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Effect of dietary organic acids supplementation on growth performance, nutrient digestibility, and gut morphology in weaned pigs

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CONFLICT OF INTEREST

The authors declare that there are no competing interests.

Abstract

This study was conducted to investigate the effects of dietary supplementation with different organic acid (OA) mixtures on growth performance, nutrient digestibility, and gut morphology in weaned pigs. A total of 56 weaned pigs (7.93 ± 1.04 kg body weight) were assigned to four dietary treatments with seven replicates per treatment for a 35-d study conducted over two phases; phase 1 (d 1 to 14) and phase 2 (d 14 to 35). Diets consisted of 1) a corn-soybean meal-basal diet without any additive (negative control, **NC**); 2) NC + formic and propionic acids (**TRT1**); 3) NC + butyric, formic, and propionic acids (**TRT2**); and 4) NC + antibiotic (positive control, **PC**). During the overall period, the gain to feed ratio of pigs fed the PC and TRT1 diets tended to be higher ($P = 0.059$) than that of the NC diet. However, OA supplementation had no effects on nutrient digestibility. Jejunal villus height to crypt depth ratio was higher ($P < 0.05$) in pigs fed the TRT1 diet than those fed the NC diet. In conclusion, dietary OA supplementation tended to increase overall feed efficiency and improved gut morphology in weaned pigs.

Keywords: growth performance, gut morphology, organic acids, weaned piglets

INTRODUCTION

Weaning is one of the most stressful event for pigs, which can cause dysfunction of the gastrointestinal tract (**GIT**) and immune system, leading to reduced growth performance and feed intake in the first week after weaning (Campbell et al., 2013). To alleviate gastrointestinal disturbances and improve microbial balance in the GIT of weaned piglets, subtherapeutic doses of in-feed antibiotic growth promoters (**AGP**) have been used (Jukes et al., 1950). However, in many jurisdictions, the use of in-feed AGP is no longer permitted due to their potential impact on antimicrobial resistance (**AMR**). Inappropriate use of antimicrobial agents accelerates the presence of AMR, and increasing AMR has been a global concern for public health because the effectiveness of antimicrobial treatment could be reduced (Smith and Coast, 2002). Thus, the development of alternatives to in-feed AGP is necessary for sustainable pig production.

Organic acids (**OA**) are potential alternatives to AGP as they are known to decrease the load of pathogenic bacteria and modulate the microbial composition in the GIT and enhance growth performance of pigs (Long et al., 2018; Xu et al., 2018). Gram-negative bacteria such as *E. coli* are more resistant to antibiotics due to their outer membrane, but OA has better antimicrobial efficacy as they can penetrate the membrane via porin protein and reduce cellular pH, thereby causing bacterial cellular dysfunction (Omonijo et al., 2018). Short-chain fatty acids such as formic acid, propionic acid, and butyric acid are the most commonly used OA (Nguyen et al., 2020). Previous studies showed that different OA follows different modes of action at each part of the GIT (Partanen, 2001; Kasprowicz-Potocka et al., 2009). Formic acid reduces gastric pH and increases enzyme activity (Mroz et al., 2000; Creus et al., 2007) and apparent ileal digestibility of nutrients (Partanen et al., 2001). Butyric acid has beneficial effects on growth performance and morphology of intestinal mucosa, although pigs fed butyric acid showed decreased feed intake due

to its irritating odor (Piva et al., 2002b). Each OA has a similar but a little different feature, therefore, the OA supplemented in this study were provided as OA mixture to maximize the acidification effects of each component. However, there is no study that used the mixture of formic and propionic acids or their mixture with fructo-oligosaccharides in weaned pigs, and less information about the effects of different OA mixtures throughout the entire nursery period. Thus, the current study was conducted to investigate the effects of dietary supplementation of different OA mixtures on growth performance, nutrient digestibility, and gut morphology in weaned pigs.

MATERIALS AND METHODS

The experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (AC11012). Pigs were handled according to the guidelines described by the Canadian Council on Animal Care (2009).

