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Running Title (within 10 words)	Supplementation clay mineral alleviate gut health in weaned piglets
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Ethics approval and consent to participate	The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-24-0013-02).

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Abstract

The objective of this study was to investigate the effects of illite (IT) and bentonite (BE) on growth performance and intestinal health in weaned pigs challenged with *Escherichia coli* (*E. coli*). A total of 24 (Duroc × Yorkshire × Landrace)weaned pigs (initial body weight: 9.61 ± 0.65 kg, 28 ± 3 days old) were assigned to six treatments with four replicates per treatment. Pigs were housed in individual pens for 17 days, including a 3-day adaptation period and 14 days after the first *E. coli* challenge. In the *E. coli*-challenged groups, all pigs were orally inoculated with a total of 10 mL of *E. coli* for three consecutive days. The experiment was conducted in a 2 × 3 factorial arrangement of treatments consisting of two challenge levels (challenged and non-challenged) and three types of clay mineral (non-supplemented, IT, and BE). IT and BE were included in the diets at 1% and 1.5%, respectively. *E. coli* challenge reduced ($p < 0.05$) ADG, ADFI, G:F during the entire experimental period and lowered ($p < 0.05$) serum interleukin-8, interleukin-10, malondialdehyde (MDA), and interferon-gamma (IFN- γ) levels on D 3. However, in the *E. coli*-challenged group, IT supplementation improved ($p < 0.05$) G:F compared to the non-supplemented group during the first week. Additionally, IT supplementation increased ($p < 0.05$) blood IFN- γ and mucin expression levels compared to the non-supplemented group in the challenged groups. At the end of the experiment, intestinal morphology and intestinal immunity were evaluated to assess intestinal health. *E. coli* challenge reduced ($p < 0.05$) villus height and tight junction protein expression while increasing ($p < 0.05$)crypt depth. In the *E. coli*-challenged group, BE supplementation increased ($p < 0.05$) villus height and the expression of tight junction proteins compared to the non-supplemented group. Additionally, IT supplementation in *E.* coli challenge increased ($p < 0.05$) mucin expression levels in the intestine compared to the non-supplemented group. Inconclusion, dietary supplementation with IT and BE mitigates the adverse effects of *E. coli* infection and suggests their potential as effective additives for managing *E. coli* challenges.

Keywords (3 to 6): weaned piglet, *E. coli* challenge, clay mineral, illite, bentonite

Introduction

Early weaning techniques are commonly used in modern intensive farming systems to boost sow productivity and economic benefits [1]. Nonetheless, weaning stress may adversely affect piglets' intestinal microbiota, physiological and biochemical functions, digestion, and absorption [2]. As a consequence of weaning stress, piglets' intestinal environments are susceptible to invasion by pathogenic microorganisms such as *Escherichia coli* (*E. coli*) [3].

Natural clay minerals (CMs) are naturally occurring rock or soil materials composed predominantly of fine-grained minerals, which exhibit high pliability when hydrated. Based on their structures and physico-chemical properties (particle size, surface charge, and adsorption capability), CMs can be used in a wide range of applications [4]. Illite (IT) is characterized by a large specific area and two tetrahedral sheets sandwiched between two octahedral sheets with an ability to absorb large amounts of water and a high capacity to exchange cations (CEC) [5]. Bentonite (BE) composed predominantly of smectite is characterized by its submicrometer crystal size, sheet-like structure, significant surface area, negative charge, and CEC [6]. Due to their characteristics, CMs are significant for gastrointestinal disease medications, anti-infective agents, and nutritional supplements [7]. According to Muniyappan et al. [8], supplementation of IT can improve feed efficiency and digestibility in pigs. Horky et al. [9] have also reported that supplementation of BE can reduce oxidative stress and protect jejunal tissue. Therefore, this study hypothesized that dietary supplementation of IT and BE could mitigate intestinal health and growth performance of nursery pigs. To test this hypothesis, effects of IT and BE on intestinal health and growth performance of nursery pigs challenged with *E. coli* were investigated.

