs was found among treatment groups (data not shown).

No significant ($P > 0.10$) difference in hematological parameters was found among treatments at day 0 (data not shown). Lymphocyte counts were improved with MCOFA diet compared with MCOFA-free diet on day 41 ($P < 0.05$; Table 5). However, SUN was not affected ($P > 0.10$) by dietary AGP or MCOFA but an interaction effect between AGP and MCOFA was observed ($P < 0.05$). Effects of MCOFA and AGP on in vitro noxious gas emissions are presented in Table 6. There were significant AGP × MCOFA interactions for NH_3, but not for H_2S and acetic acid gas emissions. Pigs fed AGP along with MCOFA supplemented diets reduced fecal NH_3 gas emissions compared with those fed control diet ($P < 0.05$). Pigs fed diet supplemented with AGP had reduced NH_3 gas emissions compared with those fed without AGP diet ($P < 0.05$). Moreover, pigs fed the diet supplemented with MCOFA had reduced NH_3 gas emissions than those fed the diet without MCOFA ($P < 0.05$). Pigs fed MCOFA diet had reduced fecal acetic acid gas emissions compared with those fed diets without MCOFA ($P < 0.05$). The fecal pH of pigs fed with MCOFA diet was lower than that of pigs fed diets without MCOFA ($P < 0.05$). No significant differences in fecal moisture was found among treatments ($P > 0.10$). The average fecal moisture were 68.43% on day 42 (data not shown).

Table 3. The effects of dietary matrix-coated organic acids blend on growth performance in growing pigs.[a]

	−AGP		+AGP		AGP		MCOFA			*P* value		
Items	−MCOFA	+MCOFA	−MCOFA	+MCOFA	(−)	(+)	(−)	(+)	SEM	AGP	MCOFA	AGP × MCOFA
ADG (g)	1.03	1.09	1.11	1.06	1.06	1.09	1.07	1.08	0.030	0.508	0.971	0.060
ADFI (g)	2974	2735	2777	2842	2855	2810	2876	2789	103.08	0.644	0.375	0.126
G:F	0.347c	0.403a	0.404a	0.374b	0.375	0.389	0.376	0.388	0.006	0.037	0.046	<0.001

Note: Values with different lowercased letters in the same row differ significantly ($P < 0.05$). ADG, average daily gain; ADFI, average daily feed intake; G:F, growth efficiency; AGP, antibiotic growth promoters; MCOFA, matrix-coated organic acids and medium-chain fatty acids blend; SEM, standard error of the mean.

[a]There were 12 replicated pens of two pigs/pen per treatment with an initial body weight (BW) of 47.71 ± 3.73 kg and final BW of 92.78 ± 6.21 kg.

Table 4. The effects of dietary matrix-coated organic acids blend on apparent total tract digestibility (%) of energy, and nutrients in growing pigs.[a]

	−AGP		+AGP		AGP		MCOFA			*P* value		
Items	−MCOFA	+MCOFA	−MCOFA	+MCOFA	(−)	(+)	(−)	(+)	SEM	AGP	MCOFA	AGP × MCOFA
CP	74.34b	87.24a	85.57a	84.79a	80.79	85.18	79.96	86.02	1.61	0.005	0.002	<0.001
DM	81.31c	89.76ab	90.89a	89.33b	85.54	90.11	86.10	89.55	0.54	<0.001	<0.001	<0.001
Fat	65.73b	73.94a	74.62a	73.02a	69.84	73.82	70.18	73.48	1.70	0.014	0.040	0.003
GE	81.22c	89.51a	90.85a	87.64b	85.37	89.25	86.04	88.58	0.74	<0.001	<0.001	<0.001
Ca	58.50	61.53	63.38	62.68	60.02	63.03	60.94	62.11	3.06	0.312	0.719	0.544
P	50.63	55.94	57.09	57.19	53.29	57.14	53.86	56.57	3.48	0.233	0.400	0.420
Ash	57.50	63.87	65.51	64.79	60.69	65.15	61.51	64.33	1.98	0.028	0.169	0.058

Note: Values with different lowercased letters in the same row differ significantly ($P < 0.05$). MCOFA, matrix-coated organic acids and medium-chain fatty acids blends; AGP, antibiotic growth promoters; SEM, standard error of the mean; GE, gross energy; DM, dry matter; CP, crude protein; Ca, calcium; P, phosphorus.

[a]There were 12 replicated pens of two pigs/pen per treatment.

