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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Short Communcation
Article Title (within 20 words without abbreviations)	Effects of dietary aluminosilicate on growth performance, frequency of diarrhea, and blood profiles of weaned pigs
Running Title (within 10 words)	Effects of dietary clay in weaned pigs
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Abstract (up to 350 words)

This study was designed with two experiments to investigate the effects of dietary aluminosilicate on growth performance, frequency of diarrhea, and blood profiles of weaned pigs. In Exp. 1, a total of 48 weaned pigs [initial body weight (BW) 7.65 ± 0.99 kg; 28 days of age) were randomly assigned to two dietary treatments (4 pigs/pen; 6 replicates/treatment) for 4 weeks in a randomized complete block design (block = initial BW and sex). In Exp. 2, a total of 48 weaned pigs [initial BW 7.85 ± 1.15 kg; 28 days of age] were randomly assigned to 2 dietary treatments (4 pigs/pen; 6 replicates/treatment) for 6 weeks in a randomized complete block design (block = initial BW and sex). Dietary treatments were a basal weaned pig diet (CON) and CON + 0.3% dietary aluminosilicate (CON + AS). Growth performance was measured in Exp.1 and 2 and the frequency of diarrhea and blood profiles in Exp. 2. All the data and sample were collected at specific time points during the experimental period. There were no differences in growth performance and frequency of diarrhea between dietary treatments. However, pigs fed CON + AS had lower hematocrit (19.13 vs 15.23%; d 42; $p < 0.10$) and hemoglobin (14.02 vs 12.40 g/dl; d 42; $p < 0.05$) than those fed CON. In conclusion, the addition of dietary aluminosilicate to the basal weaned diet had no negative effect on the growth performance, frequency of diarrhea, and blood profiles of weaned pigs.

Keywords (3 to 6): Aluminosilicate, Blood profiles, Diarrhea, Growth performance, Weaned pigs

Introduction

Weaning is one of the most critical periods in the life of a pig. During this period, the pigs are under stress of converting diet from liquid to solid, being separated from sows, immature immune system, and undeveloped gut barriers [1]. These dramatic changes reduce feed intake and increase disease susceptibility and mortality in pigs [2]. Previously, antibiotics have been used to prevent these problems. However, the use of antibiotics to promote growth has been banned in Europe and ~~many~~ other countries [3]. Therefore, dietary factors such as feed additives and feeding strategies have been increasingly used as an alternative to in-feed antibiotics. [4,5]. As Almeida [6] noted, “Clays are crystalline, hydrated aluminosilicate molecules mainly composed of phyllosilicates, containing alkali and alkaline earth cations.” Clays bind to mycotoxins, that are detrimental to animal growth and production; thus, clays are widely added to animal diets [7,8]. Specifically, clays have high adsorption properties owing to their unique layered structure and composition, and they absorb water and organic materials through interlayer spaces through cation exchange [9].

Therefore, the exchange of these cations promotes the binding of mycotoxins and is widely used in animal diets [10]. Previous studies have reported that supplementation of dietary clay plays an important role in the health of weaned pigs [11,12]. It performs three major functions in weaned pigs: (1) improvement in weight gain and feed conversion ratio [13], (2) enhancement of nutrient digestibility [14], and (3) protection of the gastrointestinal tract and anti-diarrheal and antibacterial effects [15–17]. However, the benefits of dietary clay in weaned pigs are not fully understood. Therefore, the objective of this study was to evaluate the effects of dietary aluminosilicate on growth performance, frequency of diarrhea, and blood profiles of weaned pigs.

Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Republic of Korea (approval: #202006A-CNU-090).

Experimental design and diets

In Exp. 1, a total of 48 weaned pigs [(Landrace × Yorkshire) × Duroc; 7.82 ± 0.99 kg of initial average body weight (BW); 28 days of age] were assigned to two dietary treatments (4 pigs/pen; 6 replicates/treatment): 1) a weaned diet based on corn-soybean meal (CON) and 2) CON + 0.3% dietary aluminosilicate (CON + AS) in a randomized completely block design (block= initial BW and sex). The experimental period was 4 weeks and was conducted as a preliminary experiment before proceeding with Exp. 2.

