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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