Supplementation with organic acids showing different effects on growth performance, gut morphology, and microbiota of weaned pigs fed with highly or less digestible diets[1]

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ABSTRACT: Two studies were conducted to evaluate the effects of organic acid (**OA**) supplementation in a highly digestible (Exp. 1) or a less digestible diet (Exp. 2) on the growth performance and intestinal health of weaned pigs. In Exp. 1, a total of 240 pigs weaned at day 21 were assigned to one of five dietary treatments: negative control (**NC**) (basal diet, 3,000 ppm zinc oxide (**ZnO**) in the first 2 wk only); positive control (**PC**) (NC plus 10 mg/kg zinc bacitracin, 5 mg/kg colistin sulfate, and 5 mg/kg olaquindox); OA1 (NC plus a 0.2% blend of encapsulated butyrate, medium-chain fatty acids [**MCFA**], OA, and phenolics); OA2 (NC plus 0.3% blend of free and buffered short-chain fatty acids [**SCFA**] combined with MCFA); and OA1 plus OA2 (NC plus 0.2% OA1 plus 0.3% OA2) for 49 d. All treatments in Exp. 1 used the same highly digestible basal diet. At day 28, eight pigs from each group were sacrificed, to collect intestinal and digesta samples for biochemical analysis. Growth performance and intestinal morphology were not affected by the treatments. However, pigs subjected to the OA2 treatment had lower levels of *Escherichia coli* ($P < 0.05$) in the colon. In addition, the OA1 and OA2 treatments, and their combination resulted in higher concentrations of acetate and propionic acid in the cecum and colon ($P < 0.01$) in comparison to the NC. A less digestible diet without high levels of ZnO was used in Exp. 2. A similar design was used with the exception of the replacement of OA2 with another OA blend (OA3, a blend of free and buffered OA). In comparison to the NC, supplementation with OA1 and OA3 in a less digestible diet improved the ADG and the F:G ratio in the seventh week post-weaning ($P < 0.01$); reduced the diarrhea index of pigs during the first 3 wk post-weaning ($P < 0.05$); increased the ileal villus height ($P < 0.05$), and acetic and propionic acid concentrations in colon contents ($P < 0.05$). Moreover, the genus *Prevotella* was increased in the colon and the microbial community structure was significantly altered in the OA1 + OA3 treatment. The present research indicated that dietary supplementation with OA improved intestinal health. The OA blends showed a similar growth-promoting effect as antibiotics in the less digestible diet, to which high levels of ZnO had not been added.

Key words: intestinal morphology, microbiota composition, organic acids, weaned pigs

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INTRODUCTION

Stressful factors associated with early weaning often cause digestive disorders, nutrient malabsorption, and a high incidence of diarrhea in pigs (Boudry et al., 2004; Fairbrother et al., 2005). The restricted or banned use of antibiotics and high levels of zinc oxide (**ZnO**) poses a great challenge to weaning pigs, and nutritional scientists must find effective alternative antibiotic growth promoters (**AGP**) (Kuang et al., 2015; Wang et al., 2016). The effects of the diet on intestinal enzymes and tissue development could therefore be critical during the earlier weaning stages, when abrupt stresses and undernourishment potentially are likely (Cera et al., 1988). Furthermore, diet formulation influences gastrointestinal morphology, physiology, and microbiology (van Beers-Schreurs, 1996; Pluske et al., 1997; Mikkelsen and Jensen, 2001).

Acidifiers or products based on organic acids (**OA**) seem to be the alternative of choice to replace antibiotics in postweaning pig diets. A large number of studies have shown that OA are beneficial in enhancing growth performance and nutrient digestibility, as well as modulating intestinal microbiota in pigs and broilers (Namkung et al., 2004; Abdelqader and Al-Fataftah, 2016). Studies in pigs have shown improvements in growth performance and repair of damaged intestinal tissues following the administration of butyrate, although it also evidently reduced feed intake owing to its irritating odor (Gálfi and Bokori, 1990; Piva et al., 2002; Kotunia et al., 2004; Mazzoni et al., 2008; Le Gall et al., 2009). Formic acid and its salts have been found to reduce the pH of the gastrointestinal tract and thereby enhance the activity of digestive enzymes (Partanen and Mroz, 1999; Mroz et al., 2000; Creus et al., 2007). A blend of medium-chain fatty acids (**MCFA**) and short-chain OA can be utilized by enterocytes as energy sources and can attenuate the negative effects of weaning on villus length and crypt depth (**CD**) in pigs (Lee et al., 2007).

In the present study, we evaluated the efficacy of a mixture of three commercial OA: OA1 (used in both experiments, a synergistic blend of phenolic compounds, slow release C12, target release butyrate, MCFAs, and free and buffered OA); and OA2 (used in Exp. 1, a blend of free and buffered short-chain fatty acids (**SCFA**) (mainly formic acid, acetic acid, and propionic acid) combined with MCFAs); or OA3 (used in Exp. 2, a synergistic blend of free and buffered OA based on formic acid). The carrier for OA1, OA2, and OA3 is silica. These OA were evaluated separately and in combination, to elucidate their specific effects on growth performance, gut morphology, and microbiota of weaned pigs fed highly or less digestible diets.

MATERIALS AND METHODS

The experiments followed the actual law of animal protection, were approved by the Animal Care and Use Committee of the Sichuan Agricultural University, and were performed in accordance with the National Research Council's Guideline for the Care and Use of Laboratory Animals.

In both experiments, all pigs (Duroc × Landrace × Yorkshire) were weaned at approximately 21 d of age and were housed in the fully slatted pens (1.46 × 0.30 × 0.66 m). Each pen was fitted with an adjustable stainless steel feeder and a duckbill drinker. Animals had free access to feed and water throughout the whole study period. The temperature was maintained at 24–28 °C, and the relative humidity was controlled at 60–70%.

Animal and Diets

Experiment 1. A total of 240 weaned pigs with an average weaning age of 21 d and initial BW of 7.2 ± 0.20 kg were used in a 42-d trial to determine the effects of two OA blends (OA1 and OA2) in a highly digestible diet. The basal diets of Exp. 1 included highly digestible carbohydrate ingredients (e.g., extruded corn, extruded rice, extruded wheat and whey dry) and low antinutritional factors protein ingredients (e.g., extruded soybean, Spray-Dried Plasma Protein, fishmeal). Pigs were assigned at random to one of five dietary treatments with eight replicate pens per treatment (three barrows and three gilts per pen). The dietary treatments included negative control (**NC**, basal diet), positive control (**PC**, basal diet + 10 mg/kg zinc bacitracin, 5 mg/kg colistin sulphate, and 5 mg/kg olaquindox), OA1 diet (NC + 0.2% OA1), OA2 diet (NC + 0.3% OA2), and OA1 + OA2 (NC + 0.2% OA1 + 0.3% OA2). As shown in Table 1, nutrients in the diet meet or exceed the nutritional requirements of pigs according to NRC (2012). Pig weight and feed disappearance were measured every 2 weeks to determine ADG, ADFI, and F:G. The health status of the animals was monitored throughout the study. The clinical signs of diarrhea were visually assessed every day by observers blinded to treatments, and a scoring system was applied to indicate the presence and severity of diarrhea as follows: 1 = hard feces; 2 = slightly soft feces; 3 = soft, partially formed feces; 4 = loose, semi-liquid feces; and 5 = watery, mucous-like feces. When the average score was over

Table 1. Ingredients and composition of the basal diet for Exp. 1

	Phase	
Ingredients, %	0–14 d	14–42 d
Corn	18.16	38.16
Extruded corn	18.00	10.00
Soybean meal	10.00	18.00
Extruded soybean	13.00	10.00
Extruded wheat	5.00	5.00
Extruded rice	10.00	3.00
Fish meal	5.00	4.00
SDPP	3.00	0.00
Whey powder	13.00	7.00
Soy oil	1.86	2.07
$CaHPO_4$	0.45	0.54
Limestone	0.95	0.83
Salt	0.33	0.30
L-lysine HCl (98%)	0.35	0.36
DL-Methionine	0.10	0.07
L-Threonine	0.10	0.11
L-Tryptophan	0.00	0.02
ZnO	0.30	0.04
Vitamin/trace element premix[1]	0.40	0.00
Vitamin/trace element premix[2]	0.00	0.50
Total	100.00	100.00
Nutrient composition (%)		
DE (Kcal/kg)	3,542	3,490
CP	20.56	19.63
Ca	0.8	0.7
Digestible P	0.4	0.34
d-Lys	1.35	1.24
d-Met	0.39	0.36
d-Thr	0.79	0.73
d-Trp	0.23	0.20

ZnO, zinc oxide; SDPP, spray dried animal plasma.

