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Effects of zinc oxide supported on zeolite on growth performance, intestinal microflora and permeability, and cytokines expression of weaned pigs

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ABSTRACT

Effects of zinc oxide supported on zeolite (Z-ZnO) on growth performance, intestinal microflora and permeability, and cytokines expression of weaned pigs were investigated. A total of 210 piglets, with an average weight of 6.12 ± 0.22 kg weaned at 21 ± 1 d age, were randomly allotted to five groups for two weeks. The five treatments were the control (basal diet), and the basal diet supplemented with 300, 600 or 900 mg Zn/kg from Z-ZnO or 2250 mg Zn/kg from ZnO. The results showed that incremental levels of Z-ZnO increased average daily gain (linear P=0.001; quadratic P=0.004), daily feed intake (linear P=0.006; quadratic P=0.019) and jejunal transepithelial electrical resistance (linear P=0.007; quadratic P=0.021), and decreased the postweaning scour scores (linear P<0.001; quadratic P<0.001), mucosal-to-serosal flux of fluorescein isothiocyanate dextran 4 kDa (linear P<0.001; quadratic P<0.001), the viable counts of *Clostridium* and *Escherichia coli* in small intestinal contents (linear P<0.001; quadratic P<0.001). At 7 days after weaning, on d 7 postweaning, as Z-ZnO inclusion increased, the mRNA levels of TNF-α and IFN-γ in jejunal mucosa were decreased linearly (P<0.001 and P=0.001) and quadratically (P<0.001 and P=0.001), and those of TGF-β1 and IL-10 were increased linearly (P=0.002 and P=0.010) and quadratically (P=0.009 and P=0.028). Supplementation with 600 or 900 mg Zn/kg from Z-ZnO was as efficacious as 2250 mg Zn/kg from ZnO in enhancing growth performance, alleviating postweaning diarrhea, improving intestinal microflora and barrier function of weaned pigs. The results indicated that Z-ZnO could be used as a substitute for pharmacological addition of ZnO in weanling pigs.

1. Introduction

Zinc has been reported to have many biological functions, such as anti-inflammation, anti-diarrhea and maintaining epithelial barrier integrity (Roselli et al., 2003; Patel et al., 2010). In particular, zinc oxide (ZnO) appears to have a strong protective effect in resisting intestinal diseases (Roselli et al., 2003; Patel et al., 2010). The amounts of ZnO used in these studies greatly exceeded physiological requirements (Roselli et al., 2003). Feeding pharmacological level of Zn (2000–4000 mg/kg of Zn as ZnO) to weaned pigs is widely used in the pig industry worldwide due to its proven effects on alleviating post-weaning

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; FBW, final BW; FD4, fluorescein isothiocyanate dextran 4 kDa; IBW, initial BW; SEM, standard error of the mean; TER, transepithelial electrical resistance; ZnO, zinc oxide; Z-ZnO, zinc oxide supported on zeolite.

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Table 1
Composition of the basal diet (as fed basis).

Ingredients (g/kg)	
Maize	564
Soybean meal, crude protein 466 g/kg	295
Fish meal, crude protein 615 g/kg	50
Dried whey, crude protein 124 g/kg	45
Soybean oil	15
Dicalcium phosphate	11.5
Limestone	5
Sodium chloride	3
L-Lysine HCl, 775 g/kg	1
DL-Methionine, 992 g/kg	0.5
Vitamin-mineral premix[a]	10
Composition (analyzed except for digestible energy)	
Digestible energy[b] (MJ/kg)	14.34
Crude protein (g/kg)	225.1
Lysine	14.0
Methionine	3.9
Calcium	8.9
Total phosphorus	7.2
Zn (mg/kg)	126.2

[a] Supplied per kilogram of diet: Vitamin A, 5000 IU; Vitamin D_3, 400 IU; Vitamin E, 30 IU; riboflavin, 5.0 mg; Vitamin B_{12}, 0.03 mg; pyridoxine, 3.0 mg; Vitamin k_3, 1.0 mg; biotin, 0.10 mg; thiamine, 2.0 mg; niacin, 30 mg; pantothenic acid, 20 mg; folic acid, 0.6 mg; choline, 800 mg; Zn ($ZnSO_4$), 100 mg; Fe ($FeSO_4$), 125 mg; Cu ($CuSO_4 \cdot 5H_2O$), 16 mg; Mn ($MnSO_4 \cdot H_2O$), 15 mg; I (KI), 0.2 mg; Se (Na_2SeO_3), 0.3 mg.
[b] Digestible energy was calculated from data provide by Feed Database in China (2011).