Experimental diets

Two OA commercial products consisting of a highly concentrated liquid blend of formic and propionic acids (**OA1**) and a combination of free liquid butyric, formic, and propionic acids and their salts as well as fructo-oligosaccharides (**OA2**) were provided by Anpario plc (Nottinghamshire, UK) for use in the present study. The OA1 contained 290 g/kg formic acid, 145 g/kg propionic acid, and the OA2 contained formic, propionic, and butyric acids, and inulin (fructo-oligosaccharides) as 93, 55, 73, and 64 g/kg, respectively. The four experimental diets (Table 1) used in the study were: 1) a corn-soybean meal basal diet without additives (negative control; **NC**), 2) NC + 0.15% and 0.10% OA1 during phase 1 and 2, respectively, (**TRT1**), 3) NC + 0.2% OA2 during phase 1 and 2 (**TRT2**), and 4) NC + 0.1% Aureomycin[®] 220G (Zoetis Canada

Inc., QC, Canada) that supplied 220 mg of chlortetracycline per kg of the diet (positive control; **PC**). Experimental diets were formulated to be isocaloric and isonitrogenous, and all other nutrients were provided to meet or exceed the recommendations for each phase in NRC (2012). Phase 1 and 2 diets were fed from d 0 to 14 and from d 15 to 35 after weaning, respectively. All diets contained titanium dioxide (0.3%) as an indigestible marker. The experimental diets were provided in a mash form.

Animals, housing, and experimental design

A total of 56 piglets [(Yorkshire × Landrace) × Duroc] with an average body weight (**BW**) of 7.93 ± 1.04 (mean ± SD) were used in this study. Pigs were assigned to 1 of 4 dietary treatments in a randomized complete block design based on BW to give seven replicates per treatment. Pigs were housed in pairs per pen (mixed sex; one castrated male and female). Each pen was equipped with raised plastic-covered expanded metal floors (1.2×1.5 m), a stainless-steel feeder, and a nipple drinker. Pigs had free access to feed and water throughout the 5-wk experimental period. Room temperature was maintained at $30 \pm 1^\circ\text{C}$ during the first week and then reduced by 1°C per week.

Experimental procedure and sample preparation

The individual pig BW and feed disappearance were recorded weekly to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**). On d 13 and 14, and d 34 and 35, fecal samples were collected by grab sampling from the floor after washing the pens to determine nutrient digestibility. At the end of each phase (d 14 and 35), blood samples (10 mL) were collected from the jugular vein into heparinized vacutainer tubes (Becton

Dickson, NJ, USA) from the female pig in each pen for cytokine analysis. Blood samples were centrifuged at $2,000 \times g$ for 10 min at 4°C to recover plasma samples, which were immediately stored at -80°C . After blood collection, a rectal massage was performed with a sterile swab to collect fresh fecal samples for microbial genomic DNA extraction. Collected fecal samples were stored at -80°C until further analyses.

At the end of the experiment (d 35), the female pig in each pen was euthanized to collect intestinal samples. The jejunum and ileum samples were taken from 2 m and 30 cm before the ileocecal junction, respectively, for gut morphology analysis. Samples for gut morphology were immediately stored in a 10% neutral buffered formalin. Digesta from the duodenum (30 cm away from the stomach), jejunum, and ileum were collected to determine the pH using a pH probe (AB15 plus, Fisher Scientific, ON, Canada).

Sample processing and laboratory analyses

The concentrations of interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha (TNF- α) were measured by using porcine IL-6, IL-10, and TNF- α immunoassay kits (Porcine IL-6 ELISA Kit, Porcine IL-10 ELISA Kit, and TNF alpha Porcine ELISA Kit; Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's instructions. The minimum detection levels for IL-6, IL-10, and TNF- α ELISA Kit were 41, 8, and 14 pg/mL, respectively. The inter-assay coefficient of variations was less than 12%, and the intra-assay coefficient of variations was less than 10% for all ELISA Kits. According to the manufacturer's protocol, bacterial genomic DNA was extracted from the fecal samples to determine the relative abundance of target bacteria using QIAamp DNA stool mini kits (QIAGEN, Hilden, Germany).

The fixed samples for intestinal morphology were moved from formalin and embedded in

paraffin, and then the specimens were cut into 5 μm sections. Each section was processed for dewaxing of paraffin and immersed twice in xylene, 95% ethanol, and 100% ethanol for 5 min. The sections were rinsed using water and stained with hematoxylin and eosin solution for 10 min and then mounted on slides. The slides were viewed using an Axio Scope A1 microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) with an Infinity 2 digital camera (Lemenera Corporation, ON, Canada), and villus heights (**VH**) and crypt depths (**CD**) were determined using ImageJ software (National Institutes of Health, MD).