[1]The premix provided for per kg of feed: Zn, 100 mg; Mn, 4 mg; Fe, 100 mg; Cu, 6 mg; I, 0.14 mg; Se, 0.3 mg; choline chloride, 500 mg; VA, 10500 IU; VD3, 3300 IU; VE, 22.5 IU; VK3, 3 mg; VB1, 3 mg; VB2, 7.5 mg; VB6, 4.5 mg; VB12, 0.03 mg; niacin, 30 mg; pantothenate, 15 mg; folic acid, 1.5 mg; biotin, 0.12 mg.

[2]The premix provided for per kg of feed: Zn, 80 mg; Mn, 3 mg; Fe,100 mg; Cu, 5 mg; I, 0.14 mg; Se, 0.25 mg; choline chloride, 400 mg; VA, 10500 IU; VD3, 3300 IU; VE, 22.5 IU; VK3, 3 mg; VB1, 3 mg; VB2, 7.5 mg; VB6, 4.5 mg; VB12, 0.03 mg; niacin, 30 mg; pantothenate, 15 mg; folic acid, 1.5 mg; biotin, 0.12 mg.

3, pigs were identified as having diarrhea. Diarrhea rate was calculated according to the formula reported by Sun et al., 2008:

$$\text{Diarrhea rate (\%)} = \text{the number of diarrhea pigs} \times \text{diarrhea days} / \text{the total number of pigs} \times \text{experiment days.}$$

Experiment 2. A total of 240 weaned pigs with an average weaning age of 21 d and initial BW of 6.90 ± 0.10 kg were used in a 49-d trial to determine the effects of two OA blends (OA1 and OA3) in a less digestible diet. The basal diets of Exp. 2 included lower ratio of high digestibility carbohydrate ingredients and low antinutritional factors protein ingredients than Exp. 1, and it did not use extruded wheat and extruded rice. No high levels of ZnO were included either. Pigs were assigned at random to one of five dietary treatments with eight replicate pens per treatment (three barrows and three gilts per pen). The dietary treatments included NC (basal diet), positive control (**PC**, basal diet + 40 ppm zinc bacitracin + 100 ppm olaquindox + 55 ppm kitasamycin), OA1 diet (NC + 0.2% OA1), OA3 diet (NC + 0.3% OA3), and OA1 + OA3 (NC + 0.2% OA1 + 0.3% OA3). As shown in Table 2, nutrients in the diet meet or exceed the nutritional requirements of pigs according to NRC (2012). Pig weight and feed disappearance were measured on days 0, 14, 28, 42, and 49 to determine ADG, ADFI, and F:G. The health status of the animals was monitored throughout the study. Fecal scoring was initiated on day 1 after placement through day 28 and was assessed by pen using the same methodology as described for Exp. 1

Sample Collections

In each experiment, a total of eight pigs per treatment were sacrificed at the end of experiment in a randomized order to ensure comparable conditions for sampling and sample processing. Before sampling, pigs were fasted overnight and the morning meal was provided to ensure sampling of intestinal contents approximately 4 h before slaughter. Pigs were sacrificed as previously described (Li et al., 2014). The pigs were anesthetized with an intravenous injection of pentobarbital sodium (50 mg/kg BW) before slaughtered. Immediately after death, the abdominal cavity was opened, and ileal, colonic, and caecal content samples were quickly collected as previously described (Kuang et al., 2015; Wang et al., 2016), snap-frozen in liquid nitrogen, and stored at −80 °C until analysis. Two-cm-long segments of duodenum, jejunum, and ileum were sampled as previously described and immediately fixed in phosphate-buffered paraformaldehyde (4%, pH 7.6) for histological measurements.

Measurement of the pH in the Digesta

In both experiment, digesta from the stomach, duodenum, jejunum, ileum, and colon was collected and the pH was measured with a pH electrode (InLab 410 pH-Kombinationselektrode; Mettler Toledo GmbH, Gießen, Germany) after a 2-point

Table 2. Ingredients and composition of the basal diet for Exp. 2

	Phase	
Ingredients, %	0–14 d	14–49 d
Corn	37.55	48.09
Extruded corn	18.00	15.00
Soybean meal, 47% CP	13.00	18.5
Extruded soybean	10.00	6.00
Fish meal	4.00	3.00
SDPP	3.00	0.00
Whey powder	10.00	5.00
Soy oil	1.03	1.08
$CaHPO_4$	0.78	0.66
Limestone	0.95	0.90
Salt	0.30	0.30
L-lysine HCl (98%)	0.32	0.39
DL-Methionine	0.16	0.20
L-Threonine	0.11	0.16
L-Tryptophan	0.00	0.02
ZnO	0.00	0.00
Rice bran	0.30	0.20
Vitamin/trace element premix[1]	0.50	0.00
Vitamin/trace element premix[2]	0.00	0.50
Total	100.00	100.00
Nutrient composition (%)		
DE (Kcal/kg)	3,542	3,490
CP	20.56	19.63
Ca	0.8	0.7
Digestible P	0.4	0.34
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d-Met	0.39	0.36
d-Thr	0.79	0.73
d-Trp	0.23	0.20

ZnO, zinc oxide; SDPP, spray dried animal plasma.

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[2]The premix provided for per kg of feed: Zn, 80 mg; Mn, 3 mg; Fe,100 mg; Cu, 5 mg; I, 0.14 mg; Se, 0.25 mg; choline chloride, 400 mg; VA, 10500 IU; VD3, 3300 IU; VE, 22.5 IU; VK3, 3 mg; VB1, 3 mg; VB2, 7.5 mg; VB6, 4.5 mg; VB12, 0.03 mg; niacin, 30 mg; pantothenate, 15 mg; folic acid, 1.5 mg; biotin, 0.12 mg.

calibration was performed using standard solutions of pH 4 and 7.

Intestinal Morphology Analysis

Intestinal segments were taken out from fixative solution and then dehydrated with increasing concentrations of ethanol and chloroform. The segments were processed with paraffin, and two transverse tissue samples were cut from each segment using a microtome. These parts of the tissue samples were dehydrated, embedded together in paraffin wax, and sectioned at 5 μm. One transverse tissue sample of each segment was transferred to a slide and stained with hematoxylin and eosin. Villi height (**VH**) and CD were determined as we described previously (Cao et al., 2014). Briefly, 10 intact, well-oriented crypt-villi units per sample were randomly selected and measured. The VH was measured from the tip of the villi to the base between individual villi, and CD measurements were taken from the valley between individual villi to the basal membrane.

