Effect of Supplemented Microencapsulated Zinc Oxide and Organic Acids and Pure Botanicals on Growth Performance, Nutrient Digestibility, Blood Profiles, Feces Microflora and Zinc Level of Feces in Weanling Pigs

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**Effect of Supplemented Microencapsulated Zinc Oxide and Organic Acids
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Profiles, Feces Microflora and Zinc Level of Feces in Weanling Pigs¹**

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For Review Only

Abstract

A total of 125 crossbred pigs with an initial body weight (BW) of 7.66 ± 1.30 kg were randomly distributed into 1 of 5 dietary treatments. Each treatment had 5 replicate pens with 5 pigs per pen. Treatments were as followed: 1) CON, control diet; 2) ZO, CON + 0.3% ZO; 3) ZOM, CON + 0.3% ZO + 0.1% MOP; 4) MZO, CON + 0.03% MZO; 5) MZOM, CON + 0.03% MZO + 0.1% MOP. The MZO group had higher ($P=0.03$) final BW than ZO group and also had higher ($P=0.04$, 0.01) ADG and G: F ratio compared with ZO group during d 15 to 34 and throughout the experimental period. The MZO and MZOM had higher ($P<0.05$) ATTD of DM and energy than ZO. Less ($P<0.05$) feces *E. coli* shedding was observed in the ZOM and MZOM diet compared with CON; The MZO had lowered ($P=0.001$) zinc level in feces than ZO and CON. In conclusion, results indicated that supplementation with 0.3% microencapsulated zinc oxide could improve the growth performance and ATTD of DM; inclusion of 0.1% microencapsulated organic acids and pure botanicals could decrease feces *E. coli* shedding and decrease the zinc level in feces in weanling pigs.

Keywords: Microencapsulated; Zinc Oxide; Organic Acids; Pure Botanicals; Pig (weanling);

Zinc is an essential dietary nutrient for swine. High doses of zinc oxide (2500 to 3000 mg Zn/kg of feed) added to feed have been used as a preventative measure against post-weaning diarrhea (PWD). Feeding zinc to weaned piglets has been demonstrated to reduce diarrhea incidence and to improve growth rates (Wang et al., 2009). Dietary supplementation with zinc oxide has a beneficial effect on the stability of the intestinal microflora of weaned piglets (Owusu-Asiedu et al., 2003) and morphology of their small intestine – increased height of villi in comparison with the depths of crypts (Hedemann et al., 2009). We hypothesized that microencapsulated ZO can improve the availability in the gastric intestinal tract (GIT) which may lead to the improvement of growth performance, nutrient digestibility and stimulate the microbial concentration in faeces. Even high doses of zinc have a positive effect on growth performance and improve the intestine in post-weaning pig, at meantime it has some adverse impacts on environmental pollution and destruction of cultivated land, and make animal have dependence, and high doses of zinc could affect the absorption of copper and iron. So there need to find a new form of zinc for reduce the supplementation contents.

Many studies have been conducted to identify alternatives to antibiotics as growth promoters in the animal industry (Cho et al., 2006). Recently, the use of acidifiers as potential alternatives to antibiotics in the diets of farm animals has been evaluated, and many studies have confirmed their positive effects on growth performance in post-weaning pigs (Partanen and Mroz, 1999) and poultry (Dibner and Buttin, 2002). Previous studies have demonstrated that essential oils have

beneficial effects such as antiviral (Wenk, 2003), antimicrobial (Yu et al., 2004), antioxidant (Deans et al., 1993), antiseptic (Lawrence and Reynolds, 1984), stimulating enzyme activity (Jang et al., 2004) and immune function (Wenk, 2003) in livestock. But essential oil was oxidized and volatilized easily, active substances has variability and complexity in number, so it can lead a difference result, its stability was improved by preparation process, such as vacuum freeze-drying technology and microcapsule technology.

