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Use of Lower Level of Capsulated Zinc Oxide as an Alternative to Pharmacological Dose of Zinc Oxide for Weaned Piglets

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ABSTRACT

Pharmacological level of zinc oxide is a common recommendation of swine industries, but that could produce metal toxicity to plants and soil microorganisms. The objective of this study was to evaluate the use of lower level of capsulated zinc oxide as an alternative to pharmacological level of zinc oxide for weaned piglets. In this study, 90 weaned piglets were randomly assigned into 3 groups (each group with 3 replicates, 10 piglets per replicate) and were fed with the basal diet supplemented with 0 (the control group), 3000 mg kg⁻¹ zinc from zinc oxide (the zinc oxide group) and 1500 mg kg⁻¹ zinc from capsulated zinc oxide (the capsulated zinc oxide group) for 7 days. Results indicated that lower level of capsulated zinc oxide tended to increase the average daily gain ($p = 0.062$), significantly decreased diarrhea ratio ($p < 0.01$) and significantly increased the serum concentrations of immunoglobulins and zinc ($p < 0.01$) compared to the control group. Serum concentration of insulin-like factor-I (IGF-I), mRNA levels for IGF-I in liver, jejunum and mRNA level for zonula occludens protein-1 (ZO-1) in jejunum were markedly improved by capsulated zinc oxide. There was no difference between zinc oxide group and capsulated zinc oxide group, except for the fecal zinc concentration. Lower level of capsulated zinc oxide significantly decreased the fecal zinc concentration compared to the zinc oxide group ($p < 0.01$). These results showed that lower level of capsulated zinc oxide exhibited beneficial effects on weaned piglets and could be an alternative to pharmacological level of zinc oxide in weaned piglets.

Key words: Capsulated zinc oxide, weaned piglet, growth, diarrhea, intestinal permeability

INTRODUCTION

In commercial piggeries, pigs are frequently kept in large groups with high densities and are raised rapidly to slaughter weight before they reach their physical maturity. Under these conditions, diseases are often occurred and spread quickly, especially for piglets (Wegener, 2003). Commonly, the piglets are weaned at 14 to 28 days of age, which could maximize the whole-herd production (Fang *et al.*, 2009; Kim *et al.*, 2010). However, early weaning may result in increased diarrhea ratio, the growth check and increased mortality rates (Odle *et al.*, 1996; Frydendahl, 2002). Antibiotics have been used as feed additives to enhance immunity, treat diarrheas and improve growth performance. However, widely use of antibiotics could produce bacterial resistance

and is harmful to animals or even human (Erika and Knudsen, 2001; Frydendahl, 2002; Fang *et al.*, 2009). Novel alternatives for antibiotics, such as high concentration of zinc, have attracted more attentions recently.

Dietary supplementation with pharmacological level of zinc oxide (2500-3000 ppm) could enhance growth performance and reduce weaning diarrhea rates, which has been accepted and applied in pig industry (Hill *et al.*, 2001; Case and Carlson, 2002). Researches have been conducted to investigate the possible mechanisms for the positive effects of zinc oxide (Li *et al.*, 2006; Zhang and Guo, 2009). Li *et al.* (2006) reported that high dose of zinc oxide could increase the level of IGF-I and IGF-I receptor in small intestine to benefit the intestinal integrity, function and whole-body growth. Zhang and Guo (2009) demonstrated that the tight junction proteins, which could be increased in small intestine by high level of zinc oxide, reduced intestinal permeability and improved the growth performance in weaning piglets. There is an evidence that zinc oxide, which has a lower bioavailability than zinc sulfate or zinc methionine, exhibits a better growth promoter effect than both of zinc sulfate and methionine (Wedekind *et al.*, 1994; Hollis *et al.*, 2005). These studies indicate that high doses of zinc oxide instead of zinc ions enhance the growth performance and decrease diarrhea ratios. However, dietary supplementation with high level of zinc oxide could cause increased zinc excretion. As manure with high concentration zinc is applied, zinc in soil will be accumulated and produces metal toxicity to plants and soil microorganisms and pollute the environment (L'Herroux *et al.*, 1997; Jondreville *et al.*, 2003). Therefore, lower levels of high-efficiency zinc oxide, such as capsulated zinc oxide, should be explored to be alternatives to common zinc oxide.

It has been reported that capsulated zinc oxide could prevent chemical changes of zinc oxide to maximize the positive effects (Kim *et al.*, 2010). So, this study was conducted to evaluate the effects of lower level of zinc from capsulated zinc oxide on growth performance, diarrhea ratios, serum parameters, intestinal morphology and intestinal permeability in weaned piglets.