Diet samples were ground and thoroughly mixed before chemical analysis. Fecal samples were dried in a forced-air drying oven at 60°C for 5 d and finely ground in a coffee grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT, USA) before chemical analysis. Diet and fecal samples were analyzed in duplicate for dry matter (**DM**), gross energy (**GE**), and crude protein (**CP**; nitrogen $\times$ 6.25). Titanium analysis in diets and fecal samples was also done in duplicate, according to Myers et al. (2004). The DM content was measured according to AOAC (2006) method 934.01, whereas GE was determined using a bomb calorimeter (model 6400; Parr Instrument, IL, USA). Nitrogen (**N**) was determined using the combustion method (method 990.03) with the LECO N analyzer (model CNS-2000; LECO Corp., MI, USA) to calculate the CP content ($\text{N} \times 6.25$).

Calculation and statistical analysis

The apparent total tract digestibility (**ATTD**) of nutrient (DM, GE, and CP) were determined using the following equation:

$$\text{ATTD} = 100 - [(\text{Ti}_{\text{diet}}/\text{Ti}_{\text{feces}} \times \text{N}_{\text{feces}}/\text{N}_{\text{diet}}) \times 100],$$

where Ti_{diet} and Ti_{feces} are the titanium concentrations (%) in diet and feces, respectively,

and N_{diet} and N_{feces} are the concentrations (%) of DM, GE, and CP in diet and feces, respectively.

All data were analyzed using the PROC MIXED of SAS (SAS Institute, NC, USA) with the individual pig as the experimental unit. Differences among treatment means were determined using Tukey's multiple range test. A probability of $P < 0.05$ was considered significant and $0.05 \leq P < 0.10$ was considered as a tendency.

RESULTS

All pigs adapted well to their experimental diets and conditions and readily consumed their assigned diets. Piglets fed the TRT1 and TRT2 diets had lower ($P < 0.05$) ADG than those fed the PC diet in wk 2 (Table 2). The ADG of pigs fed the TRT1 and TRT2 diets tended to be lower ($P = 0.067$) than that of pigs fed the PC diet in phase 1, whereas there were no differences in weight gain among treatments during phase 2. Overall, the ADG tended to be lower ($P = 0.069$) for the pigs fed the TRT2 diet than for the pigs fed the PC diet, whereas the ADG values among piglets fed the NC, TRT1, and PC diets were not different. The ADFI of pigs fed the TRT2 diet was lower ($P < 0.05$) than that of pigs fed the NC diet in the first week, whereas pigs fed the TRT1 diet tended to have lower ($P = 0.096$) ADFI compared to those fed NC diet in wk 5. During phase 1, the ADFI of pigs fed the TRT2 diet was lower ($P < 0.05$) than that of pigs fed the NC diet; however, no differences were observed among the other diets. Dietary treatment did not affect ADFI over the entire experimental period. The G:F of pigs fed the NC diet was lower ($P < 0.05$) than pigs consuming the PC diet in wk 2. Pigs fed the TRT1 diet tended to have higher ($P = 0.085$) G:F than those fed the NC diet in wk 3. Overall, the G:F in pigs fed the PC and TRT1 diets tended to be higher ($P = 0.059$) than that of pigs fed the NC diet. No differences were observed in the ATTD of DM, GE, and CP among dietary treatments (Table 3).

No differences were found in digesta pH among dietary treatments (Table 4). However, ileal VH in pigs fed the TRT2 and PC diets were greater ($P < 0.05$) than in those fed the TRT1 diet, and pigs fed the TRT1 and NC diets had similar ileal VH values. The CD of jejunum in pigs fed TRT2 tended to be lower ($P = 0.094$) than that of NC-fed pigs. The jejunal VH:CD ratio was greater ($P < 0.05$) in pigs fed the TRT1 diet than those fed the NC diet, and the ileal VH:CD ratio of pigs fed the TRT2 diet tended to be higher ($P = 0.066$) than that of the NC and TRT1 diets. There were no differences in blood cytokine concentrations among treatments during the overall experimental period (Table 5). For microbial abundance, pigs fed the TRT2 diet had a higher ($P < 0.05$) fecal abundance of bacteria belonging to *Bifidobacteria* than those fed the PC diet during phase 1, however, no differences among treatments were observed in phase 2 (Table 6).

DISCUSSION

The OA1 consists of a liquid blend of formic and propionic acids, and OA2 is a combination of butyric, formic, and propionic acids with fructo-oligosaccharides. Formic acid is well known for its antibacterial activity, and increased intestinal morphology and growth performance were observed when formic acid is provided with propionic acid in broilers (Senkoylu et al., 2007; Emami et al., 2017), however, no study has been conducted on nursery pigs. The inclusion of butyric acid in the OA mixture may improve intestinal health because butyric acid is a major source of energy for intestinal development and repair (Kotunia et al., 2004). Also, fructo-oligosaccharides are well reported for their benefits in gut health of animals as a prebiotic (Roberfroid, 1998), therefore, its inclusion in OA mixture is expected to increase the population of beneficial bacteria, which would help to maintain optimal gut health. However, only limited information is available for the combination of OA and fructo-oligosaccharides in weaned pigs.