SCFAs Analysis

In each experiment, SCFAs including acetate, propionate, and butyrate in digesta samples were analyzed with a modification of the previous method (Zhou et al., 2017). Briefly, 2 g of digesta samples was weighed into a 10-mL centrifuge tube and added with 5 mL of deionized water. After the tube was capped, the content was vortex mixed for 30 s, left to stand for 30 min at 4 °C and then centrifuged (1,000 × *g*, 4 °C) for 10 min. The supernatant (1.2 mL) was removed by aspiration into another 5-mL centrifuge tube, added with 0.24 mL of 25% metaphosphate, vortex mixed for 30 s and then left to stand for 30 min at 4 °C. Next, the contents were centrifuged (1,000 × *g*, 4 °C) for 10 min and then 1.2 mL of the supernatant was removed by aspiration, added with 23.3 μL of 210 mmol/L cortonic acid, and vortex mixed for 30 s. Then, 0.3 mL of the mixed solution was removed into another 2-mL tube, added with 0.9-mL carbinol, and vortex mixed for 30 s for the following gas chromatography analysis. The samples were analyzed by CP-3800 gas chromatography (Varian, Inc.) equipped with a micro-injector (10 μL), a flame ionization detector, and a capillary chromatographic column (CP-FFAP, 25 m × 0.32 mm × 0.3 μm). The injector temperature was 220 °C, detector temperature was 250 °C, hydrogen flux was 40 mL/min, and air flux was 450 mL/min. The temperature program was as follows: 100 °C hold 1 min, increase to 190 °C at 20 °C/min, increase to 190 °C hold 3 min. Peaks were identified by comparing their retention times with individual reference standard fatty acids.

Microbial Analyses

In two studies, another ileal and colonic digesta samples were used for microbial analysis. Total bacterial DNA was extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-tek, Norcross, GA) according to the manufacturer's protocols.

In Exp. 1, microbial analyses of ileum and colon digesta were accomplished by quantitative real-time PCR (**RT-PCR**). Quantitative RT-PCR of total bacteria was performed with SYBR Green PCR reagents (TaKaRa, Kyoto, Japan), whereas quantitative RT-PCR for *Lactobacillus*, and *Escherichia coli* were performed with Taq Primers and fluorescent oligonucleotide probes which were commercially synthesized (Life Technologies Ltd., Beijing, China) as listed in Table 3. The PCR reaction of total bacteria was set up in a total volume of 15 μL and contained 7.5 μL of SYBR Premix Ex TaqII, 0.6 μL of each primer, 0.6 μL of genomic DNA, and 5.7 μL of sterile deionized water, whereas the PCR reaction for *Lactobacillus* and *E. coli* were set up in a total volume of 10 μL and contained 0.15 μL of probe, 0.5 μL of each primer, 0.5 μL of probe Enhancer Solution, 4 μL of Real Master Mix, 0.5 μL of genomic DNA, and 3.85 μL of sterile deionized water. Amplification was performed using a CFX 96 System (BIO-RAD). As for total bacteria, the amplification program consisted of 1 cycle of 95 °C for 30 s and then 40 cycles of 95 °C for 5 s, 60 °C for 34 s, and 1 cycle of 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s. For *Lactobacillus* and *E. coli*, the amplification program consisted of 1 cycle of 50 °C for 2 min, 95 °C for 10 min and then 40 cycles of 95 °C for 15 s and 55.6–59.5 °C for 1 min (according to the primer set, see Table 3). Melting curve analysis and size determination of amplificates on agarose gels verified amplification of the target fragments. Standard curves were generated as previously described (Hu et al., 2016).

In Exp. 2, before sequencing, the concentration and purity of the extracted genomic DNA were measured. The integrity of the extracted genomic DNA was determined by electrophoresis on a 1% (w/v) agarose gel. Extracted colon digesta DNA samples were sent to Novogene Bioinformatics Technology (Beijing, China) to perform amplicon pyrosequencing on the Illumina HiSeq PE250 platforms. The V4 hypervariable region of the 16S rRNA gene was amplified using 515F and 806R primer (5′-GTGCCAGCMGCC GCGGTAA-3′ and 5′-GGACTACHVGGGTWT CTAAT-3′, respectively).

The effective tags were mapped to operational taxonomic unit (**OTU**) using Uparsev7.0.1001 at 97% sequence similarity. Representative sequences for each OTU were selected. The Ribosomal Database Project (**RDP**) classifier version 2.2 was used to assign a taxonomic rank to each representative sequence. The relative abundance of each OTU was examined at different taxonomic levels.

Statistical Analysis

Data are presented as means with pooled SEM, unless otherwise specified. The original data were checked by using Grubbs' test method. If $|Xp - \bar{X}| > \lambda(\alpha, n)S$, Xp was considered as the outlier. Descriptive statistics were performed to check the normality and homogeneity of variances before using parametric analyses. All of the data were tested for normal distribution. Afterward, the data were analyzed using the general linear models procedure of SAS statistical package (version 9.2; SAS Institute, Inc.) in a completely randomized design. The following statistical model was used: $Yij = \mu + Ti + eij$ where Y is the analyzed variable, μ is the overall mean, T is the effect of treatment ($i = 1 \ldots 3$), and e is the residual error ($i = 1 \ldots 5$, $j = 1 \ldots 8$). The Duncan's multiple-range test was used to compare the group means when the F test in the analysis of variance table was significant. The performance data were calculated separately before and after day 28, and the pen was recognized as a statistical unit for ADG, ADFI, F:G, and diarrhea scores. The selected pig in each pen was taken as an experimental unit for the parameters related to the intestinal morphology analysis. Before analysis, bacterial count values of Exp. 1 and relative abundance at phylum data of Exp. 2 in colonic contents were log-transformed before statistical analysis. Multicomparison was conducted by Duncan's multiple-range test. The difference was considered to be significant at $P < 0{\cdot}05$, and P value between 0.05 and 0.1 was classified as a tendency. In Exp. 2, for the microbiome data, the selected pig in each pen was taken as an experimental unit, each treatment with $n = 8$ except that PC with $n = 7$ due to a sample from PC was undetected, therefore, its values were excluded from the present study. Diversity within communities (alpha diversity) calculations and taxonomic community assessments were performed by Qiime 1.7.0. Principal coordinates analysis plots were produced using unweighted UniFrac metrics.

RESULTS

Growth Performance, Diarrhea Index, Digesta pH, Intestinal Morphology, SCFA, and Microbiota (Experiment. 1)

Growth performance and intestinal morphology were not affected by the various treatments (Table 4). In addition, the diarrhea index was not affected by the treatments overall or during any particular phase. However, OA1 and OA2 both reduced

the diarrhea index ($P < 0.05$) from days 15 to 17, when pigs were facing a change of feed (Table 5).

No significant differences were noted in digesta pH of the stomach, jejunum, and colon (Table 6). Pigs fed the OA2 diet had a higher duodenal VH than pigs fed other treatments ($P < 0.05$); whereas no significant differences ($P > 0.05$) were found in jejunal and ileal morphology (Table 7). In addition, OA1, OA2, and their combination resulted in higher concentrations of acetate and propionic acid in the cecum and colon ($P < 0.01$) in comparison to the NC (Table 8). Furthermore, pigs treated with OA2 supplementation had a lower number of *E. coli* ($P < 0.05$) in the colon (Table 9).

Growth Performance, Diarrhea Index, Digesta pH, Intestinal Morphology, and SCFAs (Exp. 2)

During the last week of the experiment, OA1, OA3, and their combination resulted in a higher ADG ($P < 0.01$) and lower F:G ($P < 0.01$) (Table 10) in comparison to the NC. In addition, during the first 3 wk post-weaning, all treatments reduced the diarrhea index in comparison to the NC ($P < 0.05$) (Table 11). However, digesta pH of the stomach, duodenum, jejunum, ileum, and colon were not affected ($P > 0.10$) by OA or the PC (Table 12).