MATERIALS AND METHODS

Experimental design: Ninety piglets (Duroc×Landrace×Yorkshire) with 8.18 kg (SEM = 0.23) body weight at 28 days of age were randomly assigned into 3 groups on the basis of weight. Each group has 3 replicates with 10 pigs per replicate. The experimental periods were 7 days. During the 7 day trial, the piglets were fed with the basal diet supplemented with 0 (the control group), 3000 mg kg⁻¹ zinc from zinc oxide (the zinc oxide group) and 1500 mg kg⁻¹ zinc from capsulated zinc oxide (the capsulated zinc oxide group). The zinc oxide and capsulated zinc oxide were both provided by Hangzhou King Techina Technology CO., Ltd. The basal diet (Table 1) was a typical corn-soybean meal-based diet which was met or exceeded (NRC, 1998).

Piglets were housed on concrete floors with a cycle of 16 h light and 8 h dark. Feed and water, which were provided by self-feeders and nipple waters, were available *ad libitum*. The duration of this feeding experiment lasted for 7 days. Experimental procedures were approved by the Zhejiang University Animal Care and Use Committee. The Average Daily Gain (ADG), Average Feed Intake (ADFI) and feed: gain ratio (F/G) were observed at d 7. The number of diarrheic piglets per repeat was recorded daily to evaluate the diarrhea ratio (Wang *et al.*, 2007):

$$\text{Diarrhea ratio (\%)} = \frac{\text{Total No. of diarrheic piglets}}{\text{Total No. of experimental piglets} \times \text{Trail days}} \times 100$$

Table 1: Ingredient and chemical composition of the basal diet on an as-fed basis

Item	Percentage
Ingredient	
Corn	52.27
Soybean meal (45% CP)	19.00
Soybean oil	1.60
Extruded full-fat soybean	12.00
Fish meal (63% CP)	4.00
Dried whey	6.00
Dicalcium phosphate	2.00
Limestone	1.00
Sodium chloride	0.25
L-lysine HCl (78%)	0.28
Methionine (98.5%)	0.60
Vitamin-mineral premix	1.00
Chemical composition as feed	
DE (MJ kg ⁻²)	14.47
Crude protein	20.45
Lysine	1.32
Met+Cyst.	0.82
Threonine	0.91
Calcium	0.91
Total phosphorus	0.64

The vitamin-mineral premix provided (kg⁻¹ feed): Vitamin A: 4000 IU, Vitamin D3: 800 IU, Vitamin E: 10 IU, Vitamin K3: 0.5 mg, Biotin: 0.05 mg, Folic acid: 0.3 mg, Niacin: 10 mg, d-pantothenic acid: 10 mg, Riboflavin: 3.6 mg, Thiamine: 1.0 mg, Pyridoxine: 1.5 mg, Cobalamin: 15 mg, Mn (MnSO₄.H₂O): 10 mg, Zn (ZnSO₄.7H₂O): 80 mg, Fe (FeSO₄.7H₂O): 80 mg, Cu (CuSO₄.5H₂O): 15.0 mg, I (KI): 0.14 mg, Se (Na₂SeO₃): 0.15 mg

Samples collection: At the end of this experiment, fecal and blood samples were collected from 2 randomly selected piglets in each replicate. Blood samples were collected by anterior vena cava puncture using heparinized and plain vacutainer tubes after fasting for 12 h. The serum, centrifuged from the blood at 3000 xg and 4°C for 15 min, were stored at -80°C until analysis. Fecal samples were frozen at -80°C until analysis of zinc concentration. The middle part of jejunum segments were collected, flushed and fixed with 10% formalin for the observation of intestinal morphology. Jejunum segments and liver samples were selected for semi quantitative RT-PCR.

Analysis of blood samples: The concentrations of serum Total Protein (TP), Urea Nitrogen (UN), zinc, Ca, P immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG) and activities of Glutamic-Oxaloacetic Transaminase (GOT) and Glutamic-Pyruvic Transaminase (GPT) were analyzed by corresponding commercial kits (Jiancheng Biochemical Reagent Co., Nanjing, China). The activity of Alkaline Phosphatase (ALP) in serum was determined by the method described by Tietz *et al.* (1983). Briefly, the serum sample was added into the warmed reaction cuvette with alkaline buffered substrate solution. Subsequently, changes in absorbance of the mixture were observed to calculate the ALP activity. Serum insulin-like growth factor-I (IGF-I) concentration was measured using the swine ELISA kit provided by Linco Research Inc. (St Charles, USA). All these parameters analyzed by kits were following the manufactures' instructions.