Reduced ADFI of pigs fed the TRT2 diet in the first week might be derived from the unpleasant odor of butyric acid (Lallès et al., 2009). A linear decrease was found in ADFI of weanling pigs as sodium butyrate increased in the diets from the work of Weber and Kerr (2008). Although an increased tendency of G:F was found in the overall period, there were only a few positive effects of OA supplementation on the growth performance of weaned pigs in the current study, and these results are related to the results of digestibility in this study. One of the modes of action of dietary OA is that they lower the pH in the stomach, which results in increased activity of digestive enzymes, especially pepsin, and therefore, improves protein digestibility and growth performance of pigs (Partanen and Mroz, 1999). Although OA supplementation did not affect the growth performance and nutrient digestibility in weaned pigs in this study, dietary OA positively affected indices of gut morphology. Higher VH is directly related to better absorption of nutrients in the GIT (Choi et al., 2017), and thus, an improved VH:CD ratio in jejunum obtained in the current study as a result of feeding a TRT1 diet may lead to positive growth performance in later life.

Piglets have an unfavorably high pH in their stomach after weaning because of their limited HCl secretion ability caused by an immature GIT (Manners, 1976). Thus, protein digestibility in weaned pigs could be reduced when they consume solid feed after weaning. Moreover, it is known that supplementation of OA reduces the passage rate of gastric digesta into the small intestine and stimulates enzyme secretion of the pancreas in pigs, leading to an increased digestibility of nutrients, especially protein (Ravindran and Kornegay, 1993; Partanen and Mroz, 1999). Thus, it was expected that the ATTD of CP in OA-supplemented diets would be higher than in the NC diet; however, no differences were observed in the ATTD of CP and GE in the current experiment. Although Kiarie et al. (2018) reported an increased ATTD of CP in nursery pigs fed

a diet with OA supplementation compared to that of the control diet, the data from Omogbenigun et al. (2003) showed no differences in apparent ileal digestibility of CP and ATTD of DM between the diets with and without OA in weaned pigs, which are in agreement with the current study. Similarly, Li et al. (2019) demonstrated that dietary supplementation with a mixture of OA did not affect the ileal digestibility of GE and CP in growing-finishing pigs; however, increased ATTD of GE and DM were found in OA-supplemented diets. These results indicate that the leading functional site of OA might be the large intestine, not the small intestine, and the lack of differences in ATTD of CP between the diets with or without OA supplementation might be due to the hindgut microbial fermentation. Bacteria use undigested protein for their metabolism and produce metabolites; thus, the digestibility of CP and amino acids should be obtained from ileal digesta, giving a more accurate estimate (Stein et al., 2007). One possible reason for the lack of effects of OA supplementation on ATTD of nutrients in the current study could be the age of pigs. Partanen and Mroz (1999) reported that OA supplementation had improved apparent ileal or ATTD of digestibility of CP and AA in growing pigs, but not in weaned pigs, which agrees with the works of Thacker et al. (1992) and Mroz et al. (1998) that demonstrated increased AID of CP and AA and increased ATTD of DM in growing pigs, respectively, but not in the works of Omogbenigun et al. (2003) and the current study using weaned piglets.

Although it is well known that OA supplementation reduces digesta pH, the results of the current study showed that dietary OA supplementation had no effects on small intestinal digesta pH. Indeed, inconsistency has been found with the effects of OA supplementation on digesta pH in pigs. For example, Radcliffe et al. (1998) reported a reduced stomach digesta pH in weanling pigs fed citric acid supplemented diets, whereas others reported no effects of dietary OA supplementation on digesta pH in weaned pigs (Namkung et al., 2004; Li et al., 2008). Dietary OA