Materials and Methods

Ethics approval and consent to participate

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-24-0013-02).

Bacterial strains, culture and challenge

E. coli KCTC 2571 was supplied from Korean Collection for Type Cultures (KCTC, Jeongeup, Korea) in a lyophilized state and suspended in sterile distilled water. The 10 μl of the suspended *E. coli* was added to luria-bertani broth (LB broth; KisanBio, Seoul, Korea) and cultured at 37℃ for 18 hours with shaking. Thereafter, the subcultured *E. coli* was smeared on MacConkey agar to confirm the bacterial enumeration. A final concentration of 1.2×10^{10} CFU/mL was used in this study.

Animals, experimental design and diets

A total of 24 (Duroc × Yorkshire × Landrace) weaned pigs (initial body weight of 9.61 ± 0.65 kg and 28 ± 3 d old), were assigned to 6 treatments with 4 replicates per treatment. Pigs were housed in individual pens for 17 days, including 3 days adaption period and 14 days after the first *E. coli* challenge (d 0). The experiment was conducted in a 2 × 3 factorial arrangement of treatments consisting of two levels of challenge (challenge and non-challenge) and three levels of CM (non-supplementation, IT and BE). Corn and soybean meal basal diets were formulated to meet or exceed the nutrient requirements for the weaned piglets as recommended by NRC (Table 1) [10]. The pigs were fed daily at 8:30 and 17:00 h and had ad libitum access to water. Feed residues were removed before the next meal and considered in the calculations. In the *E. coli* challenge treatments, all pigs were orally inoculated by dividing a total of 10 mL of *E. coli* for 3 consecutive days. Challenged piglets and non-challenged piglets were housed in a separate room. Strict biosecurity procedures were followed to avoid *E. coli* contamination of the non-challenged piglets.

Growth performance

All piglets were weighed every week during the experiment period and feed consumption was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

Nutrient digestibility

To estimate digestibility, 0.2% chromium oxide (Cr_2O_3) was supplemented with diets as an indigestible marker. Pigs were fed diets mixed with chromium oxide for 4 consecutive days from D 4 and 11, fresh excreta samples were collected in that period. At the end of the experiment, fecal samples were stored at -20℃ and dried at 70℃ for 72 h, and then, ground to pass through a 1 mm screen. All analysis items (feed and fecal) were analyzed for DM and CP. The procedures utilized for the determination of dry matter (DM) and crude protein (CP) digestibility were conducted with the methods by AOAC [11]. Chromium was analyzed with an ultraviolet absorption spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). The digestibility was calculated using the following formula: digestibility (%) = [1–(Nf × Cd)/ (Nd × Cf)] × 100, where Nf is the nutrient concentration in feces (% DM), Nd is the nutrient concentration in diet (% DM), Cd is the chromium concentration in diet (% DM), and Cf is the chromium concentration in feces (% DM).

Morphological analysis of small intestine

At the end of the experiment (D 14), pigs were anesthetized with carbon dioxide gas after blood sampling and euthanized by exsanguination. Intestinal tissues of about 10 cm from the ileum (close to the ileocecal junction) were collected and fixed in 10% neutral buffered formalin (NBF; Sigma-Aldrich, St. Louis, MO, USA). After cutting the intestine sample, it was dehydrated and dealcoholized. The samples were then installed on slides, treated with paraffin, and stained with hematoxylin and eosin. Villus height (VH) and crypt depth (CD) were measured under the light microscope (OLYMPUS DP71, BX50F-3, Olympus Optical Co. Ltd., Tokyo, Japan). VH was determined by measuring the distance between the tip of the villi to the villus crypt junction, and CD was determined by measuring the distance between adjacent villi.