Microencapsulated is a kind of micro container or packaging which has polymer wall. Microcapsule granulation technology is a kind of solid particles in the product technology that it could make the solid, liquid, or gas embedding, sealed in a tiny capsule. It can reduce the material vitality, disguise the odor and improve the material stability. And due to the microencapsulated diameter level is from millimeter to the micron, so it is better to absorb and utilize material for animals.

Therefore, this study was conducted to evaluate the effect of microencapsulated zinc oxide (MZO) on growth performance, nutrient digestibility, blood profiles, feces condition and zinc level of feces in weanling pigs compared with tradition zinc oxide(ZO), and meanwhile evaluate the effect of supplementation of microencapsulated organic acids and pure botanicals (MOP) unite with microencapsulated zinc oxide.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University.

Animals and facilities

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University. A total of 125 crossbred pigs [(Yorkshire × Landrace) × Duroc]; 21-d old] with an initial BW of 7.66 ± 1.30 kg were assigned randomly to 5 dietary treatments based on their sex and BW in this 34-d feeding trial. Each pen housed 5 pigs (2 gilts and 3 barrows), and there were 5 pens/treatment. All pigs were housed in a temperature controlled room. Each pen was equipped with a 1-sided, stainless-steel self-feeder and a nipple drinker that allowed pigs *ad libitum* access to feed and water. Individual pig BW and pen based feed intake were recorded at d 14, 34 of the experiment to determine average daily gain (ADG), average daily feed intake (ADFI), and gain: feed (G: F) ratio.

Dietary Treatments

Treatments were as followed: 1) CON, basal diet; 2) ZO, CON + 0.3% zinc oxide (ZO); 3) ZOM, CON + 0.3% ZO + 0.1% microencapsulated organic acids and pure botanicals (MOP); 4) MZO, CON + 0.03% microencapsulated zinc oxide (MZO); 5) MZOM, CON + 0.03% MZO + 0.1% MOP. Microencapsulated zinc oxide (MZO) is a product with low environmental impact zinc oxide from VetAgroSpA (product name: Zincoret S, 42100 Reggio Emilia, Italy). Microencapsulated

organic acids and pure botanicals (MOP) is a product with blends of organic acids and pure botanicals from VetAgroSpA (product name: Aviplus®-S, 42100 Reggio Emilia, Italy), containing 25% citric, 16.7% sorbic acids, 1.7% thymol and 1.0% vanillin. All nutrients in diets were formulated to meet or exceed the recommendation of NRC (2012) for weaned pigs (Table 1).

Sampling and measurements

Apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and gross energy was determined using chromic oxide (Cr_2O_3 , 2 g/kg) as an inert indicator. Pigs were fed diets mixed with chromic oxide on d 7 and 28. Fresh feces grab samples randomly collected from 2 pigs per pen (10 pigs/trt) on d 14 and 34 were mixed and pooled, and a representative sample was stored in a freezer at -20°C until analyzed. Before chemical analysis, the feces samples were thawed and dried at 50°C for 72 h, after which they were finely ground to a size that could pass through a 1-mm screen. All feed and feces samples were, then, analyzed for DM and N following the procedures outlined by the AOAC (2000). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962). The gross energy in the feed and feces was determined using a calorimeter (Mode 1241, Parr Instrument Co., USA).

At 8:00 and 20:00 on d 1 to 7, the feces score was evaluated and recorded. The feces score was determined by the average value of five pigs of each pen by using

a 5-grade score system (Hu et al., 2012). The standard of this system is as follows: 1 = hard, dry pellets in a small, hard mass; 2 = hard, formed stool that remains firm and soft; 3 = soft, formed, and moist stool that retains its shape; 4 = soft, unformed stool that assumes the shape of the container; 5 = watery, liquid stool that can be poured. Scores were recorded on a pen basis following observations of individual pig and signs of stool consistency in the pen.

At the beginning and the end of the 2th and 5th wk, 2 pigs (10 pigs/trt) were randomly chosen from each pen and bled via jugular venipuncture to obtain blood samples. White blood cell (WBC), Red blood cell (RBC) and lymphocyte counts were analyzed using an automatic blood analyzer (ADVIA 120, Bayer Corp.). All blood samples were then centrifuged for 15 minutes at $3000 \times g$ and 4 °C to separate the serum, after that the IgG was analyzed using nephelometry (Dade Behring, Marburg, Germany).