Table 5. The effects of dietary matrix-coated organic acids blend on routine hematology, and serum urea nitrogen (SUN) in growing pigs on day 41.[a]

	−AGP		+AGP		AGP		MCOFA			*P* value		
Items	−MCOFA	+MCOFA	−MCOFA	+MCOFA	(−)	(+)	(−)	(+)	SEM	AGP	MCOFA	AGP × MCOFA
Leukocytes												
WBC (10^9 L^{-1})	18.49	18.92	17.31	17.00	18.71	17.16	17.90	17.96	1.273	0.240	0.963	0.774
Segs (10^9 L^{-1})	4.55	4.16	5.12	5.35	4.36	5.24	4.84	4.76	0.431	0.055	0.863	0.482
Bands (10^9 L^{-1})	0.292	0.200	0.322	0.235	0.246	0.279	0.307	0.218	0.051	0.889	0.152	0.960
Eos (10^9 L^{-1})	0.311	0.332	0.428	0.245	0.322	0.337	0.370	0.289	0.087	0.888	0.459	0.354
Basos (10^9 L^{-1})	11.90	10.57	11.27	11.41	11.24	11.34	11.59	10.99	1.004	0.916	0.564	0.476
Lymphocyte (10^9 L^{-1})	0.43	0.92	0.51	0.57	0.68	0.54	0.47	0.75	0.129	0.283	0.035	0.098
Monos (10^9 L^{-1})	0.67	1.46	0.79	0.61	1.07	0.70	0.73	1.04	0.351	0.879	0.779	0.269
Erythrocytes												
RBC (10^{12} L^{-1})	7.69	7.55	7.42	7.46	7.62	7.44	7.56	7.51	0.123	0.140	0.680	0.486
HgB (g L^{-1})	126.75	126.75	125.66	125.72	126.75	125.69	126.21	126.24	2.324	0.641	0.990	0.989
Hct (L L^{-1})	0.413	0.410	0.409	0.410	0.412	0.410	0.411	0.410	0.006	0.749	0.853	0.710
MCV (fl)	49.51	54.40	55.19	55.02	51.96	55.11	52.35	54.71	2.318	0.186	0.308	0.287
MCH (pg)	16.52	16.79	16.94	16.83	16.66	16.89	16.73	16.81	0.256	0.373	0.745	0.477
MCHC (g L^{-1})	306.33	308.33	306.83	306.00	307.33	306.42	306.58	307.17	2.329	0.699	0.805	0.550
RDW (%)	15.98	16.20	16.36	16.24	16.09	16.30	16.17	16.22	0.119	0.091	0.677	0.152
Platelets (10^9 L^{-1})	331.58	335.70	334.63	358.25	333.64	346.44	333.11	346.98	22.238	0.523	0.489	0.626
SUN (mmol L^{-1})	5.87	5.92	6.25	6.80	5.89	6.52	6.06	6.36	0.349	0.739	0.849	0.024

Note: MCOFA, matrix-coated organic acids and medium-chain fatty acids blend; WBC, white blood cells; Segs, segmental neutrophils; Bands, band neutrophil counts; Eos, absolute eosinophil count; Basos, absolute basophil count; Monos, mononucleosis; RBC, red blood cells; HgB, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; AGP, antibiotic growth promoters; SEM, standard error of the mean.

[a]There were 12 replicated pens of two pigs/pen per treatment.

Table 6. The effects of dietary matrix-coated organic acids blend on in vitro noxious gas emissions (ppm), and fecal pH in growing pigs.[a]

	−AGP		+AGP		AGP		MCOFA			*P* value		
Items	−MCOFA	+MCOFA	−MCOFA	+MCOFA	(−)	(+)	(−)	(+)	SEM	AGP	MCOFA	AGP × MCOFA
NH_3	9.26a	8.33b	8.59b	8.40b	8.80	8.50	8.93	8.37	0.140	0.037	<0.001	0.011
H_2S	7.24	5.79	5.80	5.92	6.52	5.86	6.52	5.86	0.910	0.477	0.468	0.391
Acetic acid	3.16	1.66	2.41	1.79	2.41	2.10	2.79	1.73	0.522	0.557	0.048	0.407
pH	6.16	6.06	6.13	6.02	6.11	6.08	6.15	6.04	0.029	0.233	<0.001	0.966

Note: Values with different lowercased letters in the same row differ significantly ($P < 0.05$). NH_3, ammonia; H_2S, hydrogen sulfide; MCOFA, matrix-coated organic acids and medium-chain fatty acids blend; AGP, antibiotic growth promoters; SEM, standard error of the mean.

[a]300 g fresh fecal samples were used.