Blood profile

Blood samples were obtained from jugular vein of 6 pigs each treatment at d 0, d 3 and d 14. The samples were collected in K_3EDTA tube for complete blood count analysis and nonheparinized tubes for serum analysis, respectively. White blood cells (WBC) were analyzed using an automatic hematology analyzer (XE2100D, Sysmex, Kobe, Japan). Interleukin-10 (IL-10; P8000, R&D systems, Minneapolis, MN, USA) and interferon-γ (IFN-γ; DY985, R&D systems) were measured using commercially available ELISA kits.

Real-time quantitative RT-PCR (qRT-PCR) analysis

The Total RNA extraction kit (iNtRON Biotechnology, Seongnam, Korea) was used to extract the RNA from the intestinal mucosa. The mRNA was converted to cDNA using High-Capacity cDNA Reverse Transcription Kit

(Applied Biosystems, Waltham, MA, USA). For cDNA synthesis, the mixed solution was heat treated at 25°C for 10 min, at 37°C for 2 h, and at 85°C for 5 min. Gene amplification was performed using Fast qPCR 2×SYBR Green Master Mix (Applied Biosystems). RT-qPCR was performed in two steps. The first step was an enzyme activation step, which was performed at 95°C for 2 min for 1 cycle. The second step was a denaturation step at 95°C for 15 seconds and an annealing/extend step at 56°C for 1 min, repeating a total of 40 cycles to perform gene amplification. The target genes were zonula occludens-1 (ZO-1), claudin-1 (CLDN-1), mucin-2 (MUC2) and Glyceraldehyde-3-phosphate dehydrogenase 2 (GAPDH). Primers used in the amplification are shown in Table 2. Normalization was performed using the reference gene GAPDH. Relative gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method [12].

Statistical analysis

JMP Pro 16 (SAS Institute Inc., Cary, NC, United States) and GraphPad Prism (Version 9.1.0; GraphPad Software, San Diego, CA) were used for statistical analyses and graph visualization, respectively. All data were analyzed via two-way analysis of variance (ANOVA) using the Standard Least Squares model, with each pen as the experimental unit. The statistical model included the effect of *E. coli* challenge (C -, C +), the effect of CM supplementation (non, IT and BE) and the interaction between *E. coli* and CM.

Results

Growth performance

Effects of dietary supplementing IT and BE on growth performance in weaned piglets challenged with *E.coli* are presented in Table 3. *E. coli* challenge decreased ($p < 0.05$) final BW compared with non-challenged group. Also, *E. coli* challenge decreased ($p < 0.05$) ADG and ADFI compared with non-challenged group in whole experiment period. There was an interaction between *E. coli* challenge and CM in G:F. pigs supplemented with IT with *E. coli* challenge improved ($p < 0.05$) G:F compared to non-supplemented group with *E. coli* on 1w.

Nutrient digestibility

Effects of dietary supplementing IT and BE on nutrient digestibility in weaned piglets challenged with *E.coli* are presented in Table 4. Pigs supplemented with BE showed higher ($p < 0.05$) CP digestibility than non-supplemented group.

Intestinal morphology

Effects of dietary supplementing IT and BE on intestinal morphology in weaned piglets challenged with *E.coli* are presented in Table 5. There was an interaction between *E. coli* challenge and CM in VH. Pigs supplemented with BE with *E. coli* challenge showed higher ($p < 0.05$) VH compared to non-supplemented group with *E. coli.* Also, *E. coli* challenge decreased ($p < 0.05$) VH:CD compared with non-challenged group.

Blood profile

Effects of dietary supplementing IT and BE on blood profile in weaned piglets challenged with *E.coli* are presented in Table 6. On D3, *E. coli* challenged group showed lower ($p < 0.05$) WBC, IL-8, IL10, MDA, IFN-γ and IgG than non-challenged group. Also, there was an interaction between *E. coli* challenge and CM. pigs supplemented with IT with *E. coli* challenge showed higher ($p < 0.05$) IFN-γ than pigs challenged with *E. coli* on D3.