Feces samples were randomly collected directly via massaging the rectum of two pigs in each pen (1 gilt and 1 barrow) at the end of the experiment, and then pooled and placed on ice for transportation to the lab, where analysis was immediately carried out. One gram of the composite feces sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Viable counts of bacteria in the feces samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli*

and *lactobacillus*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The Mac Conkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *lactobacillus* colonies were counted immediately after removal from the incubator.

The feces samples were prepared for mineral analysis by a modified dry ash method (AOAC, 2000). Approximately 3.0 g of sample was ashed overnight at 600°C in an Isotemp Muffle Furnace (Fisher Scientific, Pittsburgh, PA). The ashed sample was then gently boiled for 20 minutes in 40 mL of 4N HCl (Cat. #A508; Fischer Scientific, Pittsburgh, PA). The solution was allowed to cool and then filtered (Fisherbrand P8 qualitative filter paper; Fischer Scientific) and diluted for analysis of Zn content using an atomic absorption spectrophotometer (Model S11; TJA Solutions, Franklin, MA).

Statistical analysis

Data were analyzed using a randomized complete block design following GLM procedures, with each pen being used as the experimental unit (SAS Inst. Inc., Cary, NC). The means of the treatments were compared by Duncan's multiple range test. ZO and MZO groups as well as MOP and non- MOP groups and their interaction were compared by the contrast procedure. Variability in the data were expressed as the SE and the selected level of significance at 0.05.

RESULTS

Growth performance

The result that effects of supplementation of zinc oxide, microencapsulated zinc oxide together with microencapsulated organic acids on growth performance in weanling pigs are showed in Table2.

In present study, pigs fed 0.03% MZO had higher ($P=0.03$) final BW than pigs fed 0.3% ZO and also had higher ADG ($P=0.04$) and G: F ($P=0.01$) compared with pigs fed 0.3% ZO during d 15 to 34 and throughout the experimental period. The ADFI, and feces score were unaffected ($P>0.05$) by dietary treatments throughout the experimental periods.

Nutrient digestibility

The result that effects of supplementation of zinc oxide, microencapsulated zinc oxide together with microencapsulated organic acids on nutrient digestibility in weanling pigs are showed in Table3.

Pigs fed MZO and MZOM had higher ($P<0.05$) ATTD of DM and energy than those fed ZO at 32d. Moreover, pigs fed 0.03% MZO had higher ($P=0.01$) ATTD of energy than pigs fed 0.3% ZO at the same day. There's no difference ($P>0.05$) in ATTD of nitrogen among dietary treatments.

Blood profiles

The result that effects of supplementation of zinc oxide, microencapsulated zinc oxide together with microencapsulated organic acids on blood profiles in weanling pigs are showed in Table4

There was no difference ($P>0.05$) on RBC, WBC, IgG concentrations, and lymphocyte percentage among dietary treatments at either phase of the experiment.

Feces microbial shedding and feces score

The result that effects of supplementation of zinc oxide, microencapsulated zinc oxide together with microencapsulated organic acids on feces microbial shedding and feces score in weanling pigs are showed in Table5

Feces *E. coli* shedding was decreased ($P<0.05$) in pigs fed ZOM and MZOM diets compared with pigs fed CON diet. Dietary supplemented with 0.1% MOP had lower ($P=0.01$) feces *E. coli* shedding compared with pigs fed non-MOP diet. No difference was observed in feces *Lactobacillus* counts among treatments ($P>0.05$). And supplementation of tradition or microencapsulated zinc oxide unite with microencapsulated organic acids could significantly increase the feces score in weanling pig compared with control group ($P>0.05$).

Feces zinc concentration

The result that effects of supplementation of zinc oxide, microencapsulated zinc oxide together with microencapsulated organic acids on feces zinc concentration in weanling pigs are showed in Table6

Pigs fed 0.03% MZO had a lower zinc level ($P=0.001$) in feces than pigs fed 0.3% ZO and CON.

