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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.