Tight junction protein

Effects of dietary supplementing IT and BE on TJ protein in weaned piglets challenged with *E.coli* are presented in Table 7. There was an interaction between *E. coli* challenge and CM in MUC-1, CLDN-1 and ZO-1. Pigs supplemented IT with *E. coli* challenge showed higher ($p < 0.05$) MUC-1 than pigs challenged with *E. coli*. Also,

153 Pigs supplemented BE with *E. coli* challenge showed higher ($p < 0.05$) CLDN-1 and ZO-1 than pigs challenged with

154 *E. coli*.

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Discussion

The objective of this study was to investigate effects of natural IT and BE on growth performance and intestinal health of weaned piglets challenged with *E. coli*. In the current study, *E. coli* infection significantly decreased BW, ADG, and ADFI of piglets. This result is consistent with previous studies showing that weaning stress and pathogenic challenges can severely impact growth performance [13, 14]. Reductions of growth parameters can be due to intestinal epithelial damage, decreased nutrient absorption, and increased energy expenditure for immune response [15, 16]. IT supplementation improved the G:F ratio during the first week of infection. The zinc content in IT might enhance intestinal barrier function by regulating TJ protein expression [17, 18]. Additionally, layered silicate structure of IT can adsorb toxins in the gastrointestinal tract, potentially reducing negative impacts of *E. coli* infection [19, 20]. These mechanisms may contribute to improved nutrient utilization efficiency and growth performance. BE supplementation increased CP digestibility. This could be attributed to high CEC and swelling properties [21, 22]. These characteristics may increase intestinal retention time, enhance enzyme-substrate interactions, and improve nutrient digestibility [21]. Improved protein digestion can support intestinal health and immune function, leading to enhanced growth performance [23, 24].

E. coli infection decreased the VH:CD, indicating intestinal mucosal damage. This result is consistent with previous studies showing that reduction in VH can lead to decreased nutrient absorption capacity and contribute to growth retardation [25]. BE supplementation increased VH in piglets challenged with *E. coli*. Water retention capacity of BE might protect and promote regeneration of intestinal mucosa [26].

Three days post-infection, *E. coli* challenged groups showed decreased white blood cell (WBC) counts and levels of cytokines (IL-8, IL-10, IFN-γ, IgG). These results are similar to immune suppression caused by weaning stress [27], suggesting that *E. coli* toxins might have impaired immune cell function. IT supplementation increased IFN-γ levels in piglets challenged with *E. coli*. The copper content in IT might enhance macrophage function and improve defense against pathogens [28, 29]. This indicates that IT's immunomodulatory effects may lead to improved resistance to infections.

E. coli infection is known to increase oxidative stress in the intestine. *E. coli* infection decreased TJ protein expression, consistent with previous studies showing that weaning stress and pathogenic challenges could compromise intestinal barrier function [30, 31]. Reduced TJ protein expression can increase intestinal permeability, promoting pathogen invasion and inflammation [32, 33]. IT supplementation increased MUC-1 expression, while BE supplementation increased CLDN-1 and ZO-1 expression. The manganese in IT might act as a cofactor for

enzymes to protect against DNA oxidative damage, contributing to cell [34, 35]. BE may form a protective layer on the intestinal mucosa, shielding epithelial cells from *E. coli* toxins [36, 37]. These increases in TJ protein expression can strengthen the intestinal barrier function, thus preventing pathogen invasion and reducing inflammation [30, 38].

Immune modulation mechanisms of IT and BE are not completely understood yet. For IT, its trace minerals may directly regulate immune cell functions. For example, zinc can promote T lymphocyte activation and proliferation, while copper can enhance macrophage function. For BE, its immune modulation effects are likely to be mainly indirect. BE can prevent excessive activation of the immune system by adsorbing intestinal toxins. Additionally, protective effect of BE on the intestinal mucosa may help maintain the function of gut-associated lymphoid tissues.