In Exp. 2, the experiment was conducted in the same procedure as described in Exp. 1. A total of 48 weaned pigs [(Landrace × Yorkshire) × Duroc; 7.85 ± 1.15 kg of initial average BW; 28 days of age] were assigned to two dietary treatments (4 pigs/pen; 6 replicates/treatment): 1) a weaned diet based on corn-soybean meal (CON) and 2) CON + 0.3% dietary aluminosilicate (CON + AS) in a randomized completely block design (block = initial BW and sex). The experimental period was 6 weeks. The basal diet formulated to meet or exceed the nutrient requirements of weaned pigs was estimated by the National Research Council (Table 1) [18]. The aluminosilicates used in this study were purchased from commercial company. Aluminosilicate consists of aluminum oxide (Al_2O_3, 15.84%), silicon dioxide (SiO_2, 61.74%), iron oxide (Fe_2O_3, 7.25%), sodium oxide (Na_2O, 1.92%), magnesium oxide (MgO, 1.11%), calcium oxide (CaO, 2.39%), and potassium oxide (K_2O, 1.4%). All the pigs had *ad libitum* access to feeder and water during the experimental period. Temperature and humidity were controlled by commercial facilities throughout the studies.

Data and sample collection

In Exp. 1, the BW of the pigs was measured at the beginning of the experiment (d 1) and on d 28. Residual feeds were measured and recorded at the beginning and end of each experiment. The average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were calculated on d 1 and 28. In Exp. 2, the BW of the pigs was measured on d 1 and 42. Growth performance was evaluated using the same procedure as described in Exp. 1. The fecal score of the pigs in each pen was visually checked with a score range of 1 to 5 (1 = normal feces, 2 = moist feces, 3 = mild diarrhea, 4 = severe diarrhea, and 5 = watery diarrhea) for the first 14 days. The frequency of diarrhea was calculated by counting pen days with a pen average diarrhea score of 3 or greater [19]. Blood samples were taken from the jugular vein of the randomly selected 1 pig per pen using 10 mL of tubes with ethylenediaminetetraacetic acid (EDTA) on d 7, 14, and 42 to measure blood profiles. The number of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hematocrit (HCT), hemoglobin (HGB), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were measured using an automated hematology analyzer calibrated for porcine blood (sci Vet abc hematology analyzer, sci animal care company, F-67120 Altorf, France) [20].

Statistical analysis

Data were analyzed by the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC, USA) using the PDIFF option in a randomized completely block design (block: initial BW and sex). The experimental unit was the pen. The statistical model for growth performance and blood profiles included effects of dietary treatment as a main effect and initial BW and sex as covariates. The Chi-square test was used for the frequency of diarrhea. The data were presented at LSMEAN ± SEM. Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

Results

Exp. 1

The results for growth performance are shown in Table 2. When dietary aluminosilicate was added to the diet, there were no significant differences in ADG, ADFI, and G: F between the dietary treatments throughout the study.

Exp. 2

The results for growth performance are shown in Table 3. When dietary aluminosilicate was added to the diet, there were no significant differences in ADG, ADFI, and G: F between the dietary treatments throughout the study. In addition, dietary aluminosilicate did not affect the frequency of diarrhea in pigs compared with CON (Figure 1). When aluminosilicate was added to the diet, no differences were found in WBC, PLT, RBC, MCV, MCH, and MCHC on d 7, d 14, and d 42 compared with CON (Table 4). Interestingly, pigs fed CON + AS tended to have lower HCT ($p < 0.10$) and lower HGB ($p < 0.05$) on d 42 than those fed CON (Table 4).

Discussion

The post-weaning period is the most stressful phase for piglets [21] because of the separation from their sows, mixing with unfamiliar litters in new environments, and switching from liquid milk to a solid diet [2]. Thus, the weaning situation is usually accompanied by the reduction in feed intake and growth [22] which eventually leads to immune deficiencies, changes in intestinal morphology, and even intestinal disorders [23]. Therefore, various nutritional strategies have been reported to solve these problems such as supplementation of feed additives such as enzymes, probiotics, prebiotics, functional amino acids. [24–28]. In the present study, we performed animal trials to evaluate the effects of dietary aluminosilicate on growth performance, frequency of diarrhea, and blood profiles of weaned pigs.

Previous studies have reported that the addition of 0.5-5% dietary aluminosilicate to the diet improved BW gain and feed conversion ratio of weaned pigs [12,13]. These results can be related to improved nutrient digestibility in pigs due to the characteristics of dietary aluminosilicate which increases the transit time of digesta in the digestive tract and stimulates the activity of enzymes [13,29]. However, other studies have reported that the addition of silicate minerals does not affect growth performance of weaned pigs depending on their dosages [11,14,29]. In the present studies, no differences were observed in ADG, ADFI, and G: F between the dietary treatments throughout the studies. Results of growth performance and silicate minerals could be inconsistent and vary depending on their types and amounts. Moreover, the addition of dietary aluminosilicate in the present study did not affect diarrhea frequency for the first two weeks after weaning compared with the CON. However, several studies have reported that dietary clay alleviates post-weaning diarrhea by inactivating *Escherichia coli* or inhibiting its growth [11,30]. This result may vary depending on the types and chemical structures of clay as well as pig health conditions such as clinical diseases and the environment [11,31].