Discussion

The goal of this experiment was to determine whether adding a MCOFA supplement to growing pig diets would improve performance, and reduce in vitro noxious gas emissions when the pigs consumed a diet with supplemental AGP. Growing pig diets containing in-feed AGP have been used to increase growth rate and G:F, and to control incidences of diarrhea that are often attributed to alterations or suspension of the gut flora, and their metabolic activity (Pluske et al. 1997; Nyachoti et al. 2012). However, due to increased public pressure to discontinue the use of in-feed AGP in livestock diets, the need to identify effective alternative therapies has been the focus of several recent studies including MCOFA supplementation to the diet (Herfel et al. 2013; Cho et al. 2014; Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015). The MCOFA used in the current study was a blend of OA (i.e., fumaric acid, citric acid, and malic acid) and MCFA. The MCOFA would enhance the effectiveness of acidification of OA by supplying them in a nondissociated form to the intestine, and thereby reduce bacterial resistance to acids (Bearson et al. 1997). The range of OA and MCOFA effective dose in previous studies in poultry or pigs was variable (i.e., 0.05 ~ 5 g kg^{-1}; Walsh et al. 2007; Li et al. 2008; Cho et al. 2014; Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015). In the present study, it was hypothesized that feeding growing pigs a diet supplemented with 2 g kg^{-1} MCOFA would confer beneficial effects to the growing pigs through improved growth performance, nutrient digestibility, and reduced in vitro fecal noxious gas emissions as has been reported in studies with nursery piglets (Halas et al. 2010), finishing pigs (Cho et al. 2014; Upadhaya et al. 2014*a*), and lactating sows (Kluge et al. 2006).

In our study, pigs fed AGP along with MCOFA had improved G:F compared with those fed control diet. In agreement with our results, Bagal et al. (2016) reported that OA–AGP diet improved feed intake and G:F in broiler chickens, indicating a synergistic action of AGP and OA. Results of the present study indicate that feeding grower pigs diets containing AGP had improved G:F compared with those fed diets without AGP, which is in agreement with Wang et al. (2009), who observed that dietary supplementation of a combination of 110 mg kg^{-1} aureomycin and 20 mg kg^{-1} flavomycin improved the G:F of weanling pigs by its antimicrobial and health-promoting effect. The better G:F effects observed in pigs fed diets supplemented with MCOFA than those fed diets without MCOFA were accompanied with improved nutrient digestibility, and suggested that MCOFA have ability to stabilize the pH of the GIT, perhaps linked to manipulation of intestinal microbiota related to efficacy of acidification (Ravindran and Kornegay 1993). Consistent to our findings, some researchers have shown positive effects on ADG and G:F with formic acid or sorbic acid (Partanen and Mroz 1999;

Overland et al. 2008), blends of unprotected OA (i.e., fumaric, lactic, propionic, citric and benzoic acid; Walsh et al. 2007), and blends of MCOFA (Cho et al. 2014; Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015) with doses between 2 and 5 g kg^{-1} of basal diet in different stages of pigs. On the contrary, other reports indicate none or negative responses with single acidifier such as fumaric, citric or formic acid (Radecki et al. 1988; Manzanilla et al. 2004), or blend of acidifiers such as formic acid, lactic acid, and volatile fatty acids (Lee et al. 2007). The inconsistency might be attributed to the stage of growth, diet complexity, types of acids, inclusion level of acids, different coating techniques, and the health status of the pigs (Upadhaya et al. 2014*a*, 2014*b*; Lee et al. 2015).

In the present study, pigs fed AGP along with MCOFA supplemented diets increased CP, DM, fat, and GE digestibility compared with those fed control diet. Increases in nutrient digestibility by supplemental AGP and MCOFA are often attributed to alterations of the gut flora and their metabolic activity (Upadhaya et al. 2014*a*; Hossain et al. 2016). The present results showing higher ATTD of CP, DM, fat, GE, and ash in pigs fed diets supplemented with AGP compared with those fed the control diet, are consistent with results of others showing increased digestibility of nutrients due to feeding diets containing tylosin phosphate (Van Lunen 2003; Hao et al. 2014) or apramycin (Zhang and Kim 2014; Hossain et al. 2016) to pigs. Feeding growing pigs a diet supplemented with MCOFA improved ATTD of CP, DM, fat, and GE indicating that the matrix coating helps in reducing GIT pH, which in turn reduces intestinal pathogenic microbial (i.e., *Escherichia coli, Salmonella* spp.) growth due to the their lower acid tolerance capability, thus exhibiting a positive impact on intestinal health, and nutrient absorption (Halas et al. 2010; Upadhaya et al. 2014*a*; Lee et al. 2015). In the present study, fecal and GIT microbiota were not determined to better explain the observed beneficial pig responses. Therefore, this should be an important consideration in future studies to elucidate the effects of MCOFA on pig nutrient and energy digestibility and performance. However, the observation that MCOFA supplementation improved digestibility in pigs could be explained by the fact that MCOFA improved pre-caecal digestion leading to reduced influx of bacterially fermented substrates into the hindgut (Upadhaya et al. 2014*a*, 2014*b*), which may reduce the total quantity of fecal protein, and hence increase the digestibility of nitrogen, and also of DM and GE, as an indirect consequence. Moreover, our results, along with those of Yin et al. (2001) and Luckstadt and Mellor (2011), suggested that MCOFA stimulates exocrine pancreatic secretion of enzymes and bicarbonate which enhance digestibility. In the present study, the improvements in nutrients and GE digestibility translate into improvements in G:F of the growing pigs calculated.