diarrhea and improving performance (Hahn and Baker, 1993; Ou et al., 2007; Zhang and Guo, 2009). However, the strategy is criticized because high levels of zinc are excreted into the environment and posed an environmental problem (Poulsen and Larsen, 1995; Carlson et al., 2004).

Zeolite, a typical porous mineral, has special advantages such as high surface area, high ion-exchange and adsorption capacities (Aguzzi et al., 2007). In recent years, it has been reported that zeolite could be used as a controlled-release carrier for bioactive molecule, drug and nutrients (Wheatley et al., 2006; Monte et al., 2009; Rahimi et al., 2012). Zinc oxide supported on zeolite (Z-ZnO) with novel physicochemical properties has recently been synthesized. Although there are some reports regarding the industrial uses of Z-ZnO (Sanatgar-Delshade et al., 2011; Hrenovic et al., 2012; Khatamian et al., 2012), there is no data on the biological effects of Z-ZnO. Controlling the release of ZnO in the gastrointestinal tract may improve its effectiveness (Kim et al., 2012). We hypothesized that zeolite may have controlled-release characteristic for ZnO, and Z-ZnO may be a potential substitute for pharmacological addition of ZnO in alleviating postweaning diarrhea of piglets. Therefore, the objective of this experiment was to determine whether feeding lower concentrations of Zn from Z-ZnO to weaned pigs would alleviate postweaning diarrhea and improve intestinal barrier function comparable to feeding pharmacological levels of Zn (2250 mg Zn/kg from ZnO). In this study, the effects of Z-ZnO on growth performance, intestinal microflora and cytokines expression of weaned pigs were investigated. The intestinal epithelial permeability was assessed by transepithelial electrical resistance (TER) and paracellular flux of fluorescein isothiocyanate dextran 4 kDa (FD4) by Ussing chamber technique.

2. Materials and methods

2.1. Materials

Zinc oxide supported on zeolite was synthesized using a hydrothermal method (Hrenovic et al., 2012). The raw zeolite was washed with double-distilled water and then dried at 50 °C for 24 h. Zinc acetate dehydrate was dissolved in double-distilled water, and then zeolite was added under stirring at room temperature. After 12 h stirring, aqueous solution of NaOH (5 mol/L) was slowly added dropwise to the solution under stirring until the pH of the solution reached a value of 13. The formed white precipitates were refluxed at 95 °C for 120 min. The precipitate was centrifuged and washed twice with double-distilled water and dried at 50 °C. The Zn concentration in Z-ZnO was found to be 28% on the basis of atomic absorption spectral analysis.

2.2. Experimental design and sample collection

All procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University. A total of 210 piglets (Duroc × Landrace × Yorkshire), with an average weight of 6.12 ± 0.22 kg weaned at 21 ± 1 d age, were allotted to five treatments. Each treatment had six pens of seven piglets. Dietary treatments were as follows: (1) control (100 mg/kg of supplemental Zn as $ZnSO_4$); (2) control + 300 mg Zn/kg as Z-ZnO; (3) control + 600 mg Zn/kg as Z-ZnO; (4) control + 900 mg Zn/kg as Z-ZnO; (5) control + 2250 mg Zn/kg as ZnO. Diets were formulated according to the NRC (1998) (Table 1). Procedures

of the AOAC (2000) were used to determine the concentrations of crude protein (984.13), lysine (994.12), methionine (994.12), calcium (935.13), phosphorus (964.06) and zinc (986.15).

