

# Effects of microencapsulated organic acids on growth performance, nutrient digestibility, fecal microbial counts, and blood profiles in weaning pigs

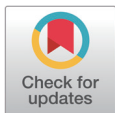
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## Abstract

This study was conducted to investigate the efficiency of a microencapsulated mixture of organic acids (MOA) with low protein in piglet feed on growth performance, diarrhea score, nutrient digestibility, fecal microbial counts, and blood profiles in weaning pigs. A total of 80 pigs [(Landrace × Yorkshire) × Duroc; 6.8 ± 0.48 kg] were randomly assigned to four dietary treatment groups: high protein (HP); low protein (LP); MOA1, LP + 0.2% MOA; and MOA2, LP + 0.3% MOA. The MOA2 group had higher average daily weight gains (during days 0–14 and days 0–28), diarrhea score (during days 0–14, during days 14–28 and days 0–28) and greater digestibility of dry matter (days 14 and 28) compared to the LP group ( $p < 0.05$ ). However, there were no significant differences ( $p > 0.05$ ) between the pigs fed diets with the MOA1 and MOA2 in blood profiles and fecal microflora. In conclusion, this study indicates that piglets fed 0.3% MOA in low protein diets maintained similar growth performance and nutrient digestibility, but alleviated the incidence of diarrhea compared to piglets fed high protein diets.

**Keywords:** Microencapsulation, Organic acids, Growth performance, Nutrient digestibility, Weaning pigs

## INTRODUCTION

After weaning, piglets face critical changes whereby changes in the method of nutrient intake causes growth depression and post-weaning diarrhea syndrome (PWD) [1]. Antibiotics and growth promoters have been commonly used to prevent diarrhea syndrome after weaning. However, antibiotics and growth promoters are now banned due to bacterial resistance [2]. Many researchers have begun to pay

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**Competing interests**

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

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**Availability of data and material**

Upon reasonable request, the datasets of this study can be available from the corresponding author.

**Authors' contributions**

Conceptualization: Lee JS, Kim TH, Song MH, Oh HJ.  
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**Ethics approval and consent to participate**

The experimental protocol for this research was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval #CBNUA-1421-20-02).

attention to 'nutrition' and 'natural antibiotic from natural sources' to solve PWD and alternatives antibiotic growth promoters [3,4]. After weaning, excessive feed intake and undigested protein have been implicated in the incidence of PWD [5,6]. Piva et al. [7] reported that low protein diets could reduce fermentation of protein by microbiota in large intestine and reduce the incidence of PWD without antibiotics. Moreover, many studies reported that low protein diets in weaning periods can reduce the possible action of organic acids (OA) involves reducing pH values in the gastrointestinal tract (GIT) blood urea nitrogen and ammonia concentrations in the small intestine [8,9].

Many studies of OA in natural antibiotics showed that they provided beneficial effects similar to those from feed with antibiotics [10,11]. Other studies reported that feeding OA to piglets was effective for growth performance [12–14]. The possible action of OA involves reducing pH values in the GIT [1], regulating the balance of microbial populations in the digestive tract, and stimulating the secretion of digestive enzymes [15,16].

Regarding the mechanism of the antibacterial action of OA, Roe et al. [17] reported that OA in a unionized state pass through the cell walls of bacteria in the GIT of livestock and are released, thereby lowering the acidity and interfering with bacterial metabolism. OA are known to improve the digestibility of proteins by reducing the pH in the stomach and improve growth performance by antimicrobial activity [18], and promote the growth and recuperation of healthy intestinal bacteria [19]. However, OA are absorbed too quickly when passing through the stomach and do not reach the desired location in the GIT, so the antibacterial activity may be limited. Microencapsulation is a technique used to deliver substances to specific parts of the GIT [20]. Microencapsulation technology allows for the slow ejection of these compounds along the intestine, allowing a considerable amount to reach the distal part of the small intestine [21]. Therefore, the object of this study was to investigate the effectiveness of a microencapsulated mixture of organic acids (MOA) with low protein diets on growth performance, diarrhea score, nutrient digestibility, fecal microbial counts, and blood profiles in weaning pigs.

## MATERIALS AND METHODS

The experimental protocol for this research was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval #CBNUA-1421-20-02).

