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Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs

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ABSTRACT

Effects of montmorillonite–zinc oxide hybrid (MMT–ZnO) on performance, diarrhea, intestinal permeability and morphology were investigated. A total of 180 piglets (Duroc × Landrace × Yorkshire, average initial weight of 7.2 ± 0.3 kg weaned at 27 ± 1 d age) were randomly allotted to five groups for two weeks, each of which has six pens with six pigs per pen. The dietary treatments were: (1) basal control diet, 100 mg/kg of supplemental Zn as $ZnSO_4$; (2) basal diet + 2.0 g/kg montmorillonite (MMT), equivalent to the MMT in the MMT–ZnO treatment; (3) basal diet + 500 mg/kg of Zn as ZnO; (4) basal diet + 500 mg/kg of Zn as MMT–ZnO; (5) basal diet + 2000 mg/kg of Zn as ZnO. The results showed that supplemental 500 mg/kg of Zn from MMT–ZnO or 2000 mg/kg of Zn from ZnO improved (P<0.05) average daily gain and daily feed intake, decreased (P<0.05) fecal scores at 7 and 14 d postweaning, reduced (P<0.05) plasma D-lactate and diamine oxidase activity, improved (P<0.05) villus height and the villus height:crypt depth ratio at the jejunal mucosa as compared with the control, MMT or 500 mg/kg of Zn from ZnO. Pigs fed with 500 mg/kg of Zn as MMT–ZnO had lower (P<0.05) plasma levels of D-lactate than those fed with 2000 mg/kg of Zn as ZnO. Pigs fed with 500 mg/kg of Zn as MMT–ZnO had lower (P<0.05) number of intestinal *Clostridium* and *Escherichia coli* than those fed with the control, MMT or 500 mg/kg of Zn as ZnO. Supplementation with 2000 mg/kg of Zn as ZnO reduced (P<0.05) the number of *Clostridium* in proximal colon as compared with the control while had no (P>0.05) influence on intestinal *E. coli*. Supplemental MMT or 500 mg/kg of Zn from ZnO had no (P>0.05) effect on growth performance, intestinal microflora, permeability and morphology as compared to the control group. The results indicated that dietary addition of 500 mg/kg of Zn from MMT–ZnO was comparable to 2000 mg/kg of Zn from ZnO while more effective than MMT or 500 mg/kg of Zn from ZnO for enhancing growth performance, alleviating diarrhea, as well as improving intestinal microflora, mucosal barrier integrity and morphology of weaned pigs.

1. Introduction

The weaning period is one of the most stressful phases and weaning process induces intestinal barrier dysfunction, digestive disorders and impaired performance (Smith et al., 2010; Peace et al., 2011; Kim et al., 2012). Supplementation

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; DAO, diamine oxidase; MMT, montmorillonite; MMT–ZnO, montmorillonite–zinc oxide hybrid; SEM, standard error of the mean.

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weaned pig diets with pharmacological levels of Zn (2000–4000 mg/kg of Zn as ZnO) alleviates postweaning diarrhea and increases growth performance (Hahn and Baker, 1993; Poulsen, 1995; Carlson et al., 1999). It also was reported that ZnO was the only inorganic form of Zn that produced these benefits (Hahn and Baker, 1993; Schell and Kornegay, 1996). Feeding high levels of supplemental Zn from ZnO results in large quantities of Zn excreted and poses an environmental problem (Poulsen and Larsen, 1995; Carlson et al., 2004). The reduction of Zn dietary supplies is one of the means to limit this environmental risk.

Montmorillonite (MMT) clay is composed of silica tetrahedral sheets layered between an alumina octahedral sheets. It has specific physical-chemical properties such as high surface area, strong adsorptive capacity, high cation exchange capacity, standout adhesive ability, and drug-carrying capability. These inherent advantages make MMT suitable as a carrier and for release of active ingredients in controlled drug delivery systems (Kollár et al., 2003; Zheng et al., 2007; Joshi et al., 2009a,b; Liu et al., 2011). In recent years, MMT intercalated by drug molecules has attracted great interest. Drug–MMT interactions and applications of MMT to carry out specific functions such as delaying and/or targeting drug release, improving drug dissolution, increasing drug stability and modifying drug delivery patterns were studied (Zheng et al., 2007; Joshi et al., 2009a,b; Liu et al., 2011).