The piglets were given *ad libitum* access to feed and water. The feeding experiment lasted 14 days. Body weight was measured at the start of the experiment (d 1), on 7 and 14 days after weaning (d 7 and d 14 postweaning). Feed intake (FI) was recorded daily. Average daily gain (ADG) and average daily feed intake (ADFI) and feed/gain ratio were calculated from d 1 to d 14 postweaning. Postweaning scour score was visually assessed according to the previous fecal scoring system from 1 to 5 (Hu et al., 2012a): 1 = hard feces, 2 = firm well formed, 3 = soft and partially formed feces, 4 = loose, semi-liquid feces, and 5 = watery feces. Individuals performing the fecal scores were unaware of the experimental treatments.

On d 7 and d 14 postweaning, six piglets from each treatment (one pig per pen) were killed. Segments of midjejunum at 14 d after weaning were harvested immediately and prepared for Ussing chamber studies. Samples of the jejunal contents at 14 d after weaning were collected for enumeration of *Clostridium* and *Escherichia coli*. Mucosal scrapings from the jejunum at 7 d and 14 d after weaning were collected, rapidly frozen in liquid nitrogen and stored at −80 °C until expression analysis of cytokines.

2.3. Intestinal microflora

The viable counts of *Clostridium* and *E. coli* in jejunal contents were analyzed by the method of Hu et al. (2012b). Bacteria were enumerated on Sulphite-Polymyxin Milk Agar (*Clostridium*), and MacConkey (*E. coli*). Single colonies were removed from selective media plates and grown in peptone yeast glucose broth. Subsequently, the bacteria was characterized to genus level on the basis of colonial appearance, gram reaction, spore production, cell morphology and fermentation end-product formation.

2.4. Ussing chamber experiment

Jejunal mucosa was stripped from the seromuscular layer in oxygenated Ringer's solution. Tissues were then mounted in EasyMount Ussing chamber system (model VCC MC6, Physiologic Instruments, San Diego, CA, USA) as described previously (Hamard et al., 2010; Overman et al., 2012). The clamps were connected to Acquire and Analyse software (Physiologic Instruments, San Diego, CA) for automatic data collection. After a 30-min equilibration period on Ussing chambers, TER was recorded at 15-min intervals over a 2-h period. The probe FD_4 (Sigma–Aldrich, St. Louis, MO) was added to the mucosal side at the final concentration of 0.375 mg/ml. The FD4 was allowed to equilibrate for 15 min after which 100 μl samples (in triplicate) were taken from the serosal side of tissues at 30-min intervals over a 2-h period. The concentrations of FD_4 were measured by fluorescence microplate reader (FLx800, Bio-Tek Instruments, Inc.). Mucosal-to-serosal flux of FD_4 (ng/cm^2 per hour) was calculated.

2.5. Cytokines mRNA by real-time PCR

The mRNA levels of cytokines (TNF-α, IFN-γ, TGF-β1 and IL-10) in jejunal mucosa were determined by quantitative real-time PCR (Liu et al., 2008). Total RNA was isolated using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and treated with RNase-free DNase I prior to cDNA synthesis following the manufacturer's guidelines. The gene bank numbers, sequences of forward and reverse primers, and fragment sizes are presented in Table 2. The qRT-PCR was performed on a StepOne Plus real-time PCR system (Applied Biosystems) using a SYBR Green Master mix ((Promega) according to the kit's instructions. Each sample was run in triplicate. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to analyze the relative expression (fold changes), calculated relative to the control group.

Table 2
Gene bank numbers, sequences of forward and reverse primers, and fragment sizes used for real-time PCR.

Target	GeneBank number	Primer sequence	Size (bp)
TNF-α	NM_214022.1	F:5′CATCGCCGTCTCCTACCA3′ R:5′CCCAGATTCAGCAAAGTCCA3′	199
IFN-γ	NM_213948.1	F:5′GAGCCAAATTGTCTCCTTCTAC3′ R:5′CGAAGTCATTCAGTTTCCCAG3′	140
TGF-β1	NM_214015.1	F:5′GGACCTTATCCTGAATGCCTT3′ R:5′TAGGTTACCACTGAGCCACAAT3′	133
IL-10	NM_214041.1	F:5′GAAGGACCAGATGGGCGACTT3′ R:5′CACCTCCTCCACGGCCCTTG3′	256
GAPDH	NM_001206359.1	F:5′ATGGTGAAGGTCGGAGTGAAC3′ R:5′CTCGCTCCTGGAAGATGGT3′	235

Table 3
Effects of Z-ZnO on growth performance and fecal scores from 0 to 14 d after weaning.