is generally considered to lower gastric pH, which is related to increased proteolytic enzyme activity and protein digestibility (Partanen and Mroz, 1999). However, variations were found in the results of digesta pH depending on the type of OA. The pK_a values indicate the pH at which 50% of the acid is dissociated (Partanen and Mroz, 1999). The OA with higher pK_a is a weaker acid with a greater portion of non-dissociated form compared to that of lower pK_a which has a greater portion of the dissociated form, therefore, the lower the pK_a values, the stronger the acid (Nguyen et al., 2020). The OA used in the current study have high pK_a values, such as 4.82 for butyric and 4.88 for propionic acids, thus, the type of OA in the current study might not be able to reduce the digesta pH. Moreover, the composition of dietary ingredients might affect the buffering capacity as well as pH. The buffering capacity is a measure of the amount of acid to reduce the pH in feedstuffs (Lević et al., 2005). Dibner and Buttin (2002) reported that cereals and cereal by-products have a low buffering capacity, and protein and mineral sources have the most contribution to buffering capacity. Therefore, the diet composition used in the current study contained a milk product and fish meal, leading to less impact of the OA supplementation. Indeed, Ravindran and Kornegay (1993) reported that simple corn-soybean meal-based diets without milk products (a low buffering capacity) had greater OA effects on weanling pigs than the complex diets using protein sources that have a high buffering capacity. The digesta pH results are consistent with the digestibility results in the current study showing that OA supplementation did not affect digesta pH, in which case enzyme activity may not have been enhanced, thus explaining, at least in part, the lack of effects on ATTD of CP in weaned pigs.

It is known that dietary OA supplementation can influence intestinal morphology, especially butyric acid and its salts showed significant improvement on gut morphology of the small intestine in weaned pigs (Kotunia et al., 2004; Long et al., 2018) because butyric acid is the

primary energy source for enterocytes (Roediger, 1980) and acts as a cell differentiation promoter which helps intestinal cells become mature in function (Scheppach, 1994). Therefore, greater VH in the ileum of pigs fed the TRT2 diet than in those fed the TRT1 diet might be due to the inclusion of butyric acid in the TRT2 diet. Previous studies also showed that supplementation of butyric acid improved VH in a different section of the small intestine in studies with both swine and broiler chickens (Kotunia et al., 2004; Adil et al., 2010). Results of the current study showed a higher VH:CD ratio in the jejunum of piglets fed the TRT1 diet than in those fed the NC diet and numerically increased jejunal VH:CD ratio in piglets fed the TRT2 and PC diets compared to those fed the NC diet. These results are in agreement with those of studies by Hou et al. (2006) and Long et al. (2018), indicating that dietary supplementation with OA showed a greater ratio of VH:CD in the small intestine of weaned pigs. Although greater VH was found in the ileum of pigs fed TRT2 containing butyric acid, pigs fed TRT2 diet showed only a tendency for increased ileal VH:CD ratio compared to the NC and TRT1 groups, which might be derived from unaffected CD. The OA supplementation only affected VH, not CD, in the current study, and this finding is consistent with the findings by Adil et al. (2010) and Wang et al. (2018) after feeding butyrate. Also, the CD of jejunum in pigs fed TRT2 tended to be lower than that of NC-fed pigs, thus, there might be a potential benefit of the use of butyric acid for the intestinal health of piglets.

Dietary OA supplementation did not change the concentration of cytokine, including TNF- α , IL-6, and IL-10 in weaned pigs in the current study, which agrees with the data of Namkung et al. (2004) and Grilli et al. (2015). This result might be because pigs were raised under good hygienic conditions, which may not stimulate an immune response; thus, OA supplementation could not have any effects on the immune response (Hernández et al., 2006). Further studies using challenge models may be needed to validate these results. Challenge models

are often applied to research using weaned pigs as Enterotoxigenic *Escherichia coli* is the primary causative agent of post-weaning diarrhea. However, there is only limited information in the literature on the immune response of pigs with OA supplementation under challenging conditions (Mohana Devi et al., 2015; Ren et al., 2019), and the results of the immune response are not consistent. The relationship between immune response and OA supplementation in weaned pigs is worthy of investigation.

Previous studies have shown that intestinal microbiota in pigs could be modulated by OA inclusion in the diets (Partanen and Mroz, 1999; Piva et al., 2002a). The *Lactobacillus*, *Bifidobacteria*, and *Clostridium* cluster IV are well-known beneficial commensal bacteria species related to improved gut health and the promotion of immune homeostasis (Kabeerdoss et al., 2013; Vlasova et al., 2013). However, the family *Enterobacteriaceae* includes pathogens, especially *Escherichia coli* strains, which cause diarrhea and GIT infections in both humans and animals (Kaper et al., 2004; Fairbrother et al., 2005). There was no effect of OA supplementation on bacteria abundance except *Bifidobacteria* in phase 1, although there were substantial numerical differences in bacteria abundance in phase 2. This could be explained by the large variability observed in this study. For example, the relative abundances of *Bifidobacteria* and *Lactobacillus* found in pigs fed the TRT2 diet were 116 and 132% higher compared to those fed the NC diet during phase 2, however, large SEM were obtained in bacteria abundance; thus, no significant differences were observed. With a larger number of replications per treatment, it is possible that these differences in the populations of bacteria would have been statistically different. Also, further analysis could be done using 16S rRNA amplicon sequencing to improve the accuracy of microbial diversity results (Gantuya et al., 2021).