Montmorillonite–zinc oxide hybrid (MMT–ZnO) has recently been synthesized by a sol–gel intercalation reaction. It was found that MMT–ZnO had novel physicochemical properties (Fatimah et al., 2011; Khaorapapong et al., 2011). In order to minimize the amount of Zn excreted into the environment, it is promising to add dietary ZnO at much lower concentration and produce similar benefits from pharmacological levels of ZnO. Therefore, an experiment was conducted to investigate whether supplementation weaned pig diets with 500 mg/kg of Zn from MMT–ZnO could alleviate diarrhea and maintain growth performance comparable to pharmacological levels of Zn (2000 mg/kg of Zn from ZnO). In this study, as compared with the basal control diet, MMT, and 500 mg/kg or 2000 mg/kg of Zn from ZnO, the effects of MMT–ZnO on growth performance, postweaning diarrhea, intestinal permeability and morphology of weaned pigs were investigated.

2. Materials and methods

2.1. Materials

Montmorillonite used in the present work was from the Inner Mongolia Autonomous Region, China. The raw material was refined according to the method of Hu et al. (2008). The content of the purified MMT was 990 g/kg and the formula was $([Na_{0.158}K_{0.082}Ca_{0.256}Mg_{0.063}]\ [Mg_{0.376}Fe^{2+}_{0.014}Fe^{3+}_{0.136}Al_{1.474}]\ [Si_{3.87}Al_{0.13}]O_{10}(OH)_2{\cdot}nH_2O)$. The cation exchange capacity (CEC) analyzed according to the method by Xia et al. (2005) was 135 mmol/100 g.

Montmorillonite–zinc oxide hybrid was composited using a sol–gel intercalation method (Khaorapapong et al., 2011). The aqueous solution of zinc chloride and sodium hydroxide was mixed at the molar ratio of Zn^{2+}:OH^- of 1:15 and vigorously stirred at 70 °C for 24 h. Then the sol solution of zinc oxide reactants was added into the colloidal suspension of MMT with continuous stirring at 70 °C and reacted for 24 h. The MMT–ZnO were separated by centrifugation at a speed of 10,000 × *g* for about 15 min, and dried at 50 °C for 3 d. The Zn concentration in MMT–ZnO was determined to be 250 g/kg by atomic absorption spectral analysis.

2.2. Experimental design and samples collection

All procedures were approved by the University of Zhejiang Institutional Animal Care and Use Committee. A total of 180 piglets (Duroc × Landrace × Yorkshire, average initial weight of 7.2 ± 0.3 kg weaned at 27 ± 1 d age) were randomly allotted to five groups for two weeks, each of which has six pens with six pigs per pen. The dietary treatments were: (1) basal control diet, 100 mg/kg of supplemental Zn as $ZnSO_4$; (2) basal diet + 2.0 g/kg montmorillonite (MMT), equivalent to the MMT in the MMT–ZnO treatment; (3) basal diet + 500 mg/kg of Zn as ZnO; (4) basal diet + 500 mg/kg of Zn as MMT–ZnO; (5) basal diet + 2000 mg/kg of Zn as ZnO. The additives were included in the diet on the expense of maize. Diets were formulated according to the NRC (1998) (Table 1). The crude protein, lysine, methionine, calcium, phosphorus, and zinc content of the basal diet were determined by methods of AOAC (2000). No antibiotic was added to all diets. All pigs were given *ad libitum* access to feed and water. Average daily gain (ADG), average daily feed intake (ADFI), and gain/feed ratio were calculated. Fecal scores on day 7 and 14 postweaning were visually assessed using a subjective score on a five-point scale ranging from 1 to 5 according to the method of Hu et al. (2012): 1 = hard feces, 2 = firm well formed, 3 = soft and partially formed feces, 4 = loose, semi-liquid feces, and 5 = watery feces.