Pigs fed the PC and OA1 + OA3 diets had a higher ileal VH than those fed the NC ($P < 0.05$

Table 3. Oligonucleotide primers and probes used for bacteriological analysis

Primers/probes		Sequence (5′–3′)	Tm (°C)	Product size (bp)
Escherichia coli	Forward	CATGCCGCGTGTATGAAGAA	57	96
	Reverse	CGGGTAACGTCAATGAGCAAA		
	Probe	AGGTATTAACTTTACTCCCTTCCTC		
Lactobacilli	Forward	GAGGCAGCAGTAGGGAATCTTC	55.7	126
	Reverse	CAACAGTTACTCTGACACCCGTTCTTC		
	Probe	AAGAAGGGTTTCGGCTCGTAAAACTCTGTT		
Total bacteria	Forward	ACTCCTACGGGAGGCAGCAG	64.5	200
	Reverse	ATTACCGCGGCTGCTGG		

Table 4. Growth performance of pigs fed the Exp. 1 diets[1]

	Diets[2]						
Item	NC	PC	OA1	OA2	OA1 + OA2	SEM[3]	*P* value
BW, kg							
Initial	7.21	7.21	7.22	7.20	7.08	0.03	0.911
Day 14	10.00	10.17	10.38	10.02	10.07	0.08	0.428
Day 28	16.03	16.38	16.27	15.84	16.08	0.15	0.808
Day 42	23.95	24.02	24.14	23.73	24.07	0.18	0.965
ADG, g/d							
Day 1–14	200	212	227	204	217	4.68	0.382
Day 15–28	429	444	424	415	430	6.92	0.795
Day 1–28	314	328	329	309	323	5.07	0.765
Day 29–42	571	586	592	578	582	6.17	0.883
ADFI, g/d							
Day 1–14	313	333	352	318	326	5.55	0.189
Day 15–28	704	718	696	673	696	9.45	0.690
Day 1–28	508	526	524	496	511	6.69	0.632
Day 29–42	896	932	941	928	916	9.12	0.620
F:G, g/g							
Day 1–14	1.57	1.59	1.55	1.58	1.51	0.02	0.852
Day 15–28	1.64	1.63	1.65	1.63	1.63	0.02	0.994
Day 1–28	1.62	1.61	1.61	1.61	1.59	0.02	0.983
Day 29–42	1.57	1.59	1.59	1.60	1.58	0.02	0.978

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA2 = NC plus organic acid 2; OA1 + OA2 = NC plus organic acid 1 and organic acid 2.

[3]Pooled SEM; $n = 8$/treatment.

Table 5. Diarrhea index of pigs fed the Exp. 1 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA2	OA1 + OA2	SEM[3]	*P* value
Diarrhea index							
Day 1–14	0.92	0.83	0.78	0.74	0.75	0.03	0.168
Day 15–17	1.14[a]	1.07[ab]	0.90[bc]	0.71[c]	1.06[ab]	0.04	0.004
Day 15–28	1.20	0.99	1.14	1.03	1.05	0.03	0.263
Day 1–28	1.06	0.91	0.96	0.89	0.90	0.02	0.146

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA2 = NC plus organic acid 2; OA1 + OA2 = NC plus organic acid 1 and organic acid 2.

[3]Pooled SEM; $n = 8$/treatment.

Table 6. Gut digesta pH of pigs fed the Exp. 1 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA2	OA1 + OA2	SEM[3]	*P* value
Stomach	4.35	4.33	4.77	4.55	3.79	0.21	0.687
Jejunum	6.50	6.06	6.51	6.45	6.38	0.07	0.169
Colon	5.91	5.92	6.06	5.92	5.93	0.06	0.935

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA2 = NC plus organic acid 2; OA1 + OA2 = NC plus organic acid 1 and organic acid 2.

[3]Pooled SEM; $n = 8$/treatment.

Table 7. Intestinal morphology of pigs fed the Exp. 1 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA2	OA1 + OA2	SEM[3]	*P* value
Duodenum							
VH[4], µm	719[b]	742[b]	742[b]	856[a]	790[ab]	15.36	0.026
CD[5], µm	412	341	353	359	367	11.85	0.420
VH/CD	1.83	2.25	2.15	2.45	2.23	0.08	0.240
Jejunum							
VH, µm	616	621	622	650	641	9.15	0.751
CD, µm	223	240	228	258	230	5.92	0.362
VH/CD	2.77	2.69	2.75	2.56	2.82	0.06	0.758
Ileum							
VH, µm	579	550	578	592	563	9.81	0.741
CD, µm	162	184	161	157	157	4.01	0.227
VH/CD	3.60	3.04	3.67	3.80	3.65	0.10	0.164

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA2 = NC plus organic acid 2; OA1 + OA2 = NC plus organic acid 1 and organic acid 2.

[3]Pooled SEM; $n = 8$/diet.

[4]VH = villus height.

[5]CD = crypt depth.

for both). The OA1 and OA1 + OA3 diets had a tendency to increase the VH of the jejunum ($P < 0.1$); however, no significant differences were noted in duodenal morphology ($P > 0.05$) (Table 13). The acetic and propionic acid concentrations in the colon contents were both increased ($P < 0.05$) by the PC and OA1 + OA3 diets. Furthermore, OA1 + OA3 had a tendency to increase the butyrate concentration in the cecum ($P < 0.1$) (Table 14).

Table 8. Volatile fatty acid concentration (µmol/g fresh matter) in the cecum and colon of pigs fed the Exp. 1 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA2	OA1 + OA2	SEM[3]	*P* value
Cecum, (µmol/g)							
Acetic	25.94^{c}	43.90^{ab}	56.80^{a}	38.95^{bc}	41.90^{abc}	2.81	0.006
Propionic	11.88^{c}	19.67^{ab}	23.33^{a}	14.75^{bc}	17.66^{abc}	1.18	0.019
Butyrate	6.25	10.04	12.83	8.94	11.26	0.76	0.059
Colon, (µmol/g)							
Acetic	23.54^{c}	40.63^{b}	49.07^{ab}	57.64^{a}	23.54^{ab}	2.91	0.000
Propionic	8.22^{b}	14.47^{a}	19.73^{a}	20.61^{a}	16.28^{a}	1.20	0.002
Butyrate	6.79^{b}	10.48^{ab}	12.65^{a}	13.32^{a}	11.59^{a}	0.69	0.008

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA2 = NC plus organic acid 2; OA1 + OA2 = NC plus organic acid 1 and organic acid 2.

[3]Pooled SEM; $n = 8$ for NC, OA1, and OA3, $n = 7$ for OA1+OA3 and PC group.

Table 9. Microbial copy numbers (log10 Cfu/g of digesta) in the ileum and colon of pigs fed the Exp.1 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA2	OA1 + OA2	SEM[3]	*P* value
Ileum							
E. coli	6.22	5.64	5.71	5.64	5.95	0.17	0.764
Lactobacilli	7.58	7.03	7.41	6.69	6.93	0.13	0.202
Total bacteria	10.05	9.82	10.15	10.27	10.04	0.07	0.491
E. coli: total bacteria	0.62	0.58	0.56	0.52	0.59	0.02	0.633
Lactobacilli: total bacteria	0.75	0.69	0.73	0.68	0.69	0.01	0.152
Colon							
E. coli	7.14^{a}	5.84^{c}	6.58^{ab}	6.43^{bc}	6.79^{ab}	0.11	0.006
Lactobacilli	8.87^{a}	7.88^{b}	8.23^{b}	8.28^{b}	8.40^{ab}	0.21	0.013
Total bacteria	11.50	11.23	10.86	11.02	11.30	0.52	0.139
E. coli: total bacteria	0.62	0.52	0.61	0.58	0.60	0.11	0.052
Lactobacilli: total bacteria	0.77	0.70	0.76	0.75	0.74	0.01	0.099

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA2 = NC plus organic acid 2; OA1 + OA2 = NC plus organic acid 1 and organic acid 2.

[3]Pooled SEM; $n = 8$/treatment.