In conclusion, antimicrobial resistance is a worldwide issue for animal production and

may jeopardize human health, thus, alternatives to antimicrobial growth promoters have been greatly investigated in decades. Organic acids represent one of the significant strategies that can replace antimicrobial growth promoters and improve the gut morphology and growth performance of weaned pigs. This study concludes that dietary organic acid supplementation in weaned pig diets has beneficial effects on feed efficiency, morphology in the small intestine, and proliferation of beneficial bacteria; however, there were no effects of dietary organic acids on nutrient digestibility and blood cytokine levels in this work. The results of this study could help sustainable pig production along with biosafety issues.

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AUTHOR CONTRIBUTION

Conceptualization, J.W.K., H.H. and C.M.N.; Methodology, J.W.K.; Formal analysis, J.L. and J.W.K.; Investigation, J.L. and J.W.K.; Writing – original draft, J.L. and J.W.K.; Writing – review & editing, H.H. and C.M.N.; Supervision, C.M.N.

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Table 1. Ingredient and nutrient composition of experimental diets (% , as-fed basis)*

	Phase 1 (d 1 - 14)				Phase 2 (d 15 - 35)			
	NC	TRT1	TRT2	PC	NC	TRT1	TRT2	PC
Ingredient, %								
Corn	50.07	50.07	50.07	50.07	53.78	53.78	53.78	53.78
Soybean meal	23.90	23.90	23.90	23.90	27.00	27.00	27.00	27.00
Whey powder	15.00	15.00	15.00	15.00	10.00	10.00	10.00	10.00
Fish meal	5.00	5.00	5.00	5.00	3.00	3.00	3.00	3.00
Vegetable oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Corn starch	0.40	0.25	0.20	0.30	0.40	0.30	0.20	0.30
Limestone	1.00	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Dicalcium phosphate	0.40	0.40	0.40	0.40	0.60	0.60	0.60	0.60

NaCl	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix [†]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine HCl	0.40	0.40	0.40	0.40	0.30	0.30	0.30	0.30
DL-Methionine	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06
L-Threonine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Organic acid (OA) blend	-	0.15	0.20	-	-	0.10	0.20	-
Antibiotic	-	-	-	0.10	-	-	-	0.10
Calculated composition [‡] , %								
NE, Mcal/kg	2.55	2.55	2.55	2.55	2.52	2.52	2.52	2.52
CP	20.9	20.9	20.9	20.9	20.7	20.7	20.7	20.7
SID AA								

Lysine	1.36	1.36	1.36	1.36	1.25	1.25	1.25	1.25
Methionine	0.39	0.39	0.39	0.39	0.36	0.36	0.36	0.36
Threonine	0.79	0.79	0.79	0.79	0.73	0.73	0.73	0.73
Tryptophan	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
STTD P	0.42	0.42	0.42	0.42	0.39	0.39	0.39	0.39
Analyzed composition [§]								
DM, %	90.6	90.7	90.7	90.4	90.5	90.7	90.4	90.3
GE, Mcal/kg	3.98	3.96	3.97	3.97	4.07	4.05	4.05	4.05
DE, Mcal/kg	3.24	3.19	3.22	3.22	3.47	3.34	3.40	3.41
CP, %	20.8	20.4	21.0	19.9	20.2	20.4	20.3	20.2

*NC = negative control (without antibiotics); TRT1 = a diet supplemented with 0.15% and 0.10% OA (formic and propionic acids) during phase 1 and 2, respectively; TRT2 = a diet supplemented with 0.2% OA (butyric, formic, and propionic acids); PC = positive control (aureomycin; 55mg/kg of actual antibiotics).