Zn source	Control	Z-ZnO[a]			ZnO	SEM[b]	*P*[c]	
Zn, mg/kg	0	300	600	900	2250		linear	quadratic
IBW[d], kg	6.11	6.17	6.09	6.12	6.09	0.094	0.90	0.97
FBW[e], kg	9.62*	9.91	10.03	10.10	10.11	0.152	0.020	0.051
ADG[f], g	251*	267	281	284	287	7.5	0.001	0.004
ADFI[g], g	324*	350	360	369	379	11.2	0.006	0.019
Feed/gain	1.29	1.31	1.28	1.30	1.32	0.03	0.92	0.89
Fecal scores	3.14*	2.71*	1.51	1.35	1.42	0.071	<0.001	<0.001

[a] Z-ZnO = ZnO supported on zeolite, contain 280 g/kg Zn.
[b] Standard error of means (n = 6 pens with 7 piglets per pen from 0 to 7 d and 6 piglets from 7 to 14 d).
[c] Effect of Z-ZnO addition by polynomial contrasts.
[d] IBW = Initial BW.
[e] FBW = Final BW.
[f] ADG = Average daily gain.
[g] ADFI = Average daily feed intake.
* Indicates a significant difference (P<0.05) with respect to the ZnO group.

2.6. Statistical analysis

Statistical analysis was performed with the SAS software package (SAS, 1996). Polynomial contrasts were used to determine the linear and quadratic effects of dietary Z-ZnO inclusion level. Differences of all the other diets against the diet supplemented with 2250 mg Zn/kg as ZnO were analyzed using Dunnett's tests. A probability of P<0.05 was considered for statements of significance.

3. Results

3.1. Growth performance and postweaning scour scores

Incremental Z-ZnO inclusion in the diet increased ADG (linear P=0.001; quadratic P=0.004) and ADFI (linear P=0.006; quadratic P=0.019), and decreased postweaning scour scores (linear P<0.001; quadratic P<0.001) (Table 3). The feed/gain ratio was not affected by dietary treatments (P>0.05). The growth performance and scour scores of pigs fed 600 or 900 mg Zn/kg as Z-ZnO did not differ from those fed 2250 mg Zn/kg as ZnO (P>0.05).

3.2. Intestinal microflora

Intestinal microflora of pigs on d 14 postweaning is presented in Table 4. The viable counts of *Clostridium* and *Escherichia coli* in jejunal contents decreased linearly (P<0.001) and quadratically (P<0.001) with increasing Z-ZnO level. The viable counts of *Escherichia coli* of pigs fed 600 or 900 mg Zn/kg as Z-ZnO were lower (P<0.05) than those fed 2250 mg Zn/kg as ZnO.

3.3. Intestinal paracellular permeability

Intestinal paracellular permeability of weaned piglets, as reflected by TER and mucosal-to-serosal flux of FD4, is presented in Table 5. The TER value showed a linear (P=0.007) and quadratic (P=0.021) increase with incremental Z-ZnO inclusion in the diet, while FD4 flux decreased linearly (P<0.001) and quadratically (P<0.001) with increasing Z-ZnO level. The TER and FD4 flux of pigs fed 600 or 900 mg Zn/kg as Z-ZnO did not differ from those fed 2250 mg Zn/kg as ZnO (P>0.05).

Table 4
Effect of Z-ZnO on intestinal microflora of pigs on d 14 postweaning.[a]

Zn source	Control	Z-ZnO[b]			ZnO	SEM[c]	*P*[d]	
Zn, mg/kg	0	300	600	900	2250		linear	quadratic
Clostridium	7.42*	l	6.13	5.97*	6.65	0.211	<0.001	<0.001
Escherichia coli	8.29	7.83	7.11*	6.98*	7.75	0.191	<0.001	<0.001

[a] Bacterial numbers are expressed as $\log_{10}$ colony-forming units per gram of DM.
[b] Z-ZnO = ZnO supported on zeolite, contain 280 g/kg Zn.
[c] Standard error of means (n = 6).
[d] Effect of Z-ZnO addition by polynomial contrasts.
* Indicates a significant difference (P<0.05) with respect to the ZnO group.