Microbiota of the Colon (Exp. 2)

Characteristics of midgut bacterial community libraries. The average total tag, average taxon tag, average unique tag, and average OTU number for each treatment are presented in Table 15. Venn diagrams were used to evaluate the distribution of OTUs among the different groups. Based on these analyses, out of a total of 2,275 OTUs, 1,071 (~47%) coexisted in all five groups (Fig. 1A). In comparison, 33, 24, 59, 86, and 90 unique OTUs were identified in the NC, PC, OA1, OA3, and OA1 + OA3 groups, respectively. As Fig. 1B shows, out of 2,038 OTUs, 1,185 (~59%) existed in five groups. Moreover, 135, 102, and 183 unique OTUs were identified in the NC, PC, and OA1 groups, respectively; and as Fig. 1C shows, out of a total of 2,082 OTUs, 1,171(~57%) existed in five groups. In addition, 134, 109, and 227 unique OTUs were identified in the NC, PC, and OA3 groups, respectively. Based on the analysis of the Venn diagrams for comparison of the OTUs in the three groups, out of 2,092 OTUs, 1,131 (~54%) existed in five groups (Fig. 1D). In addition, 158, 131, and 237 unique OTUs were identified in the NC, PC, and OA1 + OA3 groups, respectively.

Alpha and beta bacterial diversity. We compared bacterial diversity [phylogenetic diversity (**PD**) and the Shannon index] and richness (observed

Table 10. Growth performance of pigs fed the Exp. 2 diets[1]

	Diets[2]						
Item	NC	PC	OA1	OA3	OA1 + OA3	SEM[3]	*P* value
BW, kg							
Initial	6.9	6.9	6.9	6.9	6.9	0.01	0.644
Day 14	7.5	7.9	7.9	7.7	7.7	0.08	0.421
Day 28	12.2	12.9	12.5	12.2	12.9	0.15	0.302
Day 42	18.9	19.3	18.6	18.3	19.2	0.23	0.615
Day 49	22.5	23.5	22.2	22.0	23.1	0.26	0.302
ADG, g/d							
Day 1–14	43	71	75	59	52	5.98	0.440
Day 15–28	327	358	329	319	375	8.30	0.162
Day 1–28	189	214	202	189	215	5.27	0.335
Day 29–42	472	466	430	430	457	8.39	0.340
Day 43–49	439[c]	603[a]	513[bc]	533[ab]	573[ab]	14.18	0.001
ADFI, g/d							
Day 1–14	175	201	205	188	174	5.07	0.196
Day 15–28	515	548	524	510	533	10.70	0.835
Day 1–28	336	374	356	349	354	7.02	0.563
Day 29–42	821	812	765	754	784	10.82	0.216
Day 43–49	1010	1008	958	982	1018	17.89	0.835
F:G, g/g							
Day 15–28	1.62	1.57	1.62	1.65	1.44	0.05	0.759
Day 1–28	1.85	1.81	1.77	1.94	1.68	0.07	0.828
Day 29–42	1.74	1.76	1.80	1.79	1.79	0.05	0.996
Day 43–49	2.47[a]	1.68[b]	1.89[b]	1.84[b]	1.82[b]	0.08	0.010

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

[3]Pooled SEM; $n = 8$/treatment.

Table 11. Diarrhea index of pigs fed the Exp. 2 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA3	OA1 + OA3	SEM[3]	*P* value
Diarrhea index							
Day 1–7	0.89[a]	0.64[b]	0.62[b]	0.63[b]	0.67[b]	0.03	0.002
Day 8–14	0.77[a]	0.48[b]	0.52[b]	0.53[b]	0.58[b]	0.03	0.006
Day 15–21	0.53[a]	0.31[c]	0.37[bc]	0.37[bc]	0.48[ab]	0.02	0.001
Day 21–28	0.46	0.31	0.38	0.42	0.33	0.02	0.275
Day 1–28	0.68[a]	0.44[b]	0.48[b]	0.49[b]	0.53[b]	0.02	0.000

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

[3]Pooled SEM; $n = 8$/treatment.

species and the Chao index) indices for alpha diversity. The PD, Shannon index, and observed species in the OA3 group were significantly higher than those in the PC group ($P < 0.05$) (Fig. 2A–C). The OA1 supplementation increased the Shannon index in comparison to the PC ($P < 0.05$) (Fig. 2C). However, no significant differences were noted in the Chao index (Fig. 2D).

For the analysis of beta diversity, we examined the relationships among NC, PC, OA1, OA3, and OA1 + OA3 supplementation in the colon microbiome, using principal component analysis. The distribution of microbiota in the NC, OA1, and OA1 + OA3 groups was distinctly and separately clustered along a principal coordinate (Fig. 3), indicating that the PC and OA1 + OA3 groups significantly

Table 12. Gut digesta pH of pigs fed the Exp. 2 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA3	OA1 + OA3	SEM[3]	*P* value
Stomach	4.36	4.07	3.36	3.77	3.36	0.14	0.111
Duodenum	5.13	4.95	4.79	4.72	4.63	0.13	0.777
Jejunum	6.09	6.05	6.07	5.92	6.05	0.05	0.830
Ileum	7.10	6.35	6.58	6.70	6.46	0.10	0.119
Colon	6.05	6.05	6.04	6.06	5.60	0.07	0.185

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

[3]Pooled SEM; $n = 8$/treatment.

Table 13. Intestinal morphology of pigs fed the Exp. 2 diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA3	OA1 + OA3	SEM[3]	*P* value
Duodenum							
VH[4], µm	612.46	752.15	570.25	702.49	798.19	34.65	0.198
CD[5], µm	393.04	299.81	315.71	353.19	360.85	15.94	0.472
VH/CD	1.93	2.28	1.92	2.08	2.17	0.09	0.752
Jejunum							
VH, µm	525.51	613.35	646.74	496.57	636.45	21.05	0.056
CD, µm	266.75^{ab}	192.30^{b}	289.62^{a}	207.82^{b}	229.83^{ab}	10.53	0.007
VH/CD	2.37	2.70	2.28	2.45	2.74	0.08	0.231
Ileum							
VH, µm	300.00^{b}	465.22^{a}	414.51^{ab}	350.21^{ab}	453.01^{a}	19.16	0.026
CD, µm	196.61	180.09	212.53	171.17	193.32	7.19	0.364
VH/CD	2.00	2.36	2.01	2.15	2.35	0.09	0.593

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

[3]Pooled SEM; $n = 8$/treatment.

[4]VH = villus height.

[5]CD = crypt depth.

Table 14. Volatile fatty acid concentration (µmol/g fresh matter) in the cecum and colon of pigs fed the Exp. 2 diets

	Diet[2]						
Item	NC	PC	OA1	OA3	OA1 + OA3	SEM[3]	*P* value
Cecum, (µmol/g)							
Acetic	32.57	33.46	31.64	35.94	33.20	0.98	0.733
Propionic	16,70	18.26	16.50	14.26	19.67	1.00	0.522
Butyrate	6.94	5.43	6.62	5.25	7..91	0.33	0.056
Colon, (µmol/g)							
Acetic	26.25^{b}	37.10^{a}	31.16^{ab}	31.82^{ab}	36.64^{a}	1.21	0.026
Propionic	12.91^{b}	16.72^{a}	15.22^{ab}	12.00^{b}	17.70^{a}	0.60	0.004
Butyrate	5.87	6.54	7.70	5.31	7.21	0.42	0.365

Small superscript letter are different ($P < 0.05$).

[1]Values means $n = 8$ for productive performance.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

[3]Pooled SEM; $n = 8$/treatment.

affected the structure of bacterial communities in the colon, as compared to the NC.