### Animals and facilities

A total of 80 weaning pigs [(Landrace × Yorkshire) × Duroc] with Initial body weight (IBW) of  $6.8 \pm 0.48$  kg were used for the 4-week experiment. The pigs were allotted to four dietary treatments in a block design that was completely randomized based on IBW. There were five pigs per pen treatment and four replicate pens per treatment. All pigs were kept in environmentally controlled rooms, and each growing pig was provided 0.26 m<sup>2</sup> of space. Each pen was provided with a stainless-steel feeder and nipple waterer on one side. Feed and water were freely available. The individual BWs and feed intake were documented at the end of two and four weeks, and the average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G/F) was determined. The subjective diarrhea scores were recorded at 09:00 hours and 18:00 hours by the same person. This diarrhea score was based on the following: 0 = diarrhea, 1 = sloppy feces, 2 = normal feces, and 3 = well-formed feces. Scores were individually recorded and reported as an average daily diarrhea score for each pen.

### Dietary treatments

The pig treatments groups were high protein (HP); low protein (LP); MOA1, LP + 0.2% MOA and MOA2, LP + 0.3% MOA. The diets were fed during the experiment in two phases, from 0–14 days and 14–28 days (Table 1). The MOA was a microencapsulated feed additive containing botanical OA from VetAgro SpA (Tetracid® S, 42100 Regio Emilia, Italy), and it has consisted of fumaric acid (20%), citric acid (10%), malic acid (10%), and phosphoric acid (10%). All diets were formulated to meet the nutrient requirements [22] of pigs.

### Sampling and measurements

The apparent total tract digestibility (ATTD) of gross energy (GE), dry matter (DM), and crude protein (CP) was determined using chromium oxide (0.2%) as an inert indicator [23]. The pigs were fed diets mixed with chromic oxide from days 10 to 14 and days 24 to 28. On the 14th and 28th day, fresh fecal matter was collected into a plastic bag through the rectal massage of three piglets per treatment, and a representative sample was stored at  $-20^{\circ}\text{C}$  until analyzed. Before the chemical analysis, the fecal samples were defrosted and desiccated at  $70^{\circ}\text{C}$  for 72 h, after which

**Table 1. Compositions of the basal diets (as-fed basis)**

Items	0–14 days		14–28 days	
	HP	LP	HP	LP
Ingredients (%)	100.00	100.00	100.00	100.00
Corn	34.43	41.03	60.81	66.14
Extruded corn	15.00	11.69	5.00	3.20
Lactose	10.00	10.00	3.00	3.00
Dehulled soybean meal (51% CP)	13.50	10.10	13.00	9.40
Soy protein concentrate (65% CP)	10.00	10.00	10.00	10.00
Plasma powder	6.00	6.00	3.00	3.00
Whey	5.00	5.00	–	–
Soy oil	2.20	2.24	2.00	2.07
Monocalcium phosphate	1.26	1.31	1.15	1.18
Limestone	1.40	1.40	0.99	0.99
L-Lysine-HCl (78%)	0.15	0.13	0.11	0.08
DL-Methionine (50%)	0.15	0.10	0.04	0.04
Choline chloride (25%)	0.10	0.10	0.10	0.10
Vitamin premix <sup>1</sup>	0.25	0.25	0.25	0.25
Trace mineral premix <sup>2</sup>	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.30	0.30
Calculated value				
ME (kcal/kg)	3,433	3,417	3,386	3,375
CP (%)	20.76	17.69	18.78	16.08
Lysine (%)	1.35	1.33	1.15	1.12
Methionine (%)	0.39	0.35	0.30	0.28
Ca (%)	0.82	0.78	0.70	0.67
P (%)	0.62	0.63	0.60	0.59

<sup>1</sup>Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D<sub>3</sub>, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B<sub>12</sub>, 33 µg.

<sup>2</sup>Provided per kg of complete diet without Zinc: Cu (as CuSO<sub>4</sub>·5H<sub>2</sub>O), 12 mg; Mn (as MnO<sub>2</sub>), 8 mg; I (as KI), 0.28 mg; and Se (as Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O), 0.15 mg.