Table 5
Effect of Z-ZnO on jejunal barrier function of pigs on d 14 postweaning.[a]

Zn source	Control	Z-ZnO[a]			ZnO	SEM[b]	P[c]	
Zn, mg/kg	0	300	600	900	2250		linear	quadratic
TER[d], Ω cm^2	49.5*	53.4	56.5	57.1	57.8	2.08	0.007	0.021
FD4 flux[e], μg cm^{-2} h^{-1}	2.38*	2.09*	1.45	1.27	1.33	0.128	< 0.001	<0.001

[a] Z-ZnO = ZnO supported on zeolite, contain 280 g/kg Zn.
[b] Standard error of means (n = 6).
[c] Effect of Z-ZnO addition by polynomial contrasts.
[d] TER = Transepithelial electrical resistance.
[e] FD4 = Fluorescein isothiocyanate dextran 4 kDa.
* indicates a significant difference (P<0.05) with respect to the ZnO group.

Table 6
Effect of Z-ZnO on cytokines mRNA in jejunal mucosa of piglets.[a]

Zn source	Control	Z-ZnO[b]			ZnO	SEM[c]	P[d]	
Zn, mg/kg	0	300	600	900	2250		linear	quadratic
On d 7 postweaning								
TNF-α	1.00*	0.77*	0.29	0.26	0.31	0.110	<0.001	<0.001
IFN-γ	1.00*	0.67	0.32	0.36	0.33	0.120	0.001	0.001
TGF-β1	1.00*	1.65	2.50	2.63	2.58	0.404	0.002	0.009
IL-10	1.00*	1.53	2.35	2.28	2.23	0.394	0.010	0.028
On d 14 postweaning								
TNF-α	1.00	0.91	0.74	0.83	0.92	0.161	0.366	0.575
IFN-γ	1.00	1.05	0.87	0.91	0.82	0.173	0.387	0.562
TGF-β1	1.00	1.35	1.28	1.40	1.34	0.218	0.121	0.114
IL-10	1.00	1.23	1.17	1.31	1.29	0.204	0.152	0.156

[a] The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative expression (fold changes), calculated relative to the control group.
[b] Z-ZnO = ZnO supported on zeolite, contain 280 g/kg Zn.
[c] Standard error of means (n = 6).
[d] Effect of Z-ZnO addition by polynomial contrasts.
* Indicates a significant difference (P<0.05) with respect to the ZnO group.

3.4. Cytokines mRNA

Table 6 shows cytokine mRNA levels in jejunal mucosa of piglets on d 7 and d 14 postweaning. On d 7 postweaning, the mRNA levels of TNF-α and IFN-γ in jejunal mucosa were decreased linearly (P<0.001 and P=0.001) and quadratically (P<0.001 and P=0.001) with increasing Z-ZnO level. The mRNA levels of TGF-β1 and IL-10 were increased linearly (P=0.002 and P=0.010) and quadratically (P=0.009 and P=0.028) with increasing Z-ZnO level. The piglets fed DS-ZnO at 600 or 900 mg Zn/kg did not differ in cytokines mRNA from those fed ZnO at 2250 mg Zn/kg (P>0.05). However, the mRNA levels of cytokines on d 14 postweaning were not affected by dietary treatments.

4. Discussion

Post-weaning diarrhoea is one of the most common causes of mortality in weaned piglets (Zhang and Guo, 2009; Kim et al., 2012). The present results that dietary addition of 2250 mg Zn/kg as ZnO improved growth performance and alleviated postweaning diarrhea were consistent with previous reports (Hahn and Baker, 1993; Ou et al., 2007; Zhang and Guo, 2009).