DISCUSSION

Growth performance

Zinc is the second most abundant transition metal in biological systems (Suhly, 1999). Pharmacological concentrations of ZnO (2500 to 4000 ppm) have been demonstrated as effective for promoting post-weaning pig growth performance (Hill et al., 2001). Pérez et al. (2010) also showed a positive response to ZnO on ADG during the first week post-weaning. In our study, supplement tradition ZnO in diet could not affect the growth performance, but results showed that coated ZnO supplementation increased final body weight, ADG and G/F ratio compared with tradition ZnO treatment, which may be explained by the increased energy digestibility of pigs fed the coated ZnO diets. Partanen and Mroz (1999) reviewed 35 published experiments, reported that that formic acid, formate salts, fumaric acid and citric acid improved growth and feed efficiency. As acidifiers, organic acids supplementation improved growth performance in pigs (Wang et al., 2009). Grilli et al. (2010) also reported that microencapsulated mixture (organic acids and natural identical flavors) supplementation improved growth performance of weanling pigs. However, in our study, the growth performance was not affected by the microencapsulated organic acid inclusion, but it has an increased tend on low

zinc level diet. Previous studies demonstrated no effect of dietary essential oils supplementation was observed on growth performance in pigs (Janz et al., 2007).

Nutrient digestibility

It has been demonstrated that supplementation of 3000 ppm ZnO improves small intestinal health by increasing the villous height to crypt depth ratio compared to pigs fed the same diet without ZnO supplementation (Li et al., 2001). There has a similar result that added 3000 mg/kg zinc oxide could increase the villous height, decreased crypt depth and increased the ratio of villous height to crypt depth (Li et al., 2006). In this study, the low level zinc from microencapsulated could significantly increase the energy digestibility and dry matter digestibility compared with tradition high level zinc, but there was no significantly difference on nitrogen digestibility. And added microencapsulated mixture of organic acid and essential oil unite with tradition or microencapsulated zinc oxide could increase energy digestibility and dry matter digestibility, and the nitrogen digestibility has an increased tend. Because organic acids influence mucosal morphology, as well as stimulate pancreatic secretion, and they also improve protein digestibility by lowering gastric pH, which consequently increases pepsin activity as well as increasing absorption and retention of many dietary nutrients (Partanen et al., 2007). Moreover, previous studies have already proved that essential oils are capable of stimulating enzyme activity (Platel and Srinivasan, 1996). In this study, the mixture of organic acid and essential oil supplementation

improved dry matter digestibility, it is in line with Wang et al. (2009) reported that organic acids supplementation improved nutrient digestibility in pigs. Benchaar et al. (2008) also reported that essential oils are promising as feed additives to improve feed efficiency in livestock.

Blood profiles

Prasad (2002) reported that zinc supplementation could affect the blood WBC, IgG and lymphocyte concentration, thus improve the immune response in animals. Organic acids supplementation also increased blood lymphocyte and WBC concentration (Ravindran and Kornegay, 1993) in pigs. Previous studies have already proved that essential oils are capable of stimulating immune function (Wenk, 2003). However, in present study, tradition high level or microencapsulated low level zinc oxide or unite with microencapsulated mixture (organic acids and essential oils) could not blood profiles in weanling pigs. This inconsistency may be caused by different feed composition, ages of pigs, and hygiene status.