Effects of dietary OA on the relative abundance of colon microbiota at the phyla level. The relative abundance of microbiota in the top 10 samples at the phyla level is presented in Fig. 4A. The results suggest that the top five dominant phyla were *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Spirochaetes*, and *Tenericutes*. Furthermore, the top 10 phyla (>1% in at least one sample) and *Firmicutes*/*Bacteroidetes* ratio were chosen for significance analyses (Table 16). The relative abundance of *Tenericutes* was significantly higher in the OA1 and OA3 groups than in the NC or PC groups ($P < 0.05$). The PC and OA1 + OA3 supplementation reduced the relative abundance of *Cyanobacteria* ($P < 0.01$) and *Verrucomicrobia* ($P < 0.01$).

Table 15. Tags and OTUs number of five groups

	Diet[2]				
Item	NC	PC	OA1	OA3	OA1 + OA3
Total_tag	71,603	75,773	74,458	73,336	73,963
Taxon_Tag	70,709	74,865	73,245	72,026	72,996
Unique_Tag	894	908	1,213	1,310	967
OTU_num	1,048	883	1,087	1,167	1,097

OTU, operational taxonomic unit.

[1]Values means $n = 8$ for NC, OA1, OA3, and OA1 + OA3, $n = 7$ for PC group.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

Effects of dietary OA on the relative abundance of colon microbiota at the genera level. The relative abundance of microbiota in the top 10 samples at the genera level is presented in Fig. 4B. There were apparent differences in genus distribution among the five groups. The proportion of *Prevotella* was higher in the OA1 + OA3 group than in the other groups, whereas the proportion of *Escherichia–Shigella* was higher in the PC than in the other groups.

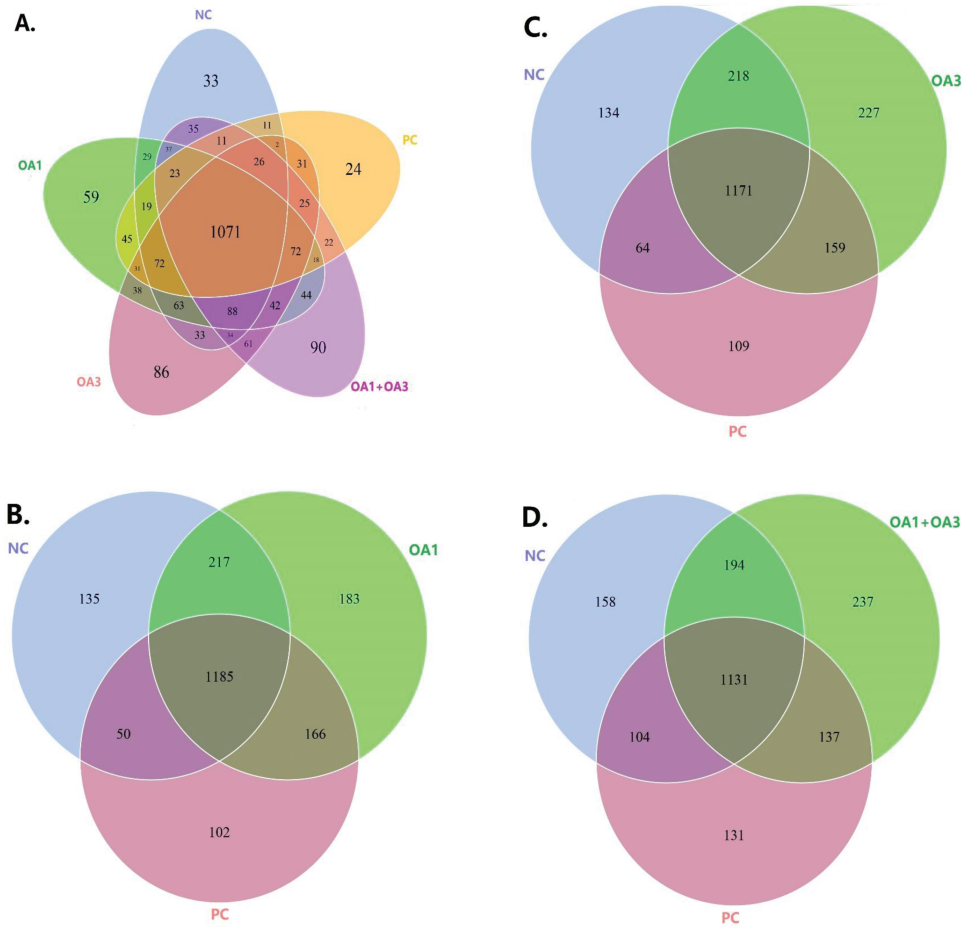

Figure 1. Microbiota comparison of the OTUs among treatments in colon. The observed OTUs sharing ≥97% sequence similarity. (A–D) Venn diagrams were generated to describe the common and unique OTUs among five or three treatments, respectively. NC = negative control, no antibiotics; OUT = operational taxonomic unit; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3; PC = positive control, antibiotics included.

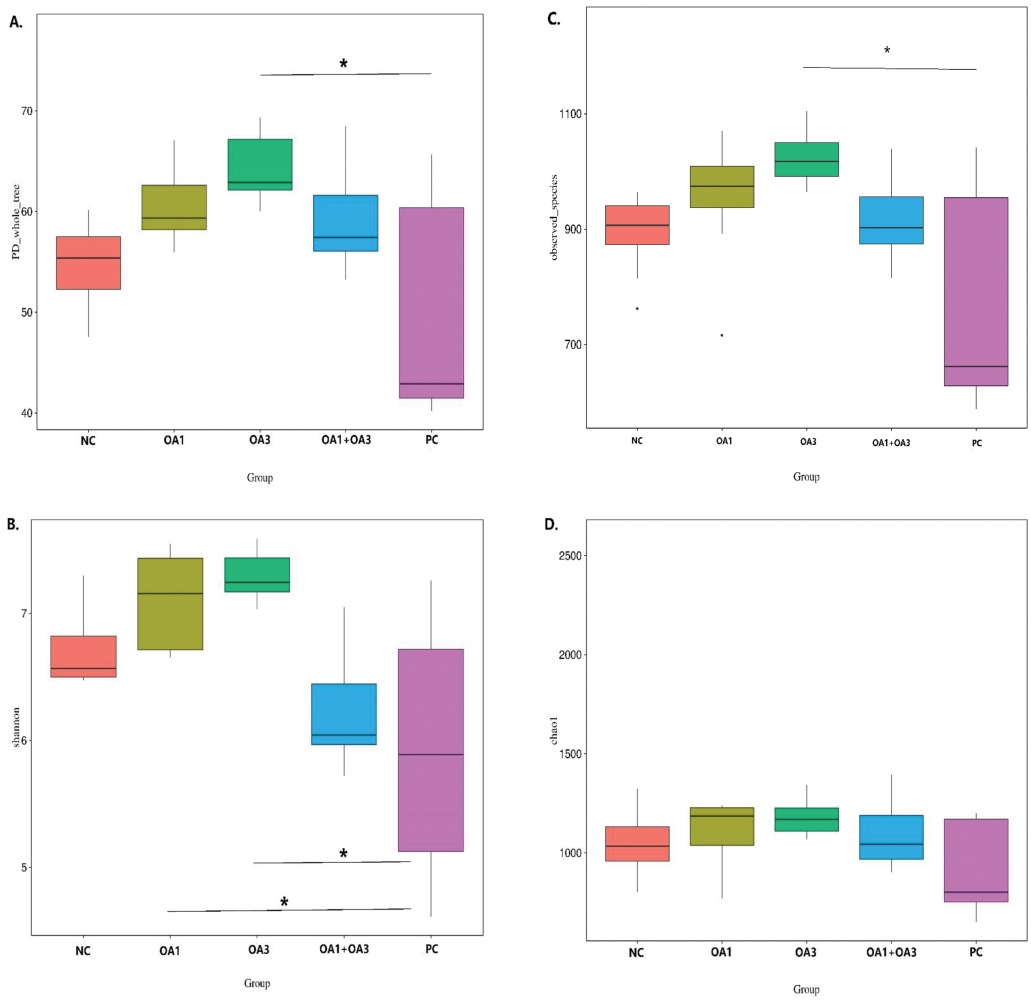

Figure 2. Microbiota alpha-diversity comparison among five groups. Pigs were regarded as the experimental units, each treatment with $n = 8$ except that PC with $n = 7$ due to a sample was eliminated. (A–C) PD, Shannon index, and Observed_species of five groups, respectively. NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