After the feeding trial, twelve pigs from each treatment (two pigs per pen) were slaughtered. Blood samples were collected from the anterior vena cava into tubes containing sodium heparin and mixed immediately to avoid coagulation. Plasma was obtained after centrifugation at 3000 × *g* for 15 min at 4 °C and then stored at −80 °C until analysis. Samples of the contents from the small intestine (from the distal end of the duodenum to the ileo-caecal junction) and proximal colon were collected for enumeration of *Clostridium* and *Escherichia coli*. The specimens from the middle part of jejunum were excised, flushed with physiological saline and fixed in 10% formalin.

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

Ingredients (g/kg)	
Maize	572.5
Soybean meal, crude protein 470 g/kg	257
Fish meal, crude protein 628 g/kg	50
Dried whey, crude protein 120 g/kg	45
Spray-dried plasma protein, crude protein 750 g/kg	25
Soybean oil	20
Limestone meal	5
Dicalcium phosphate	11
Sodium chloride	3
L-Lysine HCl, 780 g/kg	1
DL-Methionine, 990 g/kg	0.5
Vitamin-mineral premix[a]	10
Analyzed composition (g/kg)	
Digestible energy[b] (MJ/kg)	14.41
Moisture	85.2
Crude protein	230.1
Lysine	14.2
Methionine	3.8
Calcium	8.7
Total phosphorus	6.9
Zn (mg/kg)	127.9

[a] Provided per kilogram of diet: vitamin A, 6000 IU; vitamin D_3, 600 IU; vitamin E, 50 IU; vitamin K_3, 1.5 mg; thiamine, 2.0 mg; riboflavin, 8.0 mg; pantothenic acid, 20 mg; niacin, 30 mg; pyridoxine, 3.0 mg; choline, 800 mg; folic acid, 0.6 mg; biotin, 0.10 mg; vitamin B_{12}, 0.04 mg; Zn, 100 mg ($ZnSO_4$); Cu, 16 mg ($CuSO_4 \cdot 5H_2O$); Fe, 125 mg ($FeSO_4$); Mn, 15 mg ($MnSO_4 \cdot H_2O$); Se, 0.3 mg (Na_2SeO_3); I, 0.2 mg (KI).
[b] Digestible energy was calculated from data provide by Feed Database in China (2011).

2.3. Sample analysis

The viable counts of *Clostridium* and *E. coli* in the small intestine and proximal colon of weanling pigs were analyzed by the method of Xia et al. (2005). Bacteria were enumerated on Sulphite-Polymyxin Milk Agar (Mevissen-Verhage et al., 1987; *Clostridium*), and MacConkey's No.2 (Oxoid; *E. coli*). Single colonies were removed from selective media plates and grown in peptone yeast glucose broth. Subsequently, the bacteria were characterized to genus level on the basis of colonial appearance, Gram reaction, spore production, cell morphology and fermentation end-product formation.

Plasma levels of diamine oxidase (DAO; EC 1.4.3.6) and D-lactate used as indices of intestinal mucosal injury were measured using an enzymatic spectrophotometric assay. The levels of D-lactic acid in plasma were determined by porcine D-lactic acid ELISA kit (R&D Systems, Minneapolis, MN, USA). The D-lactate assay was based on the enzymatic oxidation of D-lactate with a specific D-lactic dehydrogenase coupled to reduction of NAD^+ with the spectrophotometric measurement of NADH at 340 nm (Brandt et al., 1980). Plasma DAO activities were determined as described by Zhao et al. (2011). Cadaverine dihydrochloride, o-dianisidine dihydrochloride, peroxidase from horseradish and DAO standard were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Three cross-sections for each intestinal sample were prepared after staining with hematoxylin and eosin using standard paraffin embedding procedures (Hu et al., 2007). A total of six intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height and crypt depth were determined using image processing and analysis system (Version 1, Leica Imaging Systems Ltd, Cambridge, England).

2.4. Statistical analysis

Data except the fecal scores were analyzed one-factorial-analysis of variance (ANOVA) using the general linear model procedure (SAS, 1989). The fecal scores on day 7 and 14 postweaning were analyzed according to a two factorial arrangement: main factors, time and interaction. A pen of pigs served as the experimental unit for all data. Differences among means were tested using Duncan's multiple range tests. Effects were considered significant at $P<0.05$.