Feces microbial shedding and feces score

The weaning period of pigs is frequently associated with infectious disease and diarrhea (Deng et al., 2009). Zinc is essential in the prevention of pathogen infection (Scott and Koski, 2000). Previous studies have been showed that high dietary concentrations of ZnO (inorganic mineral source) reduce post-weaning pig

mortality and diarrhea attributable to *E. coli* (King et al., 1995; Poulsen, 1989). Pharmacological concentrations of ZnO have been demonstrated as effective for reducing the incidence of diarrhea (Melin and Wallgren, 2002). Our study indicated that high level tradition zinc oxide or low level coated ZnO supplementation decreased feces *E. coli* counts, but no significantly. There is evidence suggesting that ZnO exerts antimicrobial and immunoregulatory actions in the small intestine (Tran et al., 2003), and may inhibit the growth of some intestinal bacterial species (Sawai, 2003; Højberg et al., 2005). Ou et al. (2007) suggested that supplementation with a high level of ZnO reduced the incidence of diarrhea in weanling piglets due to decreasing expression of the stem cell factor in the small intestine by ZnO supplementation.

The pH value of gastrointestinal tract was reduced advantageous to the absorption of intestinal nutrients and improved the gastrointestinal microbiota (Pettigrew, 2006). Organic acids have antibacterial and antifungal activities (Lavermicocca et al., 2003). Burt (2004) and Cho et al. (2006) reported that essential oil, which had known antimicrobial function can inhibit pathogens shedding in intestine. However, our results showed that added microencapsulated mixture (organic acids and essential oils) supplementation unite with high level tradition zinc oxide or low level microencapsulated zinc oxide could significantly reduce the population of feces *E. coli*, but there has no significantly difference on *Lactobacillus* population. Roy et al. (2002) reported that organic acid could restrain the growth of pathogen, reduced rate of diarrhea and improved animal health, it in line with

our study that microencapsulated mixture of organic acid and essential oil unite with tradition or microencapsulated zinc oxide could increase feces score and reduced rate of diarrhea.

Feces zinc concentration

High zinc excretion in animal waste poses environmental challenges (Jondreville et al, 2003). A greater bioavailability would result in better use by the pig and less excretion by the animal. In the current study, we found that low level coated ZnO led to a lower feces zinc level than pigs supplemented with 0.3% ZnO, which may reduce the pollution of zinc excretion from animal production. And unite with microencapsulated mixture of organic acid and essential oil could increased the utilization rate of zinc, reduced the discharge of zinc.

CONCLUSION

In conclusion, results indicated that supplementation with low level (0.03%) microencapsulated zinc oxide (MZO) has a better result on growth performance and ATTD of energy and decrease the zinc level in feces compared with high level (0.3%) tradition zinc oxide. and the zinc oxide from tradition or microencapsulated unite with 0.1% microencapsulated organic acids and pure botanicals (MOP) has an better result on decrease feces *E. coli* shedding and increased utilization rate of zinc in weanling pigs.

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Table 1 Compositions of basal weanling pig diets (as-fed basis).^a

Item	Phase 1 (d 0 to 14)	Phase 2 (d 15 to 28)
Ingredient, %		
Extruded corn	35.82	45.80
Biscuit meal ^b	5.00	9.00
Soybean meal, 440 g/kg CP	22.00	31.65
Fermented soybean meal	8.20	-
Fish meal	4.00	2.50
Soy oil	4.80	3.00
Lactose	6.00	-
Whey powder	10.00	6.25
Monocalcium phosphate	1.00	0.60
Sugar	2.00	-
L-Lys·HCl, 78%	0.25	0.16
DL-Met, 50%	0.15	0.14
L-Thr, 89%	0.08	-
Vitamin premix ^c	0.10	0.10
Mineral premix ^d	0.20	0.20
Limestone	0.20	0.30
Salt	0.20	0.30
Calculated composition, g/kg		
ME, Mkal/kg	3.52	3.47
CP, %	21.00	20.50
Lys, %	1.41	1.33
Met, %	0.49	0.47
Ca, %	0.78	0.75
Total P, %	0.76	0.64
Analyzed composition, g/kg		
CP, %	20.75	19.80
Lys, %	1.42	1.31
Met, %	0.52	0.45
Ca, %	0.76	0.72
Total P, %	0.79	0.59

^a Additives were added by replacing extruded corn in basal diets.

^b Biscuit meal was obtained by grinding byproducts from the bakery industry such as bread, biscuits, breakfast cereals, cakes, crisps, and snacks.