HP, high protein; LP, low protein; CP, crude protein; ME, metabolizable energy; Ca, calcium; P, phosphorus.

they were finely pulverized into a size that make it through a 1-mm screen. All fecal and feed samples were then analyzed for GE, DM, and CP ensuing the procedures outlined by the AOAC [24]. Chromium was analyzed via ultraviolet absorption spectrophotometry (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. [25]. For blood analysis, two pigs were randomly selected from each treatment on day 28 and blood samples were collected via the carotid artery. Blood samples were gathered into both non-heparinized tubes and vacuum tubes containing K<sub>3</sub>EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) to acquire serum and whole blood, respectively. After collection, the serum samples were centrifuged (3,000 g) for 15 min at 4°C. The white blood cells (WBC), red blood cells (RBC), and immunoglobulin G (IgG) concentrations in the whole blood were measured using an automatic blood analyzer (ADVIA 120, Bayer, NY, USA).

### Procedures of microbial shedding

Fecal samples were collected directly via massaging the rectum of five pigs in each treatment and then pooled and placed on ice for conveyance to the lab. One gram of the composite fecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Counts of the viable bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *Escherichia coli* and *Lactobacillus*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic circumstances. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

### Statistical analysis

All data were subjected to statistical analysis in a randomized complete block design using the general linear model procedures of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and the pen was used as the experimental unit. Mean values and standard errors of the mean were reported. Differences between the treatment means were determined using Tukey's multiple range test with a  $p < 0.05$  indicating significance.

## RESULT

### Growth performance and diarrhea score

At the end of 4 weeks, the pigs fed the MOA2 diet had higher ( $p < 0.05$ ) BW than the pigs fed the LP diet (Table 2). From weeks 0 to 2, the pigs fed the MOA2 diet had higher ( $p < 0.05$ ) ADGs than the pigs in the LP and MOA1 groups. The diarrhea score was significantly increased ( $p < 0.05$ ) in pigs fed with MOA1 or MOA2 diets compared with HP and LP treatments in same periods.

Pigs fed the MOA2 diet from 2 to 4 weeks had a higher ( $p < 0.05$ ) G/F ratio than the pigs fed the LP and MOA1 diets. The diarrhea score was significantly increased ( $p < 0.05$ ) in pigs fed with MOA2 diet compared with HP and LP treatments in 2 to 4 weeks. Pigs fed the MOA2 diet from 0 to 4 weeks had higher ( $p < 0.05$ ) ADG and G/F ratios than pigs fed the CON and MOA1 diets. In overall period, diarrhea score was significantly increased ( $p < 0.05$ ) in pigs fed with MOA1 and MOA2 diets compared with pigs fed with HP and LP diets.

### Nutrient digestibility

At weeks 2, the ATTD of the DM and GE were decreased in pigs fed the LP diet ( $p < 0.05$ ) compared

**Table 2.** Effects of microencapsulated mixture of organic acids (MOA) supplementation on growth performance in weaning pigs

Items	HP	LP	MOA1	MOA2	SE	p-value
Body weight (kg)						
Initial	6.8	6.8	6.8	6.8	0.1	0.977
2 wk	9.5	9.2	9.3	9.6	0.2	0.092
Final	15.5 <sup>a</sup>	14.6 <sup>b</sup>	15.0 <sup>ab</sup>	15.6 <sup>a</sup>	0.2	0.014
Week 0–2						
ADG (g)	191.9 <sup>a</sup>	170.4 <sup>b</sup>	176.9 <sup>b</sup>	200.4 <sup>a</sup>	6.8	0.005
ADFI (g)	283.3	268.8	282.1	283.7	8.2	0.412
G/F	0.671	0.636	0.657	0.713	0.029	0.156
Diarrhea score	1.561 <sup>b</sup>	1.606 <sup>b</sup>	1.823 <sup>a</sup>	1.784 <sup>a</sup>	0.064	0.008
Week 2–4						
ADG (g)	426.5	383.1	410.1	425.6	13.6	0.099
ADFI (g)	720.1	704.7	713.7	700.5	27.2	0.241
G/F	0.592 <sup>a</sup>	0.569 <sup>b</sup>	0.587 <sup>b</sup>	0.609 <sup>a</sup>	0.022	0.006
Diarrhea score	1.594 <sup>b</sup>	1.615 <sup>b</sup>	1.702 <sup>ab</sup>	1.809 <sup>a</sup>	0.047	0.012
Week 0–4						
ADG (g)	310.7 <sup>ab</sup>	276.9 <sup>b</sup>	293.3 <sup>ab</sup>	314.3 <sup>a</sup>	7.2	0.003
ADFI (g)	501.7	486.8	497.9	469.6	14.2	0.331
G/F	0.619 <sup>a</sup>	0.578 <sup>b</sup>	0.594 <sup>ab</sup>	0.633 <sup>a</sup>	0.013	0.001
Diarrhea score	1.582 <sup>b</sup>	1.611 <sup>b</sup>	1.787 <sup>a</sup>	1.816 <sup>a</sup>	0.048	0.003

<sup>a,b</sup>Means in the same row with different letters differ ( $p < 0.05$ ).