3. Results

3.1. Growth performance

The growth performance of weaning pigs is presented in Table 2. As compared with the control, supplemental 500 mg/kg of Zn from MMT–ZnO improved ($P<0.05$) ADG by 16.3% and ADFI by 11.5%. Supplemental 2000 mg/kg of Zn from ZnO improved ($P<0.05$) ADG by 15.1% and ADFI by 12.3% compared with the control. Feeding 500 mg/kg of Zn from MMT–ZnO was as efficacious in improving ADG and ADFI than 2000 mg/kg of Zn from ZnO. Pigs fed with 500 mg/kg of Zn from MMT–ZnO had higher ($P<0.05$) ADG and ADFI than those fed with MMT or 500 mg/kg of Zn from ZnO. Supplementation with MMT or

Table 2
Effects of montmorillonite–zinc oxide hybrid (MMT–ZnO) on average daily gain (ADG), average daily feed intake (ADFI) and gain:feed of piglets as compared with the control, montmorillonite (MMT) and ZnO.[a]

Treatment	Initial BW (kg)	Final BW (kg)	ADG (g)	ADFI (g)	Gain:feed (g/kg)
Control	7.25	11.62	312[y]	395[y]	790
MMT	7.31	11.87	326[y]	405[y]	805
500 mg/kg of Zn as ZnO	7.09	11.61	323[y]	409[y]	790
500 mg/kg of Zn as MMT–ZnO	7.15	12.23	363[x]	445[x]	816
2000 mg/kg of Zn as ZnO	7.22	12.25	359[x]	449[x]	800
Standard error of the mean	0.105	0.280	8.3	11.2	16.1
P-values	0.571	0.313	0.0003	0.005	0.763

[xy]Means within a column with different letters differ significantly (P<0.05).
[a] Data are means of six replicate pens of six pigs each.

Table 3
Effects of montmorillonite–zinc oxide hybrid (MMT–ZnO) on postweaning scour scores of weanling pigs as compared with the control, montmorillonite (MMT) and ZnO.[a]

Treatment	Fecal scores on day 7 postweaning	Fecal scores on day 14 postweaning
Control	3.83[x]	2.45[x]
MMT	3.57[y]	2.32[x]
500 mg/kg of Zn as ZnO	3.72[xy]	2.46[x]
500 mg/kg of Zn as MMT–ZnO	1.63[z]	1.31[y]
2000 mg/kg of Zn as ZnO	1.67[z]	1.24[y]
Standard error of the mean	0.080	0.059
P-values (treatment)	0.0001	
P-values (time)	0.0001	
P-values (interaction)	0.0001	

[xyz]Means within a column with different letters differ significantly (P<0.05).
[a] Data are means of six replicate pens of six pigs each.

500 mg/kg of Zn from ZnO had no (P>0.05) effect on growth performance. The gain:feed ratio was not affected by the dietary treatments.

3.2. Postweaning scour scores

The postweaning scour scores of weaning pigs are presented in Table 3. Pigs fed 500 mg/kg of Zn from MMT–ZnO or 2000 mg/kg of Zn from ZnO had lower (P<0.05) fecal scores at 7 and 14 d postweaning compared with pigs fed the control diet, MMT or 500 mg/kg of Zn from ZnO. There were no differences in fecal scores between the MMT–ZnO group and 2000 mg Zn/kg ZnO group (P>0.05). Supplemental MMT reduced fecal scores at 7 d postweaning compared with the control. Supplemental 500 mg/kg of Zn from ZnO had no (P>0.05) effect on postweaning scour scores.

3.3. Intestinal microflora

Intestinal microflora of weaning pigs is presented in Table 4. Supplementing diets with 500 mg/kg of Zn from MMT–ZnO reduced (P<0.05) the total viable counts of *Clostridium* and *E. coli* in the small intestine and proximal colon of weaned pigs as compared with the control. Supplementation with 2000 mg/kg of Zn as ZnO reduced (P<0.05) the viable counts of *Clostridium* in proximal colon as compared with the control while had no (P>0.05) effect on intestinal *E. coli*. Supplementation with MMT or 500 mg/kg of Zn as ZnO had no (P>0.05) effect on intestinal *Clostridium* and *E. coli*. Piglets fed with 500 mg/kg of Zn as