†Provided the following nutrients (per kg of diet): Vitamins: A, 2,200 IU; D3, 220 IU; E, 16 IU; K, 0.5 mg; B₁, 1mg; B₂, 3.5 mg; B₆, 7 mg; B₁₂, 17.5 µg; calcium pantothenate, 10 mg; folic acid, 0.3 mg; niacin, 30 mg; biotin, 50 µg. Minerals: Cu, 6 mg (as copper sulfate); I, 0.14 mg (as calcium iodate); Fe, 100 mg (as ferrous sulfate); Mn, 4 mg (as manganese oxide); Se, 0.3 mg (as sodium selenite); Zn, 100 mg (as zinc oxide) for phase 1 diets, and Vitamins: A, 1,750 IU; D3, 200 IU; E, 11 IU; K, 0.5 mg; B₁, 1mg; B₂, 3.0 mg; B₆, 3 mg; B₁₂, 15 µg; calcium pantothenate, 9 mg; folic acid, 0.3 mg; niacin, 30 mg; biotin, 50 µg. Minerals: Cu, 5 mg (as copper sulfate); I, 0.14 mg (as calcium iodate); Fe, 100 mg (as ferrous sulfate); Mn, 3 mg (as manganese oxide); Se, 0.25 mg (as sodium selenite); Zn, 80 mg (as zinc oxide) for phase 2 diets.

‡NE = net energy; CP = crude protein; SID = standardized ileal digestible; AA = amino acids; STTD = standardized total tract digestible; P = phosphorus.

§DM = dry matter; GE = gross energy; DE = digestible energy.

Table 2. Effects of organic acids (OA) supplementation on growth performance of weaned pigs

	Diets*					
Item†	NC	TRT1	TRT2	PC	SEM	P-value
BW, kg						
Initial	7.9	7.8	8.0	7.9	0.56	0.564
wk 1	10.7	10.6	10.5	10.8	0.18	0.604
wk 2	15.0	14.7	14.7	15.4	0.23	0.101
wk 3	19.8	20.0	19.2	20.5	0.35	0.095
wk 4	25.6	25.4	25.0	25.6	0.39	0.724
wk 5	31.8	31.5	31.2	32.3	0.46	0.401
ADG, g/d						
wk 1	396	388	354	412	23.2	0.366
wk 2	618 ^{ab}	590 ^b	595 ^b	659 ^a	13.4	0.007
wk 3	676	760	650	729	42.9	0.289
wk 4	836	772	775	716	39.1	0.234
wk 5	885	863	880	960	48.6	0.515
Phase 1 (wk 1 – 2)	507	489	474	536	15.7	0.067

Phase 2 (wk 3 – 5)	799	799	768	802	20.1	0.604
Overall (wk 1 – 5)	682	675	651	696	11.2	0.069
ADFI, g/d						
wk 1	544 ^a	481 ^{ab}	453 ^b	530 ^{ab}	19.4	0.013
wk 2	815	754	746	794	22.4	0.129
wk 3	1057	1055	971	1064	48.0	0.493
wk 4	1232	1181	1149	1111	39.5	0.204
wk 5	1762	1594	1716	1688	45.3	0.096
Phase 1 (wk 1 – 2)	679 ^a	617 ^{ab}	599 ^b	662 ^{ab}	19.5	0.027
Phase 2 (wk 3 – 5)	1351	1277	1279	1287	31.0	0.310
Overall (wk 1 – 5)	1082	1013	1007	1037	23.5	0.135
G:F, g/g						
wk 1	0.73	0.81	0.78	0.77	0.037	0.447
wk 2	0.76 ^b	0.78 ^{ab}	0.80 ^{ab}	0.83 ^a	0.016	0.002
wk 3	0.64	0.72	0.67	0.68	0.020	0.085
wk 4	0.68	0.66	0.68	0.66	0.024	0.836
wk 5	0.50	0.54	0.51	0.57	0.026	0.254
Phase 1 (wk 1 – 2)	0.75	0.80	0.80	0.81	0.018	0.111

Phase 2 (wk 3 – 5)	0.59	0.63	0.60	0.63	0.013	0.218
Overall (wk 1 – 5)	0.63	0.67	0.65	0.67	0.011	0.059

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

*NC = negative control (without antibiotics); TRT1 = a diet supplemented with 0.15% and 0.10% OA (formic and propionic acids) during phase 1 and 2, respectively; TRT2 = a diet supplemented with 0.2% OA (butyric, formic, and propionic acids); PC = positive control (aureomycin; 55mg/kg of actual antibiotics).

[†]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Table 3. Effects of organic acids (OA) supplementation on apparent total tract digestibility (%) of dry matter (DM), gross energy (GE), and crude protein (CP) of experimental diets fed to weaned pigs

	Diets*					
Item	NC	TRT1	TRT2	PC	SEM	<i>P</i> -value
Phase 1						
DM	82.8	82.0	82.3	82.5	1.35	0.980
GE	81.5	80.5	81.0	81.1	1.49	0.965
CP	77.8	76.0	76.5	77.6	1.97	0.895
Phase 2						
DM	86.0	84.0	84.6	84.9	1.02	0.587
GE	85.2	82.5	84.0	84.3	1.18	0.462
CP	82.4	80.3	80.9	81.5	1.62	0.828

*NC = negative control (without antibiotics); TRT1 = a diet supplemented with 0.15% and 0.10% OA (formic and propionic acids) during phase 1 and 2, respectively; TRT2 = a diet supplemented with 0.2% OA (butyric, formic, and propionic acids); PC = positive control (aureomycin; 55mg/kg of actual antibiotics).