HP, high protein diet; LP, low protein diet; MOA1, LP + 0.2% microencapsulated mixture of organic acid; MOA2, LP + 0.3% microencapsulated mixture of organic acid; ADG, average daily gain; ADFI, average daily feed intake; G/F, feed efficiency; Diarrhea score, 0–3 score (diarrhea-fresh fecal).

**Table 3.** Effects of microencapsulated mixture of organic acids (MOA) supplementation on nutrient digestibility to piglets

Items (%)	HP	LP	MOA1	MOA2	SE	p-value
Week 2						
DM	78.5 <sup>a</sup>	77.9 <sup>b</sup>	78.7 <sup>a</sup>	79.1 <sup>a</sup>	0.3	0.018
CP	77.0	77.3	78.0	77.8	0.3	0.392
GE	78.2 <sup>a</sup>	76.8 <sup>b</sup>	77.7 <sup>a</sup>	78.3 <sup>a</sup>	0.3	0.004
Week 4						
DM	83.4 <sup>a</sup>	81.8 <sup>c</sup>	82.8 <sup>b</sup>	83.7 <sup>a</sup>	0.2	0.001
CP	79.2	79.0	79.0	79.4	0.3	0.932
GE	81.1	81.4	82.1	82.4	0.4	0.195

<sup>a,b,c</sup>Means in the same row with different letters differ ( $p < 0.05$ ).

HP, high protein diet; LP, low protein diet; MOA1, LP + 0.2% microencapsulated mixture of organic acid; MOA2, LP + 0.3% microencapsulated mixture of organic acid; DM, dry matter; CP, crude protein; GE, gross energy.

to pigs fed the HP, MOA1 and MOA2 diets (Table 3). At week 4, the ATTD of the DM increased in pigs fed the HP and MOA2 diet ( $p < 0.05$ ) compared to pigs fed the CON and MOA1 diets.

### Fecal microflora

There was no significant difference ( $p > 0.05$ ) in the *E. coli* and *Lactobacillus* counts in the feces among in the different treatment groups (Table 4).

### Blood profiles

There was no significant difference ( $p > 0.05$ ) in the WBC, RBC, and IgG in the different

**Table 4. Effects of microencapsulated mixture of organic acids (MOA) supplementation on fecal microflora in weaning pigs**

Items ( $\log_{10}$ cfu/g)	HP	LP	MOA1	MOA2	SE	p-value
Week 4						
<i>Escherichia coli</i>	5.6	5.5	5.4	5.5	0.1	0.564
<i>Lactobacillus</i>	6.9	7	7.1	7.2	0.1	0.533

Means in the same row with different letters differ ( $p < 0.05$ ).

HP, high protein diet; LP, low protein diet; MOA1, LP + 0.2% microencapsulated mixture of organic acid; MOA2, LP + 0.3% microencapsulated mixture of organic acid.

**Table 5. Effects of microencapsulated mixture of organic acids (MOA) supplementation on the blood profiles in weaning pigs**

Items	HP	LP	MOA1	MOA2	SE	p-value
Week 4						
WBC ( $10^3/\mu\text{L}$ )	20.4	19.4	22.5	17.5	2.1	0.266
RBC ( $10^6/\mu\text{L}$ )	6.6	6.8	6.6	6.4	0.3	0.710
IgG (mg/dL)	152.7	139.9	158.9	160.4	10.0	0.266

Note: Means in the same row with different letters differ ( $p < 0.05$ ). HP, high protein diet; LP, low protein diet; MOA1, LP + 0.2% microencapsulated mixture of organic acid; MOA2, LP + 0.3% microencapsulated mixture of organic acid; SE, standard error; WBC, white blood cells; RBC, red blood cells; IgG, immunoglobulin G.

treatment groups (Table 5).