DISCUSSION

The first endpoint of the experiments was to determine the growth response of weaned pigs to supplemental OA and AGP in diets of different digestibility. The results indicated no significant benefits from the addition of OA and AGP to a highly digestible diet. However, the growth performance of weaned pigs was promoted with a less digestible diet. The results of Exp. 1 are consistent with those of previous reports (Radecki et al., 1988; Risley et al., 1991; Manzanilla et al., 2004a, b, c). Furthermore, other reports (Burnell et al., 1988; Giesting et al., 1991; Namkung et al., 2004) have shown that acids can improve growth performance, as we found in Exp. 2. The experiment showed that in comparison to the NC diet, OA diets increased ADG and reduced the F:G ratio during the last week of the study. This might have been due to the nutritional characteristics of the two different types of diet used in Exp. 1 and 2. In the diet formulation of Exp. 1, we attempted to maximize the inclusion of various carbohydrate sources and reduce antinutritional factors. This was because high-quality protein sources and a high digestibility of carbohydrate sources were necessary for weaned pigs, to avoid the negative effects associated with postweaning performance (Li et al., 1990; Healy et al., 1991; Che et al., 2012). In contrast, the diets of Exp. 2 had a lower digestibility than those of Exp. 1. Walsh et al. (2007) suggested that the effectiveness of acids improves as the pig matures, or as diet complexity is reduced. Therefore, the OA additive in the highly digestible diet was hypothesized to be less effective than in the less digestible diets. This could be due to the fact that weaned pigs could adapt to the highly digestible diet more easily and quickly.

A better intestinal morphology is beneficial for effective nutrient digestion and absorption. Moreover, the integrity of the gut barrier can be improved by the preferences and capacity for digestion and absorption in the host (Sekirov et al., 2010). The results of our study indicated

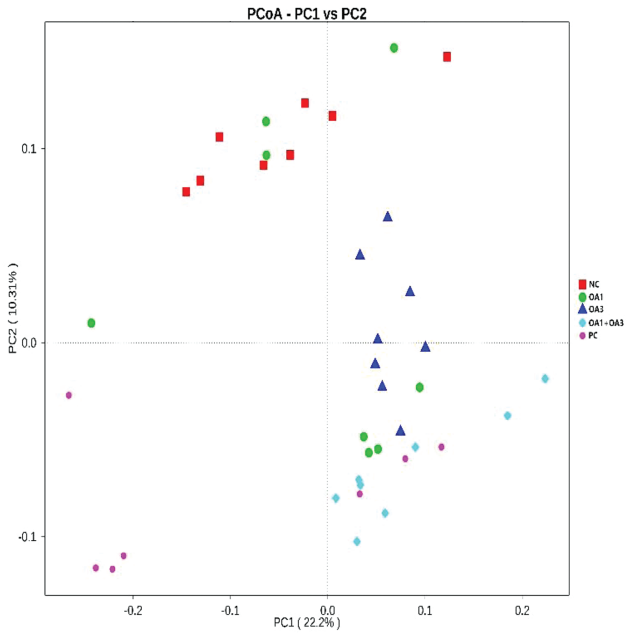

Figure 3. Comparison of the gut microbiota composition among five groups. Principal coordinate analysis to visualize the unweighted UniFrac distances of colon digesta samples from individual pig. NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

no benefit from the addition of OA and AGP to a highly digestible diet, but a positive effect in the less digestible diet on the incidence of diarrhea and intestinal morphology of weaned pigs. Consistent with the present results, in which a high digestible diet was necessary for weaned pigs to avoid the negative effects of diarrhea and less favorable intestinal morphology, previous studies have indicated that diet formulation could influence gastrointestinal morphology, physiology, and microbiology (van Beers-Schreurs, 1996; Pluske et al., 1997; Mikkelsen and Jensen, 2001). Interestingly, supplementation with OA in a less digestible diet reduced the diarrhea index and increased the ileal VH of pigs. A previous study showed that the incidence of diarrhea was significantly reduced and the ileal VH was significantly increased in pigs fed AGP and OA diets (Long et al., 2018), findings that were also reported by Tsiloyiannis et al. (2001). Guilloteau et al. (2010) indicated that reduced levels of *E. coli* in gut microbiota could reduce the incidence of diarrhea in piglets, which was consistent with the results of the present study. Similarly, increased VH in butyrate-supplemented weaned pigs have been observed in previous studies (Piva et al., 2002; Kotunia et al., 2004; Weber and Kerr, 2008). In addition, OA and their salts have been found to have a preventive effect on postweaning diarrhea in pigs (Partanen and Mroz, 1999; Franco et al., 2005; Halas et al., 2010).

The SCFAs, produced by microbial fermentation of carbohydrates in the gastrointestinal tract, are beneficial for animal (Bergman, 1990). In the previous study, mixed OA when used as feed additives, resulted in significantly higher SCFAs contents in the digesta of the cecum and colon. These findings indicated that OA caused the microbes to utilize carbohydrates to produce SCFAs. In Exp. 1, OA increased the levels of acetic and butyrate acids and reduced levels of *E. coli* in the colon. The SCFAs, especially butyric acid, produced by fermentation of carbohydrates and nonstarch polysaccharides (**NSP**) in the large intestine, have a positive effect on epithelial cell growth, blood flow, and absorptive functions in pigs (Bergman, 1990; Berni Canani et al., 2012). A novel study in a mouse model demonstrated that increased production of acetate inhibited translocation of the *E. coli* toxin from the gut lumen to the blood, which improves intestinal defense mediated by epithelial cells, and thereby protects the host against lethal infections (Fukuda et al., 2011). In addition, a reduction in the levels of *E. coli* by OA is considered to be due to the anti-inflammatory effects of some butyrate-producing bacteria (Koopmans et al., 2016). A previous study using the same commercial OA as OA1 and OA2, reported an improvement in the apparent total-tract digestibility (**ATTD**) of total carbohydrates (Long et al., 2018). This finding is partially agreed with those of Gerritsen et al. (2010), who demonstrated that a combination of 5,000 mg/kg formic acid and essential oil significantly increased the ATTD of NSPs and total carbohydrates. The ATTD of carbohydrates was improved because the reduced levels of *E. coli* in the distal gastrointestinal tract provided a more favorable environment for the fermentation of carbohydrates by bacteria (Gerritsen et al., 2010).