Determination of zinc concentration in feces: Fecal samples for zinc concentration were prepared with the method described by Armstrong *et al.* (2004). Briefly, the dried fecal sample was dissolved with nitric acid and then was digested using a microwave digestion system. Subsequently, the solution was diluted with deionized water and was analyzed with flame atomic absorption spectrophotometry (AA-6300, Shimadzu, Tokyo, Japan).

Intestinal morphology: The jejunum segments for observation of morphology were fixed with 10% formalin for 24 h. Subsequently, three cross-sections for each sample were prepared after staining with hematoxylin and eosin using standard paraffin embedding techniques (Xu *et al.*, 2003). Villous height and crypt depth were measured using light microscope with an analysis system (version 1, Leica Imaging Systems Ltd., England). Measurements of 18 (6 for each cross-section) intact, well-oriented crypt-villous units were taken for each sample.

Analysis of semi quantitative RT-PCR: RNA was isolated and reversed using the method described by Gendelman and Roth (2012) with some modifications. Briefly, total RNA was prepared from liver and jejunum using Trizol reagent (Invitrogen, USA). After extraction, the RNA was suspended in DEPC water and treated with RNase-free DNase (TaKaRa, Dalian, China) to remove DNA contamination. Subsequently, the isolated RNA was incubated at 72°C with ohgodT for 5 min and then incubated for 1 h with RT mixture (TaKaRa, Dalian, China), including 5×M-MLV-RT buffer, M-MLV reverse transcriptase and dNTPs. Finally, the reverse transcription was inactivated at 90°C for 10 min.

The primers for IGF-I, zonula occludens protein-1 (ZO-1), occludin and 18S were used in present study (Huo *et al.*, 2006; Xu *et al.*, 2012). The primer sequences, product sizes and annealing temperature were listed in Table 2.

Statistical analysis: The data were processed with ANOVA using Tukey-HSD multiple range tests of the SPSS statistical package for Windows (version 16.0; SPSS Inc., Chicago, IL). Pens were used as the experimental units for growth performance and diarrhea ratio data and individual piglet was used as the experimental unit for other indices. Probability value below 0.05 was considered as the statistical significant level and between 0.10 and 0.05 was taken to indicate a statistical tendency.

RESULTS

Growth performance and diarrhea ratio: Effects of zinc oxide and capsulated zinc oxide on growth performance and diarrhea ratio were presented in Table 3. Supplementation with

Table 2: Primer sequences

Gene	Sequence	Size (bp)	Annealing temperature (°C)
IGF-I	5-CTGAGGAGGCTGGAGATGTA-3	221	59
	5-CTCGTGCAGAGCAAAGGAT-3		
ZO-1	5-ACCCACCAAACCCACCAA-3	123	59
	5-CCATCTCTTGCTGCCAAACTATC-3		
Occludin	5-CTGGAGGAAGACTGGAT-3	244	59
	5-ATCCGCAGATCCCTTAAC-3		
18S	5-GACCAGAGCGAAAGCATT-3	375	60
	5-TCCACCAACTAAGAACGG-3		

Table 3: Effects of zinc oxide and capsulated zinc oxide on growth performance and diarrhea ratio

Item	Control	Zinc oxide	Capsulated zinc oxide	SEM (n = 3)	p-value
Initial weight (kg)	8.2367	8.1567	8.1333	0.2272	0.894
Final weight (kg)	8.7130	8.6770	8.6530	0.2423	0.969
ADG	0.0680	0.0740	0.0740	0.0024	0.062
ADFI	0.1710	0.1820	0.1770	0.0061	0.282
Feed:gain ratio	2.5180	2.4530	2.3920	0.1291	0.645
Diarrhea ratio	30.6000 ^a	14.8400 ^b	15.6500 ^b	2.6500	0.002

Means within a row with different superscripts differ significantly at $p < 0.05$, ADG: Average daily gain, ADFI: Average daily feed intake

Table 4: Effects of zinc oxide and capsulated zinc oxide on blood metabolism and zinc concentration in feces