Changes in blood cell counts are used as indicators to determine the nutritional, immunological, and physiological responses of animals [32,33]. In the present study, we evaluated the effects of dietary aluminosilicates on the blood profiles of weaned pigs. White blood cells are one of the first lines of defense in the body and their number can increase because of infection or stress [34], indicating systemic inflammatory responses in the body. The number of RBC is closely related to HGB and can be used as an indicator of oxygen transport to the organs in the body. Levels of HCT and HGB are affected by the hydration status of animals with dehydration leading to high HCT levels and weaning stress can affect the hydration status of animals [35]. In the present study, the dietary aluminosilicate supplementation in weaner diets reduced HCT and HGB levels compared with the CON. These results indicate that, although the frequency of diarrhea and systemic inflammatory responses did not differ, it was confirmed that dehydration status was not observed. However, the lower levels of HCT and HGB, which are also used as iron status indicators, may be due to the low bioavailability and absorption of iron-containing dietary clays because of their structural characteristics.

Conclusion

In summary, dietary aluminosilicate in the weaning diet based on corn-soybean meal had no appreciable effect on growth performance, frequency of diarrhea, and blood profiles of weaned pigs. Therefore, further research is needed on dietary aluminosilicate and its nutritional and strategic use as an alternative to in-feed antibiotics to prevent post-weaning diarrhea and improve growth and health of pigs.

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Table 1. Composition of experimental diet for weaning pigs (as-fed basis)

Item	Basal diet
Ingredient (%)	
Corn	55.11
Soybean meal, 44%	37.00
Tallow	2.50
Meat and bone meal	2.00
Limestone	0.95
Mono-dicalcium phosphate	0.92
L-Lysine-HCl	0.84
DL-Methionine	0.29
L-Threonine	0.19
Vitamin-Mineral premix[1]	0.20
Total	100.00
Calculated energy and nutrient contents	
Metabolizable energy (kcal/kg)	3,400
Crude protein (%)	24.31
Calcium (%)	0.85
Phosphorus (%)	0.70

[1)] Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3,2,500 IU; vitamin E, 30 IU; vitamin K3, 3mg; D-pantothenic acid, 15mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B12, 12 µg; Fe, 90mg from iron sulfate; Cu, 8.8 mg from copper sulfate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

Table 2. Effects of dietary aluminosilicate on growth performance of weaned pigs (Exp. 1)[1]

Item	CON	CON + AS	SEM	*p*-value
Day 1 to 28				
Initial BW, kg	7.82	7.82	0.52	0.995
Final BW, kg	19.60	17.86	1.36	0.388
ADG, kg/d	0.280	0.239	0.026	0.286
ADFI, kg/d	0.673	0.484	0.063	0.062
G:F, kg/kg	0.432	0.496	0.028	0.134

1) Each value is the mean value of 6 replicates (4 pigs/pen).

2) CON, basal diet based on corn and soybean meal; CON + AS, CON + 0.3% dietary aluminosilicate; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 3. Effects of dietary aluminosilicate on growth performance of weaned pigs (Exp. 2)[1]

Item	CON	CON + AS	SEM	*p*-value
Day 1 to 42				
Initial BW, kg	7.92	7.97	0.56	0.899
Final BW, kg	22.57	23.68	0.93	0.798
ADG, kg/d	0.349	0.374	0.016	0.238
ADFI, kg/d	0.590	0.589	0.047	0.857
G:F, kg/kg	0.591	0.635	0.023	0.490

1) Each value is the mean value of 6 replicates (4 pigs/pen).