In conclusion, this study demonstrates that IT and BE supplementation has the potential to improve intestinal health and growth performance of *E. coli*-challenged weaned piglets. IT and BE appear to support piglet health through distinct mechanisms. IT primarily acts through trace mineral supply to enhance immune function and toxin adsorption, while BE can improve nutrient digestibility and intestinal mucosal protection.

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Tables and Figures

Table 1. Compositions of basal diets (as-fed-basis)

Items	content
Ingredients, %	
corn	34.43
extruded corn	15.00
lactose	10.00
Dehulled soybean meal, 51% CP[a]	13.50
Soy protein concentrate, 65% CP[a]	10.00
Plasma powder	6.00
Whey	5.00
Soy oil	2.20
Monocalcium phosphate	1.26
Limestone	1.40
L-Lysine-HCl, 78%	0.06
DL-Methionine, 50%	0.15
Choline chloride, 25%	0.10
Vitamin premix[b]	0.25
Trace mineral premix[c]	0.25
Salt	0.40
Total	100
Calculated value	
ME, Kcal/kg	3433
CP, %	20.76
Lysine, %	1.35
Methionine, %	0.39
Ca	0.82
P	0.65
Analyzed value	
ME, kcal/kg	3512
CP, %	20.92

[a] Crude protein

[b]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; ribofavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B12, 33 mg

[c]Provided per kg of complete diet without Zinc: Cu (as $CuSO_4 \cdot 5H_2O$), 12mg; Mn (as MnO_2), 8mg; I (as KI), 0.28mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15mg

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Table 2. Primer sequences used for the RT-qPCR analysis with the Muc1, ZO-1, CLDN1, and GAPDH genes

Gene	Primers	Sequence (5'-3')
GAPDH	Forward	TCGGAGTGAACGGATTTGGC
	Reverse	TGACAAGCTTCCCGTTCTCC
Muc1	Forward	CCACAACCTGAAGACACAGT
	Reverse	GACCAGAATACAGACCAGCA
ZO-1	Forward	CTCTGTCCATGCAGATAAGC
	Reverse	AATAGCTCCCTGTGGGATAA
CLDN1	Forward	GCTGGGACTAATAGCCATCT
	Reverse	AAGAGAGCCTGACCAAATTC

Table 3. Effect of dietary supplementing illite and bentonite on growth performance in weaned piglets challenged with *E. coli*

Items	C+			C-			SE	Mi			C		p-value		
	-	IT	BE	-	IT	BE		-	IT	BE	+	-	Mi	C	Mi×C
BW, kg															
D-3	9.62	9.61	9.62	9.60	9.61	9.60	0.365	9.67	9.61	9.61			1.000		
D0	10.13	10.11	10.14	10.14	10.15	10.13	0.370	10.13	10.13	10.13			0.999		
D7	11.22	11.56	11.49	11.82	12.05	11.77	0.383	11.52	11.80	11.63	11.42	11.88	0.756	0.160	0.915
D14	12.93	13.32	13.17	13.86	14.05	13.70	0.385	13.40	13.69	13.43	13.14	13.87	0.718	0.032	0.873
ADG, g															
D-3 to 0	126.88	123.75	130.00	133.75	135.63	131.25	10.485	130.31	129.69	130.63			0.996		
D0 to 7	155.71	207.14	192.86	240.00	271.43	234.29	8.367	197.86b	239.29a	213.57b	185.24	238.57	<0.001	<0.001	0.061
D7 to 14	244.64	251.79	240.00	292.14	286.07	275.36	16.032	268.39	268.93	257.68	245.48	284.52	0.735	0.008	0.901
D0 to 14	200.18	229.46	216.43	266.07	278.75	254.82	9.671	233.13	254.11	235.63	215.36	266.55	0.087	<0.001	0.379
ADFI, g															
D-3 to 0	194.19	198.00	193.00	193.88	197.00	193.00	6.466	194.03	197.50	193.00			0.770		
D0 to 7	299.00	317.00	313.00	352.07	383.00	366.00	5.603	325.54b	350.00a	339.50ab	309.67	367.02	0.002	<0.001	0.427
D7 to 14	414.00	406.00	389.00	409.00	346.00	414.00	33.719	411.50	376.00	40.50	403.00	389.67	0.565	0.634	0.457
D0 to 14	356.00	361.00	351.00	380.25	402.00	390.00	7.398	368.13	381.50	370.50	356.00	390.75	0.184	<0.001	0.480
G:F, g/g															
D-3 to 0	0.65	0.62	0.68	0.69	0.69	0.68	0.053	0.67	0.66	0.68			0.924		
D0 to 7	0.52b	0.65a	0.62ab	0.68a	0.71a	0.64a	0.026	0.60b	0.68a	0.63ab	0.60	0.68	0.018	0.001	0.040
D7 to 14	0.59	0.62	0.62	0.71	0.68	0.67	0.039	0.68	0.65	0.64	0.61	0.69	0.958	0.027	0.596
D0 to 14	0.56	0.64	0.62	0.70	0.69	0.65	0.027	0.63	0.66	0.64	0.60	0.68	0.402	0.002	0.168