Examination of the proportions of blood cells is important in the conclusion and checking of infection and contamination (Hossain et al. 2017); hematological parameters were evaluated to assess the impact of dietary MCOFA supplementation in the current study. The MCOFA had increased serum lymphocyte concentrations compared with control diet in the current study, indicating its function on fighting against infections by developing antibodies that can be of use to identify future attacks by the foreign bodies (Gao et al. 2016). It is well known that SUN concentration is an indicator of protein and amino acid utilization in pigs (Eggum 1970; Zhang and Kim 2013; Oliver et al. 2014). The higher SUN from animals fed AGP along with MCOFA diets is likely due to increased amino acids that were metabolized and circulated in the blood stream before the excretion (Oliver and Wells 2013; Oliver et al. 2014), and supports the hypothesis that pigs that consumed diets with AGP or MCOFA utilized most of the dietary amino acids for protein deposition, unlike control pigs. Indeed, considering that pigs fed AGP or MCOFA diet consumed similar amounts of feed compared with control pigs, the greater SUN was expected. To the best of our knowledge, effect of feeding MCOFA–AGP supplemented diets to pigs on hematological parameters has not been reported. Thus, further research is needed to evaluate the effect of MCOFA and AGP supplementation on growing pigs especially with the serum lymphocyte counts and SUN concentrations.

The emission of odorous gases such as NH_3, H_2S, and acetic acid from pig production facilities contribute to environmental pollution, and can be hazardous to humans and animals (Zahn et al. 1997; Eriksen et al. 2010; Hossain et al. 2015). To ensure sustainable pig production, the emission of such odorous gases should be reduced by proper management and dietary interventions. In our study, pigs fed AGP along with MCOFA supplemented diets reduced fecal NH_3 gas emissions than those fed control diet. Moreover, pigs fed MCOFA reduced fecal acetic acid gas emissions compared with MCOFA-free diet in growing pigs, which is due to the increased nutrient digestibility that may have allowed less substrate for microbial fermentation in the large intestine (Upadhaya et al. 2014*b*; Hossain et al. 2015). It is suggested by Canh et al. (1998) and Song et al. (2012) that the N from ammonia is generally derived from fermentation of unabsorbed N entering the large intestine. Indeed, the reduced gas emission due to supplementation of MCOFA in growing pigs was due to the reduced fecal pH because it inhibits the invasion, and proliferation of pathogenic bacteria in the GIT, which further limits production of toxic bacterial metabolites and ammonia (Dibner and Buttin 2002; Kil et al. 2011; Upadhaya et al. 2014*a*). Upadhaya et al. (2014*a*) also reported that dietary supplementation with MCOFA at 2 g kg^{-1} reduced fecal NH_3 and acetic acid emissions compared with the basal diet in finishing pigs. Therefore,

in the current study, the decreased fecal gas emission was, perhaps, due to the improvement of nutrient digestibility and the intestinal microbial balance.

Conclusion

A supplementation of both AGP and MCOFA improved G:F and ATTD of CP, DM, fat, and GE, suggesting that MCOFA may have some growth-promoting effects similar to AGP in growing pigs. Moreover, pigs fed diets supplemented with MCOFA reduced fecal pH and increased serum lymphocyte concentrations. Similar to AGP, MCOFA reduced in vitro noxious gas emissions. These results suggest that supplementation of MCOFA can promote the performance of growing pigs subjected to an antibiotic-free feeding regimen. However, further studies on fecal microbiota are still warrant to investigate the mechanism underline.

Conflict of Interest

We have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or personal interest of any nature in any product, service and (or) company that could be construed as influencing the position presented in the review of this paper.

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