Item	Control	Zinc oxide	Capsulated zinc oxide	SEM (n = 6)	p-value
Serum					
IgG (mg mL ⁻¹)	0.697 ^b	0.937 ^a	0.950 ^a	0.042	<0.001
IgM (mg L ⁻¹)	0.113 ^b	0.192 ^a	0.187 ^a	0.017	<0.001
IgA (mg L ⁻¹)	1.135 ^b	1.477 ^a	1.398 ^a	0.074	0.001
ALP (IU L ⁻¹)	142.240	152.700	154.020	5.612	0.104
GOT (IU L ⁻¹)	68.420	69.170	68.720	2.307	0.948
GPT (IU L ⁻¹)	22.290	23.070	22.020	1.593	0.796
UN (mmol L ⁻¹)	7.802	7.895	7.788	0.203	0.851
TP (g L ⁻¹)	50.320	50.140	51.340	2.225	0.846
Ca (mmol L ⁻¹)	1.120	1.060	1.080	0.070	0.738
P (mmol L ⁻¹)	3.310	3.430	3.330	0.144	0.679
IGF-I (µg L ⁻¹)	11.370 ^b	11.930 ^a	12.020 ^a	0.165	0.003
Zinc (µmol L ⁻¹)	27.240 ^b	42.360 ^a	38.470 ^a	2.478	<0.001
Feces					
Zinc (g kg ⁻¹)	2.300 ^c	15.120 ^a	10.010 ^b	0.428	<0.001

Means within a row having different superscripts differ significantly at $p < 0.05$, IgG : Immunoglobulin G, IgM: Immunoglobulin M, IgA: Immunoglobulin A, ALP: Alkaline phosphatase, GOT: Glutamic-oxaloacetic transaminase, GPT: Glutamic-pyruvic transaminase, UN: Urea nitrogen, TP: Total protein, IGF-I: Insulin-like factor-I

3000 mg kg⁻¹ zinc from zinc oxide or 1500 mg kg⁻¹ zinc from capsulated zinc oxide did not significantly change ADFI ($p > 0.10$) and F/G ($p > 0.10$), whereas tended to increase the ADG ($p = 0.062$) in piglets. In addition, diarrhea ratios in zinc oxide and capsulated zinc oxide group were significantly lower than the control group ($p < 0.01$). There was no significant difference between the zinc oxide group and capsulated zinc oxide group ($p > 0.10$).

Blood metabolism and zinc concentration in feces: Results in Table 4 indicated that the serum concentrations of IgG, IgM and IgA were significantly increased by dietary supplemental zinc oxide or capsulated zinc oxide ($p < 0.01$). However, zinc oxide and capsulated zinc oxide did not affect the activities of ALP, GOT and GPT in serum ($p > 0.10$). In addition, the levels of UN, TP, Ca and P in serum were not influenced by zinc oxide or capsulated zinc oxide ($p > 0.10$).

Table 4 also showed that supplementation with zinc oxide and capsulated zinc oxide significantly increased the serum concentrations of IGF-I and zinc ($p < 0.01$). No significant difference was found between the zinc oxide group and capsulated zinc oxide group ($p > 0.10$). Compared with the control group, supplemental zinc oxide and capsulated zinc oxide significantly increased the zinc concentration in feces ($p < 0.01$). However, the fecal zinc concentration of piglets in capsulated zinc oxide group was significantly lower than the zinc oxide group ($p < 0.01$).

Table 5: Effects of zinc oxide and capsulated zinc oxide on morphology of jejunum and on mRNA levels

Item	Control	Zinc oxide	Capsulated zinc oxide	SEM (n = 6)	p-value
Jejunum					
Villous heigh (µm)	319.460	337.070	336.090	7.6700	0.063
Crypt depth (µm)	322.690	318.460	319.240	6.2600	0.776
Villous:Crypt	0.992 ^b	1.052 ^{ab}	1.058 ^a	0.0234	0.023
mRNA					
IGF-I (liver)	0.576 ^b	0.707 ^a	0.641 ^{ab}	0.0427	0.026
IGF-I (jejunum)	0.311 ^b	0.400 ^a	0.378 ^a	0.0195	0.001
ZO-1 (jejunum)	0.412 ^b	0.509 ^a	0.482 ^a	0.0265	0.006
Occludin (jejunum)	0.705	0.804	0.787	0.0434	0.083

Means within a row having different superscripts differ significantly at $p < 0.05$, IGF-I: Insulin-like factor-I, ZO-1: Zonula occludens protein-1