The present results that feeding 600 mg Zn/kg from Z-ZnO to weaned pigs was as efficacious as 2250 mg Zn/kg from ZnO would be beneficial for the environment. Zeolite has been researched as an effective carrier for controlled-releasing nitric oxide, vitamins and antibiotics, and can target drug release and prolong drug-releasing period (Wheatley et al., 2006; Aguzzi et al., 2007; Monte et al., 2009). It was reported that zeolite enhanced the stability of the fat-soluble vitamins in stomach with acidic pH, and increased the amount of vitamins released in intestinal condition. It was suggested that preventing the chemical change of ZnO in the stomach and increasing ZnO into the intestinal tract could improve its effectiveness (Kim et al., 2012). The effects of Z-ZnO on intestinal health of weaned pigs might due to the controlled-release of zeolite from ZnO in the intestinal tract. However, a better understanding of the release properties of Z-ZnO is needed to fully characterize this combination of products.

Previous work suggests that the protective effect of ZnO is due to its antibacterial activity (Roselli et al., 2003). *In vitro* studies show that ZnO inhibits the growth of *Staphylococcus aureus* and *E. coli* (Sawai, 2003). In human Caco-2 enterocytes, ZnO reduced bacterial adhesion and inhibited enterotoxigenic *E. coli* internalization (Roselli et al., 2003). High doses of ZnO reduced bacterial translocation from the small intestine to the ileal mesenteric lymph node in piglets (Huang et al., 1999). However, Jensen-Waern et al. (1998) reported that supplementation with 2500 mg Zn/kg as ZnO had no effect on the number of *E. coli* and *Enterococci* per g of faeces although ZnO increased the growth of the piglets during the first two weeks after

weaning. The present study showed that supplemental 2250 mg Zn/kg from ZnO decreased the viable count of *Clostridium*, while had no effect on that of *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). The current study showed that supplementation with Z-ZnO decreased the viable counts both of *Clostridium* and *E. coli*. Many reports have demonstrated that the Z-ZnO exhibited much higher antimicrobial abilities than ZnO (Hrenovic et al., 2012).

Stress associated with early weaning in pigs leads to intestinal barrier dysfunction (Moeser et al., 2007; Smith et al., 2010). Transepithelial electrical resistance (TER) is considered to reflect the opening of the tight junctions between epithelial cells and the paracellular permeability of the intestinal mucosa (Wijtten et al., 2011). A decreased TER reflects increased paracellular permeability. The flux of intact FD_4 across the intestinal epithelium occurs mainly through paracellular pathways (Hamard et al., 2010; Overman et al., 2012). An increased flux of FD_4 reflects increased paracellular permeability and impaired intestinal barrier. The present study showed that supplementation with Z-ZnO increased TER and decreased FD4 flux, indicating the intestinal mucosal barrier function was improved. Zhang and Guo (2009) reported that supplemental 2000 mg Zn/kg from ZnO reduced intestinal permeability by enhancing tight junction protein (occludin and ZO-1) expression in weaning piglets.

Weaning-associated intestinal inflammation has been reported in weaned pigs (Moeser et al., 2007). Pié et al. (2004) measured the gene expressions of inflammatory cytokines at different time point postweaning and found the proinflammatory cytokines in the intestine were up-regulated in newly weaned pigs. Overproduction of proinflammatory cytokines has an adverse effect on intestinal mucosal integrity (Liu et al., 2008). The barrier disruptive actions of TNF-α and IFN-γ have been well-established (Al-Sadi et al., 2009). The current study showed that Z-ZnO decreased mRNA levels of TNF-α and IFN-γ on d 7 postweaning, while did not influence cytokine mRNA on d 14 postweaning. The downregulation of proinflammatory cytokine in the presence of Z-ZnO indicated that weaning-induced inflammation was alleviated. Pié et al. (2004) found that weaning induced a transient up-regulation of inflammatory cytokine on d 3–4 postweaning and most cytokines rapidly returned to preweaning values after d 9 postweaning. This might be why the mRNA levels of cytokines on d 14 postweaning were not affected by dietary treatments. The present results that Z-ZnO and ZnO increased mRNA levels of TGF-β1 and IL-10 on d 7 postweaning were consistent with Roselli et al. (2003), who found that ZnO upregulated the mRNA level of TGF-β in enterotoxigenic *E. coli* infected cells. It has been reported that TGF-β and IL-10 are anti-inflammatory cytokines and can protect the intestinal barrier function (Madsen et al., 1997; Howe et al., 2005).