^c Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D3, 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 B12, 33 µg.

^d Provided per kg of complete diet: Cu (as CuSO₄·5H₂O), 12 mg; Zn (as ZnSO₄), 85 mg; Mn (as MnO₂), 8 mg; I (as KI), 0.28 mg; and Se (as Na₂SeO₃·5H₂O), 0.15 mg.

Table 2 Effect of supplementation of ZO, MZO together with MOP on growth performance in weanling pigs.^a

Items	CON	ZO		MZO		P-value			SE ^b
		-MOP	+MOP	-MOP	+MOP	ZO	-MOP	ZO*MOP	
						vs.	vs.	vs	
BW, kg									
Initial	7.68	7.68	7.66	7.65	7.64	0.27	0.31	0.43	0.02
d 14	11.59	11.54	11.67	11.94	11.60	0.48	0.63	0.58	0.22
d 34	21.44	20.27	20.45	21.86	21.69	0.03	1.00	0.04	0.61
d 0-14									
ADG, kg	0.279	0.276	0.286	0.306	0.283	0.431	0.704	0.683	0.016
ADFI, kg	0.336	0.329	0.343	0.342	0.326	0.922	0.965	0.874	0.021
G:F	0.831	0.838	0.835	0.896	0.868	0.374	0.720	0.587	0.056
d 15-34									
ADG, kg	0.493	0.437	0.439	0.496	0.505	0.041	0.850	0.037	0.029
ADFI, kg	0.730	0.726	0.730	0.719	0.711	0.535	0.934	0.663	0.020
G:F	0.675	0.601	0.601	0.690	0.710	0.012	0.781	0.021	0.036
d 0-34									
ADG, kg	0.405	0.370	0.376	0.418	0.413	0.032	0.981	0.043	0.018
ADFI, kg	0.568	0.562	0.571	0.564	0.552	0.611	0.923	0.832	0.016
G:F	0.713	0.659	0.659	0.741	0.749	0.011	0.932	0.022	0.030

Note: Means within a column not sharing a lowercased italic letter differ significantly at the P<0.05 level.

^a Abbreviations: ZO: zinc oxide; MZO: microencapsulated zinc oxide; MOP: microencapsulated organic acids and pure botanicals,

BW=body weight, ADG=average daily gain, ADFI=average daily feed intake, G:F=gain:feed ratio.

^b Standard error.

Table 3 Effect of supplementation of ZO, MZO together with MOP on nutrient digestibility in weanling pigs.
^a

		<u>ZO</u>		<u>MZO</u>		<u>P-value</u>			
Items, %	CON	-MOP	+MOP	-MOP	+MOP	ZO	-MOP	ZO*MOP	SE ^b
						vs. MZO	vs. +MOP	vs MZO*MOP	
14 d									
Dry matter	84.66	83.46	83.19	83.19	83.04	0.43	0.42	0.63	1.26
Nitrogen	88.53	88.16	88.60	88.59	87.75	0.68	0.69	0.78	1.00
Energy	81.83	81.62	81.64	81.81	81.07	0.53	0.23	0.54	2.30
34 d									
Dry matter	80.78 ^{ab}	80.00 ^b	82.04 ^{ab}	81.79 ^a	84.66 ^a	0.10	0.08	0.253	1.01
Nitrogen	80.65	79.80	81.35	82.01	81.22	0.15	0.59	0.831	0.69
Energy	81.06 ^{ab}	80.18 ^b	81.33 ^{ab}	82.19 ^a	83.28 ^a	0.01	0.22	0.127	1.49

Note: Means within a column not sharing a lowercased italic letter differ significantly at the P<0.05 level.

^a Abbreviations: ZO: zinc oxide; MZO: microencapsulated zinc oxide; MOP: microencapsulated organic acids and pure botanicals, BW=body weight, ADG=average daily gain, ADFI=average daily feed intake, G:F=gain:feed ratio.

^b Standard error.