Table 4
Effects of montmorillonite–zinc oxide hybrid (MMT–ZnO) on intestinal microflora of weanling pigs as compared with the control, montmorillonite (MMT) and ZnO.[a]

Treatment	Small intestine		Proximal colon	
	Clostridium	*Escherichia coli*	*Clostridium*	*Escherichia coli*
Control	6.48[x]	7.82[x]	7.95[x]	8.81[x]
MMT	6.11[x]	7.47[x]	7.61[xy]	8.51[x]
500 mg/kg of Zn as ZnO	6.35[x]	7.85[x]	7.92[x]	8.78[x]
500 mg/kg of Zn as MMT–ZnO	5.47[y]	6.71[y]	6.64[z]	7.62[y]
2000 mg/kg of Zn as ZnO	5.97[xy]	7.64[x]	7.05[yz]	8.63[x]
Standard error of the mean	0.205	0.246	0.261	0.276
P-values	0.017	0.019	0.005	0.032

[xy]Means within a row with different letters differ significantly (P<0.05).
[a] Data are the means of six replicates of two pigs per replicate. Bacterial numbers are expressed as $\log_{10}$ colony-forming units per gram of DM.

Table 5
Effects of montmorillonite–zinc oxide hybrid (MMT–ZnO) on plasma D-lactate and diamine oxidase of weanling pigs as compared with the control, montmorillonite (MMT) and ZnO.[a]

Treatment	D-Lactate (mg/L)	Diamine oxidase (U/ml)
Control	9.38^{w}	1.64^{w}
MMT	8.57^{x}	1.53^{w}
500 mg/kg of Zn as ZnO	9.01^{wx}	1.57^{w}
500 mg/kg of Zn as MMT–ZnO	2.91^{z}	0.91^{x}
2000 mg/kg of Zn as ZnO	4.12^{y}	0.85^{x}
Standard error of the mean	0.185	0.050
P-values	0.0001	0.0001

^{wxyz}Means within a column with different letters differ significantly (P<0.05).
[a] Data are means of six replicates of two pigs per replicate.

Table 6
Effects of montmorillonite–zinc oxide hybrid (MMT–ZnO) on jejunal mucosa morphology as compared with the control, montmorillonite (MMT) and ZnO.[a]

Treatment	Villus height (μm)	Crypt depth (μm)	Villus height:crypt depth
Control	695^{y}	453^{x}	1.53
MMT	726^{y}	440^{xy}	1.65
500 mg/kg of Zn as ZnO	703^{y}	452^{x}	1.56
500 mg/kg of Zn as MMT–ZnO	808^{x}	409^{y}	1.98[a]
2000 mg/kg of Zn as ZnO	803^{x}	408^{y}	1.97[a]
Standard error of the mean	24.5	12.9	0.060
P-values	0.004	0.038	0.0001

^{xy}Means within a column with different letters differ significantly (P<0.05).
[a] Data are means of six replicates of two pigs per replicate.

MMT–ZnO had lower (P<0.05) number of intestinal *Clostridium* and *E. coli* than those fed with MMT or 500 mg/kg of Zn as ZnO.

3.4. Intestinal permeability

Intestinal permeability of weaned piglets, as reflected by plasma levels of D-lactate and DAO, are presented in Table 5. Supplementation with 500 mg/kg of Zn from MMT–ZnO or 2000 mg/kg of Zn from ZnO reduced (P<0.05) plasma D-lactate and DAO compared with the control, MMT or 500 mg/kg of Zn from ZnO. Supplementation with MMT or 500 mg/kg of Zn as ZnO had no (P>0.05) effect on plasma D-lactate and DAO. Pigs fed with 500 mg/kg of Zn as MMT–ZnO had lower (P<0.05) plasma levels of D-lactate than those fed with 2000 mg/kg of Zn as ZnO.

3.5. Morphology of jejunal mucosa

Morphological measurements of the jejunal mucosa of pigs are presented in Table 6. Dietary addition of MMT–ZnO (500 mg/kg of Zn) or ZnO (2000 mg/kg of Zn) had higher (P<0.05) villus height and the villus height:crypt depth ratio at the jejunal mucosa as compared with the control or MMT or 500 mg/kg of Zn. Supplementation with MMT or 500 mg/kg of Zn from ZnO had no (P>0.05) effect on morphology of jejunal mucosa.