Morphology and the mRNA levels for IGF-I, ZO-1 and occludin: The morphology and mRNA levels for IGF-I, ZO-1 and occludin were shown in Table 5. The results indicated that compared with the control, supplemental zinc oxide and capsulated zinc oxide did not affect crypt depth ($p > 0.10$), whereas tended to increase the villous height ($p = 0.063$). Moreover, compared with the control, dietary supplementation with capsulated zinc oxide significantly increased the villous height: crypt depth ratio ($p < 0.05$). However, no significant difference was found between zinc oxide group and the control. In addition, dietary supplemental zinc oxide significantly enhanced the mRNA levels for IGF-I in both liver and jejunum ($p < 0.01$), significantly increased the mRNA level for ZO-1 ($p < 0.01$) and tended to increase the mRNA level for occludin in jejunum ($p = 0.083$). There was no significant difference between zinc oxide group and capsulated zinc oxide group ($p > 0.10$).

DISCUSSION

Early weaning of piglets commonly resulted in increased diarrhea ratio, depressed feed intake and growth performance (Frydendahl, 2002). Pharmacological dose of zinc oxide (2500-3000 mg kg⁻¹) and antibiotics were used as additives to promote the growth performance. It has been proposed that pharmacological dose of zinc oxide could enhance the synthesis of ghrelin, increase the feed intake, reduce the inflammation and improve growth performance (Hill *et al.*, 2001; Ou *et al.*, 2007; Yin *et al.*, 2008; Shelton *et al.*, 2011). However, in present study supplementation with 3000 mg kg⁻¹ zinc derived from zinc oxide or 1500 mg kg⁻¹ zinc from capsulated zinc oxide did not significantly affect ADFI, whereas tended to increase the ADG. The reason for no significant effect on growth performance in present study may be that the duration of the feeding experiment (7 days) was not long enough.

Diarrhea is one of the most important problems in post-weaning piglets, which is often caused by pathogens or indigestion (Wittig *et al.*, 1995). Several reports indicated that high dose of zinc oxide could effectively alleviate or prevent the diarrhea in piglets (Huang *et al.*, 1999; Owusu-Asiedu *et al.*, 2003). Ou *et al.* (2007) revealed that high level of zinc oxide could decrease the stem cell factor expression and the number of mast cells in small intestine, attenuate histamine release and prevent diarrhea. The present study also found that high dose of zinc oxide (3000 mg kg⁻¹) had a beneficial effect on diarrhea in weaned piglets. In addition, lower level of zinc (1500 mg kg⁻¹) from capsulated zinc oxide had similar effects on diarrhea to the high dose of zinc oxide, which was partly in line with the reports by Kim *et al.* (2010).

In the present study, the serum concentrations of immunoglobins (IgA, IgG and IgM) were determined to evaluate effects on the immune status in weaned piglets. The immunoglobins play an important role in immunity, which could inhibit the bacterial adherence and preserve the host from infections (Smith *et al.*, 1997; Li *et al.*, 2007). Results indicated that 3000 mg kg⁻¹ zinc from zinc oxide and 1500 mg kg⁻¹ zinc from capsulated zinc oxide could significantly increase the serum concentrations of immunoglobins (IgA, IgG and IgM). These results indicated that high dose of zinc oxide and lower level of capsulated zinc oxide could enhance the immune status in weaned piglets. The reason for increased immunoglobulin levels may be that zinc oxide could participate in the action of many enzymes or influence the cytokine responses (Hahn and Baker, 1993; Kuscher *et al.*, 1997). However, the possible mechanisms for the increased immunoglobins are still needed further investigation.

Activities of GOT, GPT, ALP and levels of Ca, P in serum were analyzed to evaluate the potential zinc oxide toxicity and assess the mineral status. Results of the present study showed that 3000 mg kg⁻¹ zinc from zinc oxide and 1500 mg kg⁻¹ zinc from capsulated zinc oxide did not affect these serum components. Serum levels of UN and TP were an indicator of metabolism of protein (Fukawa *et al.*, 1982). In this study, serum concentrations of UN and TP were also not significantly influenced, which indicated that dietary supplementation with 3000 mg kg⁻¹ zinc from zinc oxide and 1500 mg kg⁻¹ zinc from capsulated zinc oxide were not involved in the protein synthesis in piglets, which was different from the reports by Rubio *et al.* (2010). The disparity between findings in present study and those of Rubio *et al.* (2010) might be explained by the different duration of the feeding experiments.