C, challenge; Mi, clay mineral; IT, illite; BE, bentonite; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake G:F, feed efficiency; SE, standard error.

a,b Values within a row with different superscripts are significantly different.

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Table 4. Effect of dietary supplementing illite and bentonite on nutrient digestibility in weaned piglets challenged with *E. coli*

Items	C+			C-			SE	Mi			C		p-value		
	-	IT	BE	-	IT	BE		-	IT	BE	+	-	Mi	C	Mi×C
1w															
DM	79.75	80.28	80.48	80.53	80.70	80.65	0.647	80.14	80.49	80.56	80.17	80.63	0.786	0.394	0.893
CP	70.33	71.94	72.57	72.89	73.84	74.78	0.807	71.61b	72.89ab	73.67a	71.61	73.84	0.049	0.002	0.919
GE	81.12	81.41	81.14	81.81	82.40	81.91	0.564	81.47	81.91	81.53	81.23	82.04	0.695	0.087	0.967
2w															
DM	79.36	79.64	79.53	79.58	79.90	79.83	0.774	79.47	79.77	79.68	79.51	79.77	0.923	0.691	0.998
CP	71.40	72.15	71.81	73.68	72.89	72.85	0.124	72.54	72.52	72.33	71.79	73.14	0.983	0.189	0.806
GE	80.99	82.17	81.61	81.06	82.27	81.57	0.850	81.03	82.22	81.59	81.59	81.64	0.386	0.944	0.997

C, challenge; Mi, clay mineral; IT, illite; BE, bentonite; DM, dry matter; CP, crude protein; GE, gross energy; SE, standard error.

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Table 5. Effect of dietary supplementing illite and bentonite on intestinal morphology in weaned piglets challenged with *E. coli*

Items	C+			C-			SE	Mi			C		p-value		
	-	IT	BE	-	IT	BE		-	IT	BE	+	-	Mi	C	Mi×C
VH	318.02c	362.00bc	366.00ab	394.34ab	408.75a	385.34ab	10.856	356.18b	385.37a	375.6ab	348.67	396.14	0.035	<0.001	0.045
CD	183.74	187.35	198.47	172.80	179.48	179.32	6.112	178.27	183.41	188.90	189.85	177.20	0.237	0.017	0.639
VH:CD	1.74	1.94	1.85	2.31	2.29	2.16	0.092	2.02	2.12	2.00	1.84	2.25	0.444	<0.001	0.315

C, challenge; Mi, clay mineral; IT, illite; BE, bentonite; VH, villus height; CD, crypt depth; VH:CD, villus height to crypt depth ratio

a-c Values within a row with different superscripts are significantly different.