Furthermore, the gut microbiota plays a critical role in animal growth and production. Differences in the microbial profile following the administration of different treatments are expected to affect the efficiency of host digestion and intestinal function (Frese et al., 2015). High bacterial diversity is favorable for the overall health and productivity of animals (Hildebrand et al., 2013). In Exp. 2, the OA, administered as a growth promoter and alternative supplement to the animals, possibly promoted growth performance by modulating the microbiota in the digestive tract of the host. The supplementation of OA increased the bacterial diversity and richness, in comparison to the PC group. Analysis of the Venn diagrams showed that the OA group had more unique OTUs than either the NC or

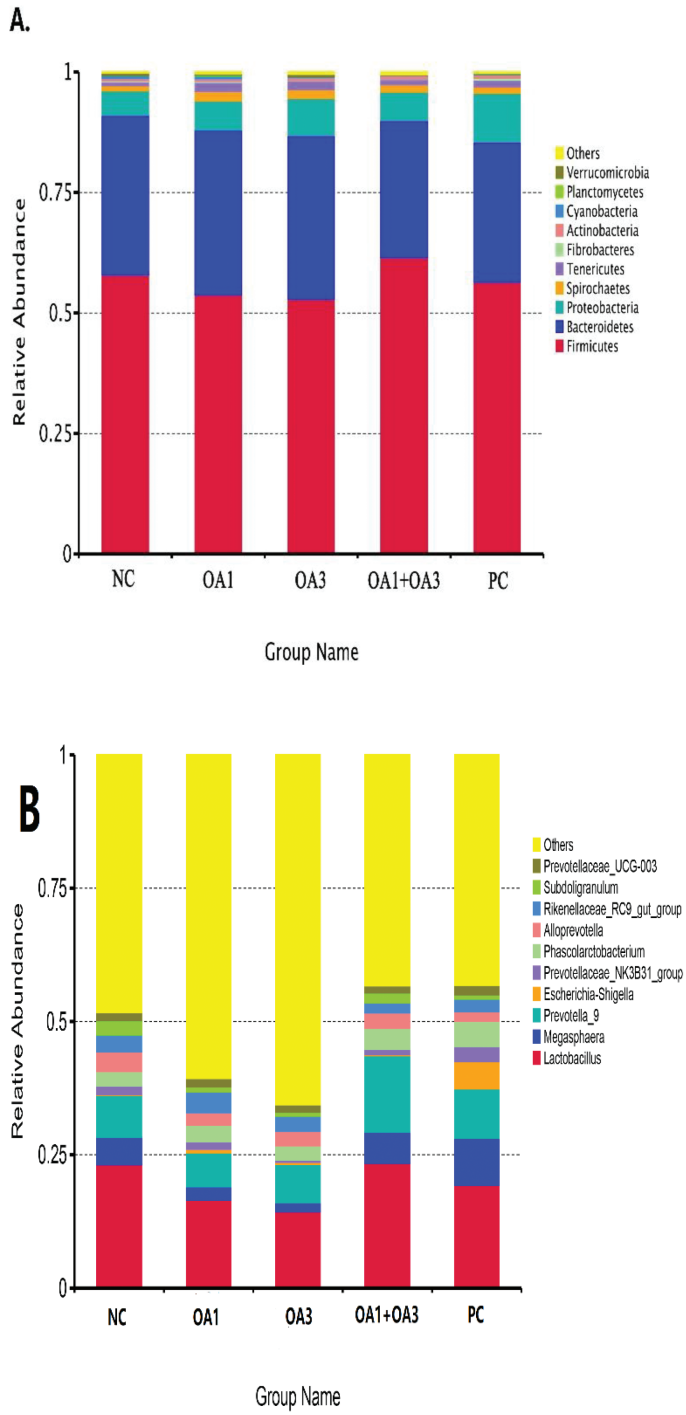

Figure 4. (A) 16S rRNA gene analysis reveals phyla level differences in microbiota of five groups. (B) 16S rRNA gene analysis reveals genus level differences in microbiota of five groups. NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

PC group. Similarly, an earlier study showed that in-feed antibiotic supplementation in weaned pigs reduced the microbiota diversity of colonic digesta (Yu et al., 2017). Furthermore, beta diversity analysis showed that the community structure was distinctly different among the OA1 + OA3, PC, and NC treatments, indicating that either the OA1 + OA3 or the PC treatments modulated the microbiota of the pigs. Collectively, our results demonstrated that OAs modulated the intestinal microbiota of pigs by a different mechanism from that used by the PC.

It is noteworthy that the condition of the digestive system is very important for early-weaned pigs, because of the sudden switch from protein-rich milk to plant-fiber diets. Disruption of the pathways associated with the animal digestive tract will cause digestive disorders, nutrient malabsorption,

Table 16. The relative abundance of ten phyla (%, >1% in at least one sample) and Firmicutes/Bacteroidete ratio in colon of pigs fed the experimental diets[1]

	Diet[2]						
Item	NC	PC	OA1	OA3	OA1 + OA3	SEM[3]	*P* value
Firmicutes	57.93	56.46	53.81	52.93	61.57	1.72	0.676
Bacteroidetes	33.09	29.03	34.16	33.93	28.37	1.73	0.354
Proteobacteria	5.10	10.14	6.05	7.70	5.94	0.73	0.239
Spirochaetes	1.07	1.33	1.97	1.84	1.52	0.25	0.558
Tenericutes	0.83^{b}	1.38^{b}	1.91^{a}	1.77^{a}	1.11^{ab}	0.12	0.014
Fibrobacteres	0.16	0.40	0.20	0.08	0.10	0.05	0.220
Actinobacteria	0.54	0.67	0.55	0.61	0.73	0.06	0.728
Cyanobacteria	0.55^{a}	0.23^{c}	0.55^{ab}	0.24^{bc}	0.13^{c}	0.05	0.001
Planctomycetes	0.02	0.01	0.18	0.01	0.03	0.03	0.604
Verrucomicrobia	0.51^{a}	0.08^{b}	0.33^{a}	0.46^{a}	0.01^{b}	0.04	0.000

[1]Values means n = 8 for NC, OA1, OA3, and OA1 + OA3, n = 7 for PC group.

[2]NC = negative control, no antibiotics; PC = positive control, antibiotics included; OA1 = NC plus organic acid 1; OA3 = NC plus organic acid 3; OA1 + OA3 = NC plus organic acid 1 and organic acid 3.

[3]Pooled SEM; n = 8 per treatment except that n = 7 for PC group.

and a high incidence of diarrhea (Yu et al., 2017). Furthermore, alteration of gut microbiota has been proven to be one mechanism by which antibiotics can enhance the growth of livestock (Schwarz and Chaslus-Dancla, 2001; Dibner and Richards, 2005). Our results indicated that the PC modulated the microbiota of pigs and yielded high levels of the genera *Escherichia–Shigella*, whereas the OA1 + OA3 group yielded a high level of the genus *Prevotella*. Zhao et al. (2015) reported that *Escherichia–Shigella* is a dominant bacterial group in the small intestine, where it mainly takes part in digestion. *Prevotella*, which has a unique capability to degrade the mucin glycoprotein (Wright et al., 2000; Rho et al., 2005), could exploit these conditions, and thereby improve growth and survival. Our results consistently showed that the OA1 + OA3 treatment increased the levels of acetic acid in the colon. Pajarillo et al. (2014) showed that *Prevotella* becomes one of the most abundant genera in pigs after weaning. This increase in the relative abundance of *Prevotella* during the post-weaning period is supposedly linked to the ability of these bacteria to degrade hemicelluloses, such as xylans in plant-based feed (Hayashi et al., 2007; Lamendella et al., 2011). Previous reports have shown a strong association between *Prevotella* and carbohydrates from fiber-rich diets or long-term carbohydrate diets (De Filippo et al., 2010; Wu et al., 2011). A higher abundance of *Prevotella* is reportedly a dominant feature of the fecal microbiota in healthy pigs, as compared to post-weaning diarrheic pigs (Dou et al., 2017). Thus, dietary supplementation of OA1 + OA3 stimulated an increase in the population of *Prevotella* to facilitate the breakdown of carbohydrates, and thus improve intestinal immunity and reduce the incidence of diarrhea.

CONCLUSIONS

The present research indicated that dietary supplementation with OA in a highly digestible diet for weaned pigs did not improve growth performance, whereas intestinal health was improved by increasing the levels of SCFAs and reducing the abundance of *E. coli*. However, OA blends evidently use a different mechanism by which the colonic microbiota is modulated, but OA blends showed a similar growth-promoting effect to that of antibiotics in the less digestible diet to which high levels of ZnO were not added.

Conflict of interest statement. None declared.

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