4. Discussion

4.1. Growth performance and postweaning diarrhea

Digestive disorders, postweaning diarrhea and impaired performance are common problems among weanling piglets (Smith et al., 2010; Peace et al., 2011; Kim et al., 2012). Pharmacological concentrations of ZnO are commonly added to nursery pig diets during the weaning period because of the improved growth response and alleviated diarrhea often elicited (Hahn and Baker, 1993; Poulsen, 1995; Carlson et al., 1999). The present results that feeding 2000 mg/kg of Zn as ZnO to weaned pigs improved growth performance and decreased postweaning scour scores concurred with previous research. The present finding that supplementing 500 mg/kg of Zn as ZnO resulted no growth benefit were consistent with previous investigators, who reported no improvement in growth when supplementing ZnO at levels less than 1000 mg/kg (Davis et al., 2004; Hollis et al., 2005).

Montmorillonite is non-toxic to humans and has good adsorption ability and high cation exchange capacity, which permits the intercalation of drugs (Liu et al., 2011). These inherent advantages make MMT suitable as controlled-release carrier for various drug molecules and nutrient substance, such as timolol maleate (Joshi et al., 2009a), ibuprofen (Zheng et al., 2007), amino acids (Kollár et al., 2003) and vitamins B_6 (Joshi et al., 2009b). In the present study, montmorillonite–zinc oxide hybrid (MMT–ZnO) was synthesized by a sol–gel intercalation between the exfoliated montmorillonite nanosheets and the

sol solution of zinc oxide reactants. Zinc oxide was reported to be intercalated between the interlayer spaces of MMT and also adsorbed on the surface of MMT (Fatimah et al., 2011; Khaorapapong et al., 2011). The present experiment demonstrated that supplementing weaned pig diets with 500 mg/kg of Zn from MMT–ZnO gave equal performance to 2000 mg/kg of Zn from ZnO. The superior performance of MMT–ZnO may be attributed to ZnO–MMT interactions. Montmorillonite served as controlled-release carrier that might modify the rate and/or site of ZnO release, and target ZnO release. Zheng et al. (2007) investigated the intercalation of ibuprofen with MMT and found that the release rate of MMT–ibuprofen in simulated intestinal fluid was noticeably higher than that in simulated gastric fluid.

Animal feed containing 20–30 g/kg MMT has been shown to reduce the detrimental effects of aflatoxin-contaminated diets (Slamova et al., 2011). Montmorillonite has high surface area and standout adhesive ability. These inherent advantages make MMT effectively act by attaching to the mucus to reinforce the intestinal mucosal barrier and help in the regeneration of the epithelium (Albengres et al., 1985). In human medicine MMT has been applied as an antidiarrheal remedy (Ahmed et al., 1993; Chang et al., 2007). In the present study, supplementation with 2.0 g/kg MMT reduced postweaning scour scores at 7 d postweaning. This was consistent with previous research, which reported that the oral treatment of 1 ml 20% dioctahedral smectite suspension three times a day in piglet could control diarrhea in piglets (Jung et al., 2010).

4.2. Intestinal microflora

It has been suggested that the successful prophylactic use of ZnO in preventing diarrhea may be due to its antibacterial activity (Roselli et al., 2003). This was based on observations that ZnO inhibited the growth of bacterial species, such as *Enterococcus faecalis*, *E. coli*, *Staphylococcus aureus*, *Peptostreptococcus micros*, *Streptococcus intermedius* and *Porphyromonas gingivalis* (Podbielski et al., 2000; Sawai, 2003). In piglets, high doses of ZnO have been associated with reduced bacterial translocation from the small intestine to the ileal mesenteric lymph node (Huang et al., 1999), and with increased stability and homogeneity in coliform populations (Katouli et al., 1999). However, Jensen-Waern et al. (1998) reported that dietary supplementation with 2500 mg/kg of Zn as ZnO had no effect on the number of *E. coli* and *Enterococci* per gram of feces although the zinc supplementation increased the growth of the piglets during the first two weeks after weaning. Our results demonstrated that dietary addition of 2000 mg/kg of Zn as ZnO reduced the viable counts of *Clostridium* in proximal colon compared with the control while had no effect on intestinal *E. coli*. This finding was in agreement with the fact that gram-positive bacteria are more susceptible to ZnO than gram-negative bacteria (Sawai, 2003).

