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Research paper

The effect of dietary bentonite on post-weaning diarrhoea, growth performance and blood parameters of weaned piglets

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ABSTRACT

The aim of this study was to investigate the effects of dietary supplementation of weaned piglets with natural bentonite on post-weaning diarrhoea (PWD), intestinal colonization by enterotoxigenic *Escherichia coli* (ETEC) and intestinal histopathology, growth performance, and haematological and serum biochemical parameters. The piglets in our study were obtained from a traditional farm with a high prevalence of PWD. The effects of dietary bentonite were tested on 50 weaned piglets in two trials. Piglets were allocated to two dietary groups, control and bentonite, receiving a basal diet with or without bentonite supplementation at levels of 1% and 2% for 21 days. In vitro testing of antibacterial activity of bentonite against ETEC O149:F4:LT showed that it had no ability to reduce bacterial plate counts. Dietary bentonite has not been effective in reducing the occurrence and duration of diarrhoea or mortality rates, although faecal ETEC shedding decreased ($P = 0.004$) in bentonite fed piglets. Histopathological examinations of the intestines demonstrated that 2% bentonite exerted a protective effect on the small intestinal mucosa, the inflammation of which was milder. Also, a beneficial effect on regenerative processes following intestinal infections was observed. A significantly lower leukocyte count ($P = 0.048$) in peripheral blood indicated that the infection in bentonite-fed piglets was less severe. Exposure of piglets to bentonite did not significantly affect their post-weaning growth performance. The addition of bentonite to diet had no adverse effect on biochemical blood parameters in piglets. The present study shows that the use of natural untreated bentonite as a feed additive for weaned piglets can be considered safe, but its final efficacy in preventing the occurrence and severity of PWD is low.

1. Introduction

Post-weaning diarrhoea (PWD), which is caused by enterotoxigenic *Escherichia coli* (ETEC), is by far the most common disease in weaned pigs and is a major economic problem worldwide. Pathogenic strains colonize the mucosa of the small intestine using specific adhesion factors (fimbriae) and produce two enterotoxins responsible for the clinical manifestations of the infections. The fimbrial adhesins F4 and F18 are commonly found in pathogenic *E. coli* isolated from weaned piglets (Bertschinger, 1999; Frydendahl, 2002). ETEC strains are known to produce heat stable enterotoxins (STa or STb) and/or heat labile enterotoxin (LT) which induce diarrhoea associated with water and electrolyte loss from the small intestine (Bertschinger, 1999; Frydendahl, 2002; Zajacova et al., 2012). Verotoxigenic *E. coli* (VTEC) producing verotoxin (VT2e) can also occur after weaning. Some porcine *E. coli* strains can produce both VT2e and enterotoxins and these isolates may be associated with diarrhoea and/or oedema disease (Nagy et al., 1997).

Dietary supplementation of antibiotic growth promoters used to be the most effective means of keeping the disease under control. In the EU, the ban on the use of antibiotics as growth promoters has caused a higher incidence of PWD leading to impaired growth performance and high mortality of piglets. Therefore, research efforts have been focused on development of alternatives to antibiotics in order to prevent infections on farms and maintain animal health and performance.

One prevention strategy to control PWD is the use of clays and clay minerals in animal diets (Slamova et al., 2011; Trckova et al., 2004; Vondruskova et al., 2010). Clays have been used for medicinal purposes in the treatment of human diarrhoea (Carretero, 2002; Gomes and Silva, 2007). Beneficial effects of clay minerals have been attributed to antibacterial activity (Haydel et al., 2008; Williams and Haydel, 2010; Williams et al., 2008, 2011), adsorption of luminal antigens (Hassen et al., 2003; Ramu et al., 1997; Schell et al., 1993a, 1993b; Shi et al., 2007; Xu et al., 2004; Yu et al., 2008, 2009), increasing amount of mucin production in the intestine and improving gastrointestinal mucus resistance to various aggressors (Albengres et al., 1985; Droylefaix et al., 1985; Gonzalez et al., 2004). However, the efficiency of different clays may vary depending on geochemical composition (Haydel et al., 2008; Tateo et al., 2006; Williams and Haydel, 2010; Williams et al., 2008) and physical features (Hassen et al., 2003).

Bentonite is essentially impure clay consisting mostly of montmorillonite (Mt) with minor amounts of illite, chlorite, biotite, pyrophyllite, cristobalite, albite, sanidine and quartz, among others. Mt and the

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other clay minerals from the smectite group have a vast specific surface area and a high cation exchange capacity. Bentonite, also referred to as Mt, is reported to be one of the most powerful healing clays.

Although only a few studies have focused on the effects of modified forms of Mt, with metal cations (Ag^{+} and Cu^{2+}) on the surface, on intestinal microflora and diarrhoeal infections of weaned piglets (Hu and Xia, 2006; Hu et al., 2004; Xia et al., 2004, 2005; Zhou et al., 2004), no studies to date have investigated the efficacy of natural untreated bentonite.