Table 4. Effects of organic acids (OA) supplementation on pH of digesta and morphology of small intestine of weaned pigs

		Diets*				SEM	P-value
		NC	TRT1	TRT2	PC		
pH							
	Duodenum	5.90	6.09	5.73	5.91	0.166	0.465
	Jejunum	5.73	5.60	5.70	5.66	0.106	0.809
	Ileum	6.30	6.45	6.51	6.47	0.187	0.822
Villus height, μm							
	Jejunum	543	676	583	623	33.2	0.067
	Ileum	567 ^{ab}	532 ^b	630 ^a	635 ^a	23.3	0.013
Crypt depth, μm							
	Jejunum	378	319	279	307	25.6	0.094
	Ileum	305	288	285	310	10.9	0.284
Villus height: crypt depth							
	Jejunum	1.49 ^b	2.18 ^a	2.13 ^{ab}	2.09 ^{ab}	0.156	0.026
	Ileum	1.87	1.86	2.25	2.07	0.108	0.066

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

*NC = negative control (without antibiotics); TRT1 = a diet supplemented with 0.15% and 0.10% OA (formic and propionic acids) during phase 1 and 2, respectively; TRT2 = a diet supplemented with 0.2% OA (butyric, formic, and propionic acids); PC = positive control (aureomycin; 55mg/kg of actual antibiotics).

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Table 5. Effects of organic acids (OA) supplementation on concentrations of blood cytokines of weaned pigs (pg/mL)

	Diets*					
Item†	NC	TRT1	TRT2	PC	SEM	P-value
Phase 1						
TNF- α	48.8	64.6	47.4	53.9	6.69	0.289
IL-6	182	211	192	198	103.6	0.998
IL-10	62.2	72.1	71.3	65.0	19.51	0.980
Phase 2						
TNF- α	49.7	57.2	59.3	44.4	6.48	0.367
IL-6	61.4	13.8	9.1	20.7	23.98	0.417
IL-10	35.2	23.1	17.6	32.4	9.65	0.556

^{*}NC = negative control (without antibiotics); TRT1 = a diet supplemented with 0.15% and 0.10% OA (formic and propionic acids) during phase 1 and 2, respectively; TRT2 = a diet supplemented with 0.2% OA (butyric, formic, and propionic acids); PC = positive control (aureomycin; 55mg/kg of actual antibiotics).

[†]TNF- α = tumor necrosis factor-alpha; IL-6 = interleukin-6; IL-10 = interleukin-10.

Table 6. Effects of organic acids (OA) supplementation on bacteria abundance ($\log_2$) in the feces of weaned pigs

	Diets*					
Item	NC	TRT1	TRT2	PC	SEM	P-value
Phase 1						
<i>Bifidobacteria</i>	1.85 ^{ab}	6.00 ^{ab}	9.24 ^a	0.09 ^b	2.063	0.020
<i>Clostridium</i> cluster IV	1.22	2.65	1.67	1.11	0.532	0.193
<i>Enterobacteriaceae</i>	2.89	3.43	0.93	1.76	1.848	0.702
<i>Enterococcus</i>	1.91	2.46	1.78	0.30	1.027	0.534
<i>Lactobacillus</i>	0.95	0.70	0.96	1.08	0.400	0.921
Phase 2						
<i>Bifidobacteria</i>	1.84	2.24	3.97	3.40	1.358	0.742
<i>Clostridium</i> cluster IV	2.04	1.95	1.43	0.93	0.601	0.572
<i>Enterobacteriaceae</i>	0.59	1.11	3.10	2.34	0.772	0.209
<i>Enterococcus</i>	2.71	1.17	3.36	1.51	1.191	0.551
<i>Lactobacillus</i>	1.60	1.42	3.71	0.75	1.669	0.631

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

*NC = negative control (without antibiotics); TRT1 = a diet supplemented with 0.15% and 0.10% OA (formic and propionic acids) during phase 1 and 2, respectively; TRT2 = a diet

supplemented with 0.2% OA (butyric, formic, and propionic acids); PC = positive control (aureomycin; 55mg/kg of actual antibiotics).

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