In the present study, dietary supplementation with 3000 mg kg⁻¹ zinc from zinc oxide and 1500 mg kg⁻¹ zinc from capsulated zinc oxide increased the serum zinc concentration, which was in line with the results of Wang *et al.* (2009). Increased serum zinc suggested that dietary supplementation with zinc oxide and capsulated zinc oxide could promote the zinc absorption. The absorbed zinc might activate many enzymes, which could partly explain the increased serum immunoglobulin concentrations (Hahn and Baker, 1993). Moreover, the fecal zinc in capsulated zinc oxide group significantly lower than that of zinc oxide group, which was partly consistent with the reports by Kim *et al.* (2010) suggested that the capsulated zinc oxide could deliver the zinc to the ideal location and decrease the zinc concentration in feces, which was beneficial to the environment. The intestinal tract is an important physical and immunological barrier which protects the animals and human from harmful materials, such as pathogens. However, weaning piglets undergoing numerous stresses (for example the transition from the liquid to the solid diet) exhibit many marked changes in small intestine, such as reduced villous height and high intestinal permeability (Pluske *et al.*, 1997; Osek, 1999; Bruewer *et al.*, 2003). It has been documented that supplementation with high levels of zinc oxide could promote intestinal wound healing in porcine and increase the villous height (Li *et al.*, 2001, 2006).

It was speculated that the beneficial effects of high dose of zinc oxide was associated with the IGF-I, which could increase the villous height and inhibit the apoptosis of intestinal cells (Alexander and Carey, 1999; Mylonas *et al.*, 2000). Carlson *et al.* (1999) reported that supplementation with 2500 mg kg⁻¹ zinc oxide could significantly increase the serum concentration of IGF-I. Li *et al.* (2006) suggested that additional 3000 mg kg⁻¹ zinc oxide enhanced the mRNA level for IGF-I in the small intestine and increased the villous height of weaned piglets. Consistent with those results, the present study showed that high-level zinc oxide (3000 mg kg⁻¹) and lower level capsulated zinc oxide (1500 mg kg⁻¹) increased the serum concentration of IGF-I, enhanced the IGF-I expression in both liver and jejunum, tended to increase villous height. In addition, lower

level encapsulated zinc oxide also significantly improved the villous height: crypt depth ratio. Since IGF-I, as one of insulin-like growth factors, could regulate growth, differentiation and survival in a multitude of cells and tissues, it was speculated that beneficial effects of high dose of zinc oxide were, at least partly, related to the increased IGF-I expression (Yu *et al.*, 2005).

In the present study, the mRNA levels for ZO-1 and occludin in jejunum were determined to evaluate effects of zinc oxide on the intestinal permeability. The intestinal permeability was an indicator of intestinal barrier, which was regulated by the tight junction (Kucharzik *et al.*, 2001). ZO-1 is an important linker protein like a bridge between the membrane and cytoskeleton proteins (Furuse *et al.*, 1996). Occludin is an integral membrane protein maintaining the integrity of the tight junction (Fanning *et al.*, 1998). Zhang and Guo (2009) reported that high dietary levels of zinc oxide increased the expression of ZO-1 and occludin and reduced the intestinal permeability in small intestine of weaned piglets. In addition, Finamore *et al.* (2008) documented that zinc deficiency induced membrane barrier damage by decreasing the expression of ZO-1 and occludin in Caco-2 cells. In line with those reports, results of the present study indicated that high level of zinc oxide and lower level of encapsulated zinc oxide both increased the mRNA level for ZO-1 and tended to increase the mRNA level for occludin. These results suggested that the intestinal permeability were decreased by high level zinc oxide (3000 mg kg⁻¹) and lower level encapsulated zinc oxide (1500 mg kg⁻¹), which could partly explain the decreased diarrhea ratio in weaned piglets fed with diet supplemented with zinc oxide and encapsulated zinc oxide.

CONCLUSION

Dietary supplementation with high-level zinc oxide (3000 mg kg⁻¹) and lower level of encapsulated zinc oxide (1500 mg kg⁻¹) tended to increase the growth performance, significantly decreased the diarrhea ratio, enhanced the immune status and decreased the intestinal permeability. These beneficial effects may be, at least partly, associated to the improved IGF-I expression, which resulted in the increased mRNA levels for ZO-1 and protected the intestinal barrier from damage. There was no difference between zinc oxide group and encapsulated zinc oxide group, except for the fecal zinc concentration. Fecal zinc concentration in encapsulated zinc oxide group was significantly lower than that of the zinc oxide group. These results suggested that lower level of encapsulated zinc oxide could take place of high level of zinc oxide in weaned piglets to alleviate the environmental pollution. However, the optimal level of encapsulated zinc oxide for weaned piglets and possible mechanisms for the apparent effects are still required more investigations.

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