Our results showed supplemental 500 mg/kg of Zn from MMT–ZnO reduced number of intestinal *Clostridium* and *E. coli* of weanling pigs as compared with MMT or 500 mg/kg of Zn as ZnO. The adsorption of MMT with ZnO enhanced the antibacterial activity. A similar phenomenon was reported by Xia et al. (2005), who found that copper bearing montmorillonite (Cu-MMT) reduced the number of intestinal *Clostridium* and *E. coli*, while equal amounts of MMT or $CuSO_4$ had no effect on intestinal microflora. They suggested that electrostatic attraction and the antibacterial effect of high density of Cu^{2+} ions on the mineral surface are two ways of the antimicrobial action of Cu-MMT.

4.3. Intestinal permeability

The integrity of the intestinal barrier is fundamental to the proper functioning of the epithelial cells and to preventing the entry of pathogenic bacteria that cause inflammation. Injured intestinal barrier increased the epithelial permeability. Stress associated with early weaning in pigs leads to impaired mucosal barrier function and increased intestinal permeability (Smith et al., 2010; Peace et al., 2011; Kim et al., 2012). Plasma DAO and D-lactate has been proposed as a quantitative and sensitive circulating marker for monitoring the extent of intestinal mucosal injury (Brandt et al., 1980; Zhao et al., 2011). Diamine oxidase is found exclusively in the villi of the upper small intestine. When intestinal mucosal integrity is damaged, DAO is released into blood, indicating that DAO in blood reflects the destruction of the intestinal mucosal epithelial cell layer and intestinal mucosa barricade (Wolvekamp and de Bruin, 1994). Plasma D-lactate is the end product of intestinal bacteria such that almost all D-lactate appearing in the circulation is derived from the intestinal tract. Mammals do not have the enzyme systems to metabolize D-lactate rapidly (Brandt et al., 1980). An imbalance in intestinal flora may result in proliferation of local pathogens and excessive production of D-lactate which, in turn, is released into the blood stream through the damaged mucosa (Zhao et al., 2011). The present study showed that dietary addition of 500 mg/kg of Zn from MMT–ZnO reduced plasma D-lactate and diamine oxidase (DAO), indicating protecting the barrier function of the intestinal mucosal. It was reported that zinc played a role in maintaining epithelial barrier integrity and function. Zinc deficiency altered the barrier function of porcine endothelial cells, whereas treatment of these cells with zinc prevents TNF-induced disruption of the cell monolayer (Hennig et al., 1993). Zinc supplementation improved mucosal repair and decreased paracellular permeability in experimental colitis (Sturniolo et al., 2002). Roselli et al. (2003) reported that ZnO protected intestinal cells from *E. coli* K_{88} infection by inhibiting the adhesion and internalization of bacteria, preventing the increase of tight junction permeability and modulating cytokine gene expression.

4.4. Small intestinal morphology

Stressors that are present in the digesta can lead relatively quickly to changes in the intestinal mucosa. A shortening of the villus decreases the surface area for nutrient absorption. The crypt is the area where stem cells divide to permit the renewal

of the villus, and a large crypt indicates fast tissue turnover and a high demand for new tissue. The short villus height and long crypt depth observed in the control treatment was in agreement with established literature, which suggested a drastic deterioration of intestinal morphology following weaning and consumption of dry diets (Kim et al., 2012). In the present study, supplementation with 500 mg/kg of Zn from MMT–ZnO or 2000 mg/kg of Zn from ZnO had higher villus height and the villus height:crypt depth ratio at the jejunal mucosa as compared with the control or MMT or 500 mg/kg of Zn.

5. Conclusion

The results showed that supplementing weaned pigs diets with 500 mg/kg of Zn from MMT–ZnO was as efficacious as 2000 mg/kg of Zn from ZnO in promoting growth performance, alleviating diarrhea, improving intestinal microflora and barrier function. Pigs fed with 500 mg/kg of Zn from MMT–ZnO had higher performance and intestinal barrier function than those fed with MMT or 500 mg/kg of Zn from ZnO.

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