One of the major concerns arising from the dietary use of clays is their potential interaction with the dietary compounds, such as minerals, due to their high adsorption property and ion-exchange capacity (Alexopoulos et al., 2007; Hooda et al., 2002; Patterson and Staszak, 1977). Although some studies found no evidence of any adverse effect of other clays on haematological and blood chemistry profiles (Schell et al., 1993a, 1993b; Kyriakis et al., 2002; Narkeviciute et al., 2002; Papaioannou et al., 2002; Xu et al., 2004; Alexopoulos et al., 2007; Trckova et al., 2009), it is necessary to be aware of the potential risks of natural bentonite to the health of piglets.

The purpose of the present study was to investigate the effect of feeding natural bentonite as a supplement to piglets on post-weaning diarrhoea, ETEC colonization and histopathology of the intestine, growth performance, haematological and serum biochemical parameters in weaned piglets.

2. Material and methods

2.1. Experimental material

The natural bentonite used in this study was extracted by surface mining (Hnusta, Slovakia). It was composed dominantly of Mt (90%) with small amounts of sanidine, biotite, albite, pyrophyllite and quartz. The clay was calcined at 600 °C, ground and homogenized. It held <20 μm particle diameter (50% of the particles). The specific surface area determined by methylene blue adsorption was 342.4 mg/g. The swelling capacity was 29.0 cc. Chemical composition of the clay is illustrated in Table 1.

2.2. Antibacterial activity of bentonite in vitro

Antibacterial activity of bentonite was tested on ETEC serotype O149:F4, positive for LT enterotoxin (12260). A bacterial suspension was prepared by inoculation of ETEC O149:F4:LT into a nutrient broth (Imuna Pharm, Sarisske Michalany, Slovakia) and was cultured at 37 °C for 16 h to match the turbidity of 3.3 McFarland turbidity standard. Antibacterial activity tests were conducted in triplicate. 200 or 400 mg of bentonite was dispersed in 20 ml of nutrient broth, i.e. at concentrations of 1% and 2%. One tube containing only nutrient broth without any bentonite served as control. To imitate gastric and intestinal conditions in piglets, pH of nutrient broth suspensions was adjusted to 2.5 by adding HCl for 30 min and then to 7.5 by adding NaOH. Suspensions were sterilized by autoclaving at 120 °C for 20 min. 20 μl aliquots of the ETEC O149:F4:LT suspensions were added to the tubes with 20 ml of nutrient broth suspensions containing bentonite. All tubes were agitated in an orbital shaker at 150 rpm at 37 °C. After 4 and 8 h of incubation, all samples were diluted in phosphate buffered saline (PBS) within the logarithmic range. Then, 100 μl of each dilution and 10 ml of agar were added to Petri dishes and incubated at 37 °C for 24 h. The numbers of bacterial colonies that grew on each plate were counted.

Table 1
Chemical composition of bentonite.

Chemical compound	%
SiO_2	59.50
Al_2O_3	18.80
MgO	5.66
Na_2O	2.70
Fe_2O_3	2.46
CaO	1.91
K_2O	0.86
TiO_2	0.15
MnO	0.15
SO_3	0.09
BaO	0.07
P_2O_5	0.05
ZrO_2	0.02
SrO	0.02
Cr_2O_3	0.01
ZnO	0.01

2.3. Animal management

The effect of feeding bentonite as a supplement was tested on 50 weaned piglets (equal numbers of barrows and gilts) in two trials. Animal handling followed the EU directive 86/609/EEC concerning animal care. Piglets of the breed Pietrain × (Large White × Landrace), weaned at 28 days, were obtained from a traditional farm with a high prevalence of PWD. Piglets were identified by individual ear tags and housed in indoor pens with concrete floors and straw bedding.

2.3.1. Trial 1

Twenty weaned piglets were allocated to two dietary treatments, with 2 pens (5 piglets per pen) for each treatment. The dietary treatments were: (1) control group, fed a basal diet; (2) bentonite group, fed a basal diet + 10 g/kg of bentonite (1% bentonite supplementation).

2.3.2. Trial 2

Thirty weaned piglets were allocated to two dietary treatments, with 2 pens (7 and/or 8 piglets per pen) for each treatment. The dietary treatments were: (1) control group, fed a basal diet; (2) bentonite group, fed a basal diet + 20 g/kg of bentonite (2% bentonite supplementation).

The basal diet (Table 2) was formulated according to animal requirements (National Research Council, NRC, 1998). No antibiotics were included in the diets. Piglets were fed twice a day ad libitum, water was provided by automatic waterers. The dietary treatments were maintained for 21 days.

Table 2
Ingredient and chemical composition of diet (as-fed basis).

Item	Basal diet
Ingredient (g/kg)	
Wheat	397.2
Barley	303.0
Soybean meal, 47% CP	184.0
Dry whey and soy protein concentrate	50.0
Soybean oil	23.0
Limestone, ground	12.0
Dicalcium phosphate	11.0
Salt	3.0
Sodium carbonate	1.0
L-Lysine HCl	0.2
L-Threonine	0.3
DL-Methionine	0.3
Vitamin, amino acid, trace mineral premix	15.0
Calculated chemical composition	
ME (MJ/kg)	12.93
Crude protein (g/kg)	182.99
Lysine (g/kg)	12.73
Threonine (g/kg)	8.28
Methionine (g/kg)	3.84
Tryptophan (g/kg)	2.35
Ca (g/kg)	8.16
P (g/kg)	3.88