Table 4 Effect of supplementation of ZO, MZO together with MOP on blood profiles in weanling pigs. ^a

Items	ZO			MZO		P-value			SE ^b
	CON	-MOP	+MOP	-MOP	+MOP	ZO vs. MZO	-MOP vs. +MOP	ZO*MOP vs MZO*MOP	
Lymphocyte, %									
0 d	42.10	41.76	42.74	42.04	41.50	0.80	0.91	0.54	1.92
14 d	41.60	44.12	42.18	43.12	39.28	0.59	0.43	0.33	3.60
34 d	52.92	53.36	53.66	54.98	57.72	0.45	0.68	0.45	3.73
RBC, 10 ⁶ /μl									
0 d	6.29	6.20	6.28	6.19	6.24	0.60	0.28	0.62	0.05
14 d	6.21	6.41	6.36	6.31	6.36	0.79	0.99	0.77	0.18
34 d	6.24	6.27	6.40	6.28	6.35	0.91	0.50	0.74	0.14
WBC, 10 ³ /μl									
0 d	13.12	12.96	13.22	13.18	13.00	0.99	0.95	0.87	0.57
14 d	17.02	17.70	16.82	18.64	17.94	0.38	0.49	0.22	1.16
34 d	17.22	17.82	18.50	18.08	17.78	0.83	0.86	0.85	1.02
IgG, mg/dL									
0 d	185.0	191.6	187.6	190.8	189.2	0.97	0.81	0.75	11.8
14 d	197.6	222.0	212.6	215.4	210.4	0.86	0.77	0.45	25.3
34 d	212.5	224.8	242.0	227.4	223.8	0.71	0.75	0.66	21.0

Note: Means within a column not sharing a lowercased italic letter differ significantly at the P<0.05 level.

^a Abbreviations: ZO: zinc oxide; MZO: microencapsulated zinc oxide; MOP: microencapsulated organic acids and pure botanicals, BW=body weight, ADG=average daily gain, ADFI=average daily feed intake, G:F=gain:feed ratio.

^b Standard error.

Table 5 Effect of supplementation of ZO, MZO together with MOP on feces microbial shedding and feces score in weanling pigs. ^a

Items, log10cfu/g	CON	ZO		MZO		P-value			SE ^b
		-MOP	+MOP	-MOP	+MOP	ZO	-MOP	ZO*MOP	
						vs. MZO	vs. +MOP	vs MZO*MOP	
<i>Lactobacillus</i>	7.47	7.59	7.60	7.54	7.58	0.83	0.94	0.53	0.08
<i>E. coli</i>	6.41 ^a	6.37 ^{ab}	6.24 ^b	6.36 ^{ab}	6.20 ^b	0.94	0.01	0.83	0.05
Feces score	2.34 ^a	3.13 ^b	3.11 ^b	3.09 ^b	3.12 ^b	0.61	0.78	0.66	0.02

Note: Means within a column not sharing a lowercased italic letter differ significantly at the P<0.05 level.

^a Abbreviations: ZO: zinc oxide; MZO: microencapsulated zinc oxide; MOP: microencapsulated organic acids and pure botanicals, BW=body weight, ADG=average daily gain, ADFI=average daily feed intake, G:F=gain:feed ratio.

^b Standard error.

Table 6 Effect of supplementation of ZO, MZO together with MOP on feces zinc concentration in weanling pigs.^a

Items, %	CON	ZO		MZO		P-value			SE ^b
		-MOP	+MOP	-MOP	+MOP	ZO vs. MZO	-MOP vs. +MOP	ZO*MOP vs MZO*MOP	
Zinc level	1.10 ^a	1.33 ^a	1.27 ^a	0.81 ^b	0.66 ^b	0.001	0.18	0.001	0.07

Note: Means within a column not sharing a lowercased italic letter differ significantly at the P<0.05 level.

^a Abbreviations: ZO: zinc oxide; MZO: microencapsulated zinc oxide; MOP: microencapsulated organic acids and pure botanicals, BW=body weight, ADG=average daily gain, ADFI=average daily feed intake, G:F=gain:feed ratio.

^b Standard error.