5. Conclusion

Supplementation with 600 or 900 mg Zn/kg from Z-ZnO was as efficacious as 2250 mg Zn/kg from ZnO in increasing growth performance, alleviating postweaning diarrhea, improving intestinal microflora and barrier function of weaned pigs. The results indicated that Z-ZnO could be used as a substitute for pharmacological addition of ZnO in weanling pigs.

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References

Aguzzi, C., Cerezo, P., Viseras, C., Caramella, C., 2007. Use of clays as drug delivery systems: possibilities and limitations. Appl. Clay Sci. 36, 22–36.
Al-Sadi, R., Boivin, M., Ma, T., 2009. Mechanism of cytokine modulation of epithelial tight junction barrier. Front. Biosci. 14, 2765–2778.
AOAC International, 2000. In: Horwitz, W. (Ed.), Official Methods of Analyses, 17th ed. AOAC Int., Gaithersburg, MD.
Carlson, M.S., Boren, C.A., Wu, C., Huntington, C.E., Bollinger, D.W., Veum, T.L., 2004. Evaluation of various inclusion rates of organic zinc either as a polysaccharide or proteinate complex on the growth performance, plasma, and excretion of nursery pigs. J. Anim. Sci. 82, 1359–1366.
Feed Database in China, 2011. Table of Feed Composition and Nutritive Value in China: 22nd ed., China Feed.
Hahn, J.D., Baker, D.H., 1993. Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. J. Anim. Sci. 71, 3020–3024.
Hamard, A., Mazurais, D., Boudry, G., Le Huërou-Luron, I., Sèvea, B., Le Floc'ha, N., 2010. A moderate threonine deficiency affects gene expression profile, paracellular permeability and glucose absorption capacity in the ileum of piglets. J. Nutr. Biochem. 21, 914–921.
Howe, K.L., Reardon, C., Wang, A., Nazli, A., McKay, D.M., 2005. Transforming growth factor-beta regulation of epithelial tight junction proteins enhances barrier function and blocks enterohemorrhagic *Escherichia coli* O157:H7-induced increased permeability. Am. J. Pathol. 167, 1587–1597.
Hrenovic, J., Milenkovic, J., Daneu, N., Kepcija, R.M., Rajic, N., 2012. Antimicrobial activity of metal oxide nanoparticles supported onto natural clinoptilolite. Chemosphere 88, 1103–1107.
Hu, C.H., Gu, L.Y., Luan, Z.S., Song, J., Zhu, K., 2012a. Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim. Feed Sci. Technol. 177, 108–115.
Hu, C.H., Song, J., You, Z.T., Luan, Z.S., Li, W.F., 2012b. Zinc oxide-montmorillonite hybrid influences diarrhea, intestinal mucosal integrity and digestive enzyme activity in weaned pigs. Biol. Trace Elem. Res. 149, 190–196.
Huang, S.X., McFall, M., Cegielski, A.C., Kirkwood, R.N., 1999. Effect of zinc supplementation on *Escherichia coli septicemia* in weaned pigs. Swine Health Prod. 7, 109–111.
Jensen-Waern, M., Melin, L., Lindberg, R., Johannisson, A., Petersson, L., Wallgren, P., 1998. Dietary zinc oxide in weaned pigs-effects on performance, tissue concentrations, morphology, neutrophil functions and faecal microflora. Res. Vet. Sci. 64, 225–231.
Khatamian, M., Divband, B., Jodaei, A., 2012. Degradation of 4-nitrophenol (4-NP) using ZnO nanoparticles supported on zeolites and modeling of experimental results by artificial neural networks. Mater. Chem. Phys. 134, 31–37.
Kim, J.C., Hansen, C.F., Mullana, B.P., Pluske, J.R., 2012. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. Anim. Feed Sci. Technol. 173, 3–16.

Liu, Y.L., Huang, J.J., Hou, Y.Q., Zhu, H.L., Zhao, S.J., Ding, B.Y., Yin, Y.L., Yi, G.F., Shi, J.X., Fan, W., 2008. Dietary arginine supplementation alleviates intestinal mucosal disruption induced by *Escherichia coli* lipopolysaccharide in weaned pigs. Br. J. Nutr. 100, 552–560.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and $2^{-\Delta\Delta Ct}$ method. Methods 25, 402–408.

