Amelioration of Enterotoxigenic *Escherichia coli*-induced disruption of intestinal epithelium by Manno-oligosaccharide in weaned pigs

En Yu
Institute of Animal Nutriton, Sichuan Agricultural University
Daiwen Chen
Institute of Animal Nutrition, Sichuan Agricultural University
Bing Yu
Institute of Animal Nutrition, Sichuan Agricultural University
Yuheng Luo
Institute of Animal Nutrition, Sichuan Agricultural University
Ping Zheng
Institute of Animal Nutrition, Sichuan Agricultural University
Heng Yin
Dalian Institute of Chemical Physics, Chinese Academy of Sciences
XiangBing Mao
Institute of Animal Nutrition, Sichuan Agricultural University
Zhiqing Huang
Institute of Animal Nutrition, Sichuan Agricultural University
Jie Yu
Institute of Animal Nutrition, Sichuan Agricultural University
Junqiu Luo
Institute of Animal Nutrition, Sichuan Agricultural University
Hui Yan
Institute of Animal Nutrition, Sichuan Agricultural University
Jun He (hejun8067@163.com)
Institute of Animal Nutrition, Sichuan Agricultural University https://orcid.org/0000-0002-1963-9766

Research

Keywords: Manno-oligosaccharide, Weaned pigs, Intestinal barrier, Inflammation response, Apoptosis

Posted Date: December 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-120366/v1

Abstract

Background: Enterotoxigenic *Escherichia coli* (ETEC) is one of the major bacterial causes leading to diarrhea and disruption of intestinal epithelium in neonatal animals. Manno-oligosaccharide (MOS) is a prebiotic deprived from natural plants or yeasts. Here, we explored the protective effect of MOS on intestinal epithelium in weaned pigs upon ETEC challenge.

Methods: Thirty-two pigs were randomly assigned into four treatments and fed with a basal diet or basal diet containing 0.3% MOS. On day 19, pigs were challenged by ETEC or culture medium.

Results: MOS supplementation reduced diarrhea incidence in the pigs upon ETEC challenge (P<0.05). ETEC-challenge elevated the serum concentrations of D-lactate and diamine oxidase (DAO), however, MOS significantly decreased their concentrations in the serum (P<0.05). Moreover, MOS significantly decreased serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the ETEC-challenged pigs (P<0.05). Interestingly, MOS enhanced the expression and localization of zonula occludens-1 (ZO-1) protein in the duodenal and jejunal epithelium. Moreover, MOS decreased the cell apoptosis rate (P<0.05), but significantly elevated the content of secretory immunoglobulin A (sIgA), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in the jejunal mucosa (P<0.05). Importantly, MOS decreased the expression levels of critical genes involving in mucosal inflammatory responses (TNF-α, IL-1β, TLR4, and NF-κB) and the apoptosis (Caspase 3, Caspase 9, and Bax) in the jejunum upon ETEC challenge (P<0.05). Moreover, MOS up-regulated the expression of mucosa functional genes such as the heme oxygenase-1 (HO-1) and nuclear factor E2-related factor 2 (Nrf2) in the jejunum and ileum (P<0.05), and elevated the expression level of β-defensin 114 (PBD-114) in the duodenum upon ETEC challenge.

Conclusions: These results suggested that MOS can alleviate ETEC-induced disruption of intestinal barrier in weaned pigs, which was associated with suppressed inflammation and epithelial cell apoptosis, and improved antioxidant capacity and intestinal barrier functions.

Introduction

Intestinal epithelium is the main site of nutrient digestion and absorption for mammalian animals. Importantly, it can secrete a variety of immunoglobulins and bioactive components, and serve as a physical barrier for the host against various pathogens in the intestinal lumen [1, 2]. Disruption of the intestinal epithelium not only led to severe diarrhea, but also led to overproduction of inflammatory cytokines and reactive oxygen species (ROS) [2–6]. Previous study indicated that bacterial infection is one of the major causes leading to diarrhea and disruption of intestinal epithelium for neonatal pigs [7]. Pathogenic bacteria such as the enterotoxigenic *Escherichia coli* (ETEC) strains can adhere to intestinal epithelial cells and produce enterotoxins that act on the small intestine, leading to secretion of fluids and electrolytes [7]. In last decades, antibiotics were widely used to prevent various bacteria-induced infection and diarrhea in pig production industry. However, long-term utilization or overdose utilization of antibiotics may result in drug

residues in animal products and the developing of antibiotic resistant bacteria [8–10]. Therefore, developing of substitutes for traditionally used antibiotics is solely needed.

Oligosaccharides have been looked as one of prominent alternatives for antibiotics, as they can selectively stimulate the growth of beneficial microorganisms in the gut, and improve the intestinal health [11–13]. Manno-oligosaccharides (MOS) is a non-digestible prebiotic derived from natural plants or the cell wall of yeast containing a mannan and glucan component [14]. Previously studies suggested that most prebiotics including MOS cannot be hydrolyzed in upper small intestine, but can be fermented by various bacteria in large intestine to produce short chain fatty acids (acetate, butyrate and propionate), which can facilitate the tight-junction assembly and reduce apoptosis of intestinal epithelial cells [13, 15, 16].

Importantly, MOS was found to enhance the growth performance and attenuate the gastrointestinal inflammatory response in weaned pigs and other animal species [17–19]. Although, a number of studies indicated a beneficial effect of MOS on growth performance and gut health in animals [17, 20, 21], the mechanisms of action of MOS is just beginning to be explored. Moreover, few studies investigated the protective effect of MOS on intestinal epithelium in weaned pigs exposure to ETEC challenge. The present study was conducted to determine the effect of MOS on inflammatory response, integrity and function of intestinal epithelial barrier, antioxidant capacity, and the related signaling pathways in weaned pigs upon ETEC challenge.

Materials And Methods

Animal diets and experimental design

All experimental protocols used in the animal experiment were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (No.20181105). Thirty-two male pigs (Duroc × Landrace × Yorkshire) weaned at 21 