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Table 6. Effect of dietary supplementing illite and bentonite on blood profile in weaned piglets challenged with *E. coli*

Item	C+			C-			SE	Mi			C		p-value		
	-	IT	BE	-	IT	BE		-	IT	BE	+	-	Mi	C	Mi×C
D0															
IL-8	2044.29	2061.94	2062.17	2057.01	2077.15	2071.31	203.862	2050.65	2069.55	2066.74	2056.13	2068.49	0.995	0.941	1.000
IL-10	43.68	44.61	43.49	45.43	44.85	42.98	2.912	44.56	44.73	43.23	43.93	44.42	0.855	0.837	0.925
MDA	70.77	84.89	63.31	72.89	71.94	83.49	11.091	71.83	78.42	73.40	72.99	76.11	0.826	0.732	0.336
IFN-γ	190.57	164.99	192.10	187.51	189.35	175.70	21.662	189.04	177.17	183.90	182.56	184.19	0.860	0.927	0.634
D3															
IL-8	2231.93	3021.26	2706.83	1885.84	1800.97	1827.35	222.309	2058.89	2411.11	2267.09	2653.34	1838.05	0.292	<.0001	0.153
IL-10	49.90	64.81	54.59	46.33	44.60	48.25	4.226	48.11	54.71	51.42	56.44	46.39	0.306	0.006	0.121
MDA	123.71	130.34	117.87	74.60	82.82	85.95	10.029	99.15	106.58	101.91	123.97	81.12	0.757	<.0001	0.642
IFN-γ	242.16b	341.81a	283.42b	177.17c	175.40c	175.27c	13.443	209.66b	258.61a	229.35ab	289.13	175.95	0.003	<.0001	0.002
D14															
IL-8	1915.27	2005.14	2261.69	2090.19	1953.23	1995.16	170.927	2002.73	1979.19	2128.43	2060.70	2012.86	0.647	0.734	0.441
IL-10	51.67	49.61	48.39	48.63	52.80	52.79	2.522	50.15	51.21	50.59	49.89	51.41	0.916	0.465	0.296
MDA	84.19	95.62	88.91	77.37	75.92	73.07	11.557	80.78	85.77	80.99	89.58	75.46	0.888	0.142	0.850
IFN-γ	183.84	174.37	161.38	155.29	139.03	154.77	20.934	169.57	156.70	158.08	173.20	149.70	0.797	0.176	0.774

C, challenge; Mi, clay mineral; IT, illite; BE, bentonite; WBC, white blood cell; IgG, immunoglobulin G; IL-8, interleukin-8; IL-10, Interleukin-10; MDA, malondialdehyde; IFN-γ, interferon γ; SE, standard error.

a-c Values within a row with different superscripts are significantly different.

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Table 7. Effect of dietary supplementing illite and bentonite on tight junction in weaned piglets challenged with *E. coli*

Item	C+			C-			SE	Mi			C		p-value		
	-	IT	BE	-	IT	BE		-	IT	BE	+	-	Mi	C	Mi×C
MUC	0.82b	1.25a	1.17ab	1.00ab	0.90ab	0.85b	0.075	0.91	1.07	1.01	1.08	0.92	0.140	0.020	0.007
CLDN-1	0.82b	1.18ab	1.23a	1.00ab	0.87ab	0.83b	0.081	0.91	1.03	1.03	1.08	0.90	0.270	0.021	0.008
ZO-1	0.84b	1.15ab	1.24a	1.00ab	0.90ab	0.86b	0.075	0.92	1.02	1.05	1.08	0.92	0.232	0.023	0.008

C, challenge; Mi, clay mineral; IT, illite; BE, bentonite; MUC, mucin-1; CLDN-1, claudin-1; ZO-1, zonula occludens-1; SE, standard error.
a,b Values within a row with different superscripts are significantly different.