Madsen, K., Lewis, S.A., Tavernini, M.M., Hibbard, J., Fedorak, R.N., 1997. Interleukin 10 prevents cytokine-induced disruption of T84 monolayer barrier integrity and limits chloride secretion. Gastroenterology 113, 151–159.

Moeser, A.J., Ryan, K.A., Nighot, P.K., Blikslager, A.T., 2007. Gastrointestinal dysfunction induced by early weaning is attenuated by delayed weaning and mast cell blockade in pigs. Am. J. Physiol. Gastrointest. Liver Physiol. 293, G413–G421.

Monte, M.B.M., Middea, A., Paiva, P.R.P., Bernardi, A.C.C., Rezende, N.G.A.M., Baptista, M., Silva, M.G., Vargas, H., Amorim, H.S., de Souza-Barros, F., 2009. Nutrient release by a Brazilian sedimentary zeolite. An. Acad. Bras. Cienc. 81, 641–653.

National Research Council, 1998. Nutrient Requirements of Swine, 10th ed. National Academy Press, Washington, DC.

Ou, D.Y., Li, D.F., Cao, Y.H., Li, X.L., Yin, J.D., Qiao, S.Y., Wu, G.Y., 2007. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J. Nutr. Biochem. 18, 820–826.

Overman, E.L., Rivier, J.E., Moeser, A.J., 2012. CRF induces intestinal epithelial barrier injury via the release of mast cell proteases and TNF-α. PLoS ONE 7, e39935.

Patel, A., Mamtani, M., Dibley, M.J., Badhoniya, N., Kulkarni, H., 2010. Therapeutic value of zinc supplementation in acute and persistent diarrhea: a systematic review. PLoS ONE 5, e10386.

Pié, S., Lallès, J.P., Blazy, F., Laffitte, J., Sève, B., Oswald, I.P., 2004. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. J. Nutr. 134, 641–647.

Poulsen, H.D., Larsen, T., 1995. Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. Livest. Prod. Sci. 43, 235–242.

Rahimi, M., Mobedi, H., Behnamghader, A., Baygi, A.N., Mivehchi, H., Biazar, E., 2012. Fat-soluble vitamins release based on clinoptilolite zeolite as an oral drug delivery system. Lett. Drug Des. Discov. 9, 213–217.

Roselli, M., Finamore, A., Garaguso, I., Britti, M.S., Mengheri, E., 2003. Zinc oxide protects cultured enterocytes from the damage induced by *Escherichia coli*. J. Nutr. 133, 4077–4082.

Sanatgar-Delshade, E., Habibi-Yangjeh, A., Khodadadi-Moghaddam, M., 2011. Hydrothermal low-temperature preparation and characterization of ZnO nanoparticles supported on natural zeolite as a highly efficient photocatalyst. Monatsh. Chem. 142, 119–129.

SAS, 1996. SAS User's Guide (Release 6. 12 ed.). SAS Institute, Inc., Cary, NC, USA.

Sawai, J., 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO MgO and CaO) by conductimetric assay. J. Microbiol. Methods 54, 177–182.

Smith, F., Clark, J.E., Overman, B.L., Tozel, C.C., Huang, J.H., Rivier, J.E., Blisklager, A.T., Moeser, A.J., 2010. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. Am. J. Physiol. Gastrointest. Liver Physiol. 298, G352–G363.

Wheatley, P.S., Butler, A.R., Crane, M.S., Fox, S., Xiao, B., Rossi, A.G., Megson, I., Morris, R.E., 2006. NO-releasing zeolites and their antithrombotic properties. J. Am. Chem. Soc. 12, 502–509.

Wijtten, P.J.A., van der Meulen, J., Verstegen, M.W.A., 2011. Intestinal barrier function and absorption in pigs after weaning: a review. Br. J. Nutr. 105, 967–981.

Zhang, B.K., Guo, Y.M., 2009. Supplemental zinc reduced intestinal permeability by enhancing occluding and zonula occludens protein-1 (ZO-1) expression in weaning piglets. Br. J. Nutr. 102, 687–693.