## DISCUSSION

In present study, the growth performance was no significantly difference between HP and MOA diets. OA have attracted attention in animal nutrition in recent years due to their natural antimicrobial and growth-promoting effects [20,26]. In this study, the MOA2 diets showed higher BW in pigs at week 4 of the experiment than pigs fed the LP diet. Additionally, the MOA2 diet improved the ADG and G/F ratio from 0–2 weeks, 2–4 weeks, and over the entire experimental period. The results of this study were similar to the results of Li et al. [27]. In the other study, the ADG and final body weight were improved when weaning pigs were fed 0.3% OA supplemented diets [28]. In addition, dietary supplementation with 0.1% to 0.4% OA improved the growth performance of older pigs such as growing and finishing pigs [2,29]. These positive effects on growth performance seemed to be because OA promoted nutrient retention by lowering the pH of the GIT [30]. Also, fumaric acid is an available energy source that can have a positive effect on the mucous membrane of the small intestine, and it has been reported to improve the absorbent surface and capacity of the small intestine through the rapid recovery of gastrointestinal epithelial cells [31]. In contrast, some researchers reported that adding 1.8% OA to the diets did not influence the ADG or ADFI [32]. Other researchers showed that supplementation with single acidifiers such as citric acid, fumaric acid, and a blend of acidifiers such as lactic acid and formic acid did not affect the growth performance of weaning pigs [33,34]. These contradictory results were due to various factors such as the OA levels and acid type contained in the feed.

In this study, dietary supplementation with MOA treatment had reduced the incidence of diarrhea compared with pigs fed with HP and LP diets. Lei et al. [35] reported that the supplementation of mixture of MOA shown reduced the incidence of diarrhea with *E. coli* K88 challenges. According to Knarreborg et al. [36], the antimicrobial activity of MOA and the function of lowering the gastrointestinal pH prevent diarrhea. This results indicated that the effect of

preventing diarrhea and improving growth performance through the supplementation of MOA to low protein diets is more effective than high protein diets.

In this study, the ATTD of the DM at 2 and 4 weeks and the GE at 2 weeks increased when weaning pigs were fed diets containing 0.2 and 0.3% MOA. These results are similar to previous studies where dietary supplementation with 0.1% to 0.2% OA blends improved nutrient digestibility in weaning to finishing pigs [29,37–40]. These positive changes in nutrient digestibility were due to lowering the pH of the gastrointestinal tract, which improved the solubility of minerals [41]. However, other researchers have shown no significant difference in nutrient digestibility by the addition of 0.2% to 0.4% OA, which was fed as blends or single fumaric acid in the diet of weaning or growing pigs [2,42]. These inconsistent results were due to various factors such as the single or blended acid type and the levels contained in the diets. In this study, dietary MOA supplementation did not affect fecal *E. coli* and *Lactobacillus* counts in the weaning pigs. However, other studies showed that weaning pigs fed diets containing 0.2% to 0.3% OA reduced *E. coli* and *Salmonella* [41,43] and increased *Lactobacillus* counts [44]. These changes in bacteria were because dietary supplements with OA can reduce the upper GIT pH levels by increasing the fermentation of *Lactobacillus*, which reduces the growth of pathogenic bacteria such as *E. coli* in weaning pigs [45]. However, 0.3% MOA used in this study was not enough to increase *Lactobacillus*, which changes the pH of the digesta. Therefore, no change in the counts of the two bacteria was seen. Thus, the effect of adding 0.3% OA to pig feed on the changes in intestinal *Lactobacillus* and *E. coli* in piglets requires further study. Our results indicated that there was no significant difference in blood profile components such as WBC, RBC, and IgG concentration among the pigs in the different treatment groups during the total experimental period. This result is consistent with the results of Upadhaya et al. [29] who found no difference in blood characteristics such as RBC and WBC when pigs were fed diets containing 0.2% OA. However, some researchers reported that 0.2% to 0.5% OA supplementation stimulated changes in WBC counts, IgG concentration, and lymphocyte percentage in the host immunity-related profile [39,46,47]. Further studies on the effects of OA addition on WBC, RBC, and IgG concentration in pigs are needed because there have been no other studies performed to investigate the relationship between OA and the blood profile.

## CONCLUSION

Supplementation with dietary MOA enhanced growth parameters such as BW, ADG, and G/F, diarrhea score and the nutrient digestibility of DM and GE in weaning pigs. Specifically, MOA2 was similar effect on growth performance with HP diet, and it was more effective in improving growth performance and nutrient digestibility than the LP and MOA1 diets.

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