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Research paper

Effects of dietary supplementation with palygorskite on intestinal integrity in weaned piglets

Junmin Zhang [a,*], Yunfeng Lv [b], Chaohua Tang [a], Xiuqi Wang [b]

[a] State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[b] Department of Animal Science, South China Agricultural University, Guangzhou 510642, China

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ABSTRACT

The effects of dietary supplementation of palygorskite on intestinal integrity in weaned piglets were investigated in this study. Pigs weaned at 24 days of age (n = 27) were allocated to three groups and fed the same basal diet supplemented with 0 mg/kg (control), 2000 mg/kg, or 3000 mg/kg palygorskite for 42 days. Three pigs from each treatment group were slaughtered for assessment of intestinal integrity on days 21 and 42. The results showed that both the feed/gain and the rate of diarrhea were decreased ($P<0.05$) with supplementation of 2000 mg/kg palygorskite, and there were no significant differences between the two palygorskite-treated groups. Pigs fed palygorskite showed lower ($P < 0.05$) plasma endotoxin and diamine oxidase concentrations on day 21. Compared with control, the villus diameters of duodenum (2000 mg/kg group) and ileum (3000 mg/kg group) were increased ($P<0.05$) on day 21, and villus height of the ileum was improved in the groups given palygorskite ($P < 0.05$) on day 42. Lymphocyte numbers in the jejunum were increased ($P < 0.05$) with dietary supplementation of 2000 mg/kg palygorskite on day 42, compared with control. Palygorskite was shown to be beneficial to the intestinal integrity, which resulted in improving growth performance in weaned piglets.

1. Introduction

Palygorskite is a layered magnesium aluminum silicate that is present in nature as a fibrillar silicate clay mineral with reactive —OH groups on the surface (Huang et al., 2007). The unique chain-layered crystal structure of palygorskite confers on it the properties of absorption, cation exchange capacity, and adhesive ability, therefore it is used widely in industry. Theoretical calculations of the internal and external surface areas of palygorskite from Florida and Georgia gave 600 m^2/g and 300 m^2/g, respectively (Serna and Vanscoyoc, 1979). Given that palygorskite has a large specific surface area, it has the potential to adsorb pathogenic bacteria and toxins and to be used as an antidote to protect the intestines of weaned piglets against disease, which is considered to be the main cause of death in postweaned pigs. Palygorskite is used as an antidiarrheal drug (Zaid et al., 1995) and as a toxin binder in pigs and broiler chickens (Bailey et al., 2006). However, information about the effects of palygorskite on intestinal function in weaned piglets is limited. The present study was performed to evaluate the effects of dietary supplementation of palygorskite on growth performance, rate of diarrhea, and intestinal morphology and barrier functions in weaned piglets.

2. Materials and methods

2.1. Animals and palygorskite

The palygorskite used in this study was of 94% purity and provided by our laboratory. The physico-chemical properties and heavy metal content of the palygorskite used are shown in Table 1. Determination of mineral composition was carried out by X-ray diffraction, using a Dmax 12 kw powder diffractometer. The measurement of cation exchange capacity and ethylene blue absorption was performed according to the method described by Yongduo and Gaoxiang (2004). The contents of Pb and Cd were determined by the flame atomic absorption spectrometry method, Hg by the atomic absorption spectrometry method, Cr by the colorimetric method. All the measurements for heavy metals were under the limits of *General Rules of Natural Mineral Feed* (GB/T 22144—2008) in China for animal feed. Twenty-seven castrated male weaned piglets (Large White × Great Yorkshire) with a weaning age of 24 days and an average initial weight of 7.32 kg ± 0.12 kg were housed in metabolism cages.

2.2. Diets and experimental design

All procedures were performed according to the guidelines for animal experiments of the National Institute of Animal Health. The animals were allocated to three treatments (each pig was considered to be a replicate): the control group was fed the basal diet without palygorskite, and two treatment groups were supplemented with 2000 mg/kg and 3000 mg/kg palygorskite, respectively, to replace the

* Corresponding author. Tel.: +86 10 62815852; fax: +86 10 62815537.
E-mail address: zhjmxms@sina.com (J. Zhang).

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Table 1
Physico-chemical properties and heavy metal content of the palygorskite.

CEC (mmol/100 g)	EEBA (mmol/100 g)	pH	As (mg/g)	Pb (mg/g)	Cd (mg/g)	Hg (mg/g)	Cr (mg/g)
24.24	50	8.08	0.67	5.32	0.028	0.01	<1

CEC = cation exchange capacity; EBA = ethylene blue absorption.

same amount of powdered rice hulls in the basal diet. The animals were fed for 42 days, with ad libitum access to feed and drinking water. All experimental diets without antibiotics were formulated to meet the National Research Council (1998) requirements (Table 2).

2.3. Slaughter and sampling

On days 21 and 42, blood samples were collected by jugular venipuncture from three piglets (the closest to the mean body weight in that group) in each treatment group, and subsequently the same piglets were killed to sample their intestinal tissue and digesta. The intestines were divided into three parts (duodenum, jejunum, ileum) according to the method described by Hopwood et al. (2004), washed with PBS, fixed by immersion in formalin (4%) and stored at 4 °C prior to analysis of the morphology. The cecum and colon were ligated and excised from the distal end of the jejunum for collection of their digesta. The digesta were placed immediately into liquid nitrogen before storage at −80 °C. Blood samples were collected in vacutainer tubes with or without anticoagulant (lithium heparin, Greiner Bio-One GmbH), and centrifuged at 3000 ×*g* for 15 min at 4 °C to separate serum and plasma, respectively. Plasma and serum samples were stored at −80 °C before analysis.