2) CON, basal diet based on corn and soybean meal; CON + AS, CON + 0.3% dietary aluminosilicate; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 4. Effects of dietary aluminosilicate on blood profiles of weaned pigs (Exp. 2)[1]

Item	CON	CON + AS	SEM	*p*-value
WBC, $\times 10^3/\mu L$				
Day 7	21.92	20.95	3.16	0.464
Day 14	24.87	25.12	3.01	0.677
Day 42	37.17	26.20	4.50	0.126
RBC, $\times 10^6/\mu L$				
Day 7	7.78	7.09	0.35	0.405
Day 14	7.71	8.06	0.34	0.773
Day 42	4.37	3.40	0.34	0.130
PLT, $\times 10^3/\mu L$				
Day 7	480.83	401.00	82.98	0.745
Day 14	359.50	446.25	66.87	0.406
Day 42	179.17	177.67	25.97	0.966
HCT, %				
Day 7	40.17	36.20	1.78	0.318
Day 14	39.18	40.03	1.60	0.874
Day 42	19.13	15.23	1.29	0.099
HGB, g/dL				
Day 7	13.07	11.92	0.59	0.401
Day 14	12.75	13.21	0.46	0.582
Day 42	14.02	12.40	0.46	0.040
MCV, fL				
Day 7	51.67	50.83	0.90	0.646
Day 14	50.83	49.83	0.79	0.539
Day 42	43.83	46.67	1.65	0.416
MCH, pg				
Day 7	16.78	16.75	0.36	0.693
Day 14	16.62	16.39	0.31	0.391
Day 42	32.27	43.57	5.97	0.365
MCHC, g/dL				
Day 7	32.47	32.87	0.26	0.257
Day 14	32.68	32.98	0.34	0.433
Day 42	73.50	89.35	8.25	0.367

1) Each value is the mean value of 6 replicates (4 pigs/pen).

2) CON, basal diet based on corn and soybean meal; CON + AS, CON + 0.3% dietary aluminosilicate; WBC, white blood cells; RBC, red blood cells; PLT, platelet; HCT, hematocrit; HGB, hemoglobin; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

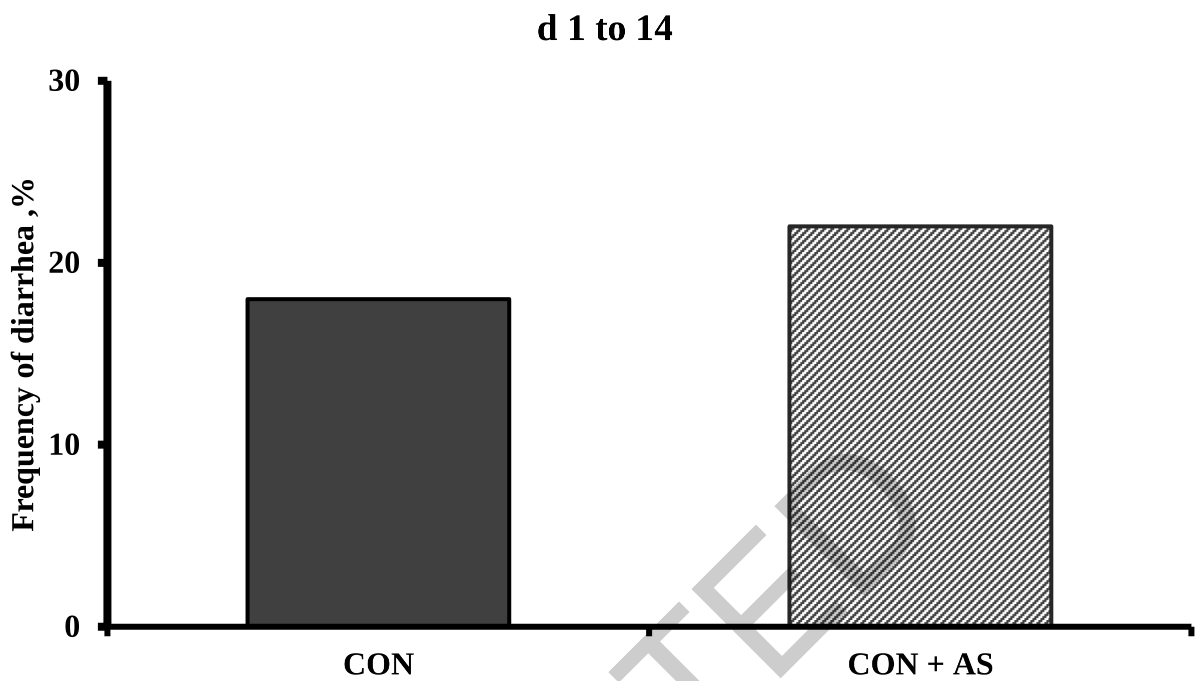

Figure 1**.** Frequency of diarrhea in weaned pigs for the first two weeks after weaning (Exp. 2). CON, basal diet based on corn and soybean meal; CON + AS, CON + 0.3% dietary aluminosilicate. Frequency of diarrhea (%) = (number of diarrhea with score 3 or greater / number of pen days) × 100. Data were analyzed using the Chi-square test.