Table 2
Composition of the experimental diets on a dry-matter basis.

Ingredient (g/kg)	Control	Palygorskite group 2000 mg/kg	Palygorskite group 3000 mg/kg
Corn	600	600	600
Soybean meal	240	240	240
Expanded soybean	40	40	40
Fish meal	40	40	40
Dried whey	40	40	40
Limestone	10	10	10
Calcium hydrogen phosphate	10	10	10
L-lysine	3	3	3
L-threonine	1	1	1
Sodium chloride	3	3	3
Powdered rice hulls	3	1	0
Palygorskite	0	2	3
Premix[a]	10	10	10
Calculated nutrient composition (g/kg)[b]			
Digestible energy (MJ/kg)	13.81	13.81	13.81
Crude protein	20.13	20.13	20.13
Calcium	0.89	0.89	0.89
Total phosphorus	0.66	0.66	0.66
Available phosphorus	0.47	0.47	0.47
Lysine	1.36	1.36	1.36
Methionine + cystine	0.64	0.64	0.64
Threonine	0.88	0.88	0.88
Tryptophan	0.23	0.23	0.23

[a] The following amounts of vitamin and minerals were provided per kilogram of diet: vitamin A 9360 IU; vitamin D_3 2080 IU; vitamin E 21.3 mg; vitamin K_3 3.0 mg; thiamin 1.2 mg; riboflavin 5 mg; pyridoxine 3.1 mg; vitamin B_{12} 0.02 mg; D-calcium pantothenate 12.5 mg; nicotinamide 21.2 mg; folic acid 1 mg; biotin 0.08 mg; choline chloride 500 mg; Fe 120 mg; Cu 200 mg; Mn 35 mg; Zn 123 mg; I 0.2 mg; Se 0.3 mg.
[b] The nutrient composition was calculated from data provided by the Feed Database in China.

2.4. Growth performance and rate of diarrhea

Experimental piglets underwent a 12-hour fast before being weighed individually on day 0 (at weaning), day 21 and day 42 at 8:00 in the morning, after introduction of the experimental diets. Feed intake was recorded at every feed during the whole experiment. Fecal scores were monitored only during the first 21 days and quantified using a scale ranging from 0 to 3 as described by Marquardt et al. (1999), with 0 = normally shaped feces, 1 = shapeless (loose) feces, 2 = thick, liquid (soft) feces, and 3 = thin, liquid feces and watery diarrhea. A piglet with a score greater than 1 was regarded as having diarrhea.

2.5. Serum and plasma analysis

The detection of endotoxin in plasma was performed using the quantitative chromogenic tachypleus amebocyte lysate method with endotoxin test kits (Chinese Horseshoe Crab Reagent Manufactory, Co., Ltd., China). And the measurement of diamine oxidase in plasma was performed using enzymatic kinetic spectrophotometry method with diamine oxidase test kits (Nanjing Jian Cheng Technology, Co., Ltd., China). The enzymatic kinetic spectrophotometry method with a D-lactate test kit (Genmed Scientifics Inc. USA) was used to analyze the concentrations of D-lactate in serum.

2.6. Intestinal histology

Intestinal tissue samples were stained (hematoxylin and eosin and periodic acid–Schiff stains) following standard paraffin embedding procedures (Uni et al., 1998; Xu et al., 2003) and examined using a microscope with an ocular micrometer. Ten intact crypt villi in each sample were selected to measure the villus height, crypt depth, villus diameter and mucosa depth. In addition, five integral villi in each sample were chosen to determine the number of intestinal epithelium lymphocytes and goblet cells per 100 intestinal epithelium cells. The villus height and crypt depth were calculated as described by Nabuurs et al. (1993), the villus diameter was measured at the widest part of the villus and the mucosa depth was calculated as the vertical dimension from the epithelial mucosa to the muscularis mucosae (these layers were included).

2.7. Intestinal microbiology

The contents of the cecum and colon sampled for microbial analysis were thawed at room temperature, 0.5 g of sample was serially diluted with physiological saline (dilutions 10^{-2} to 10^{-4}), and subsequently 0.1 ml of each diluted sample was inoculated on eosin methylene blue agar (Beijing Land Bridge Technology Co., Ltd., China) and bismuth sulfite agar (Beijing Land Bridge Technology Co., Ltd., China). The number of colonies of *Escherichia coli* and *Salmonella* was counted after 24 h (36 °C) and 48 h (36 °C) of incubation, respectively.

2.8. Statistical analysis

The data in the tables are presented as the arithmetic mean ± standard error (SE). Statistical analysis was performed by one-way analysis of variance (ANOVA) using SPSS for Windows (Version 16, Chicago, USA). Duncan's multiple-range test was used, with differences considered to be significant at $P<0.05$.

3. Results

3.1. Growth performance and rate of diarrhea

As shown in Table 3, the feed/gain in the 2000 mg/kg palygorskite group was lower ($P < 0.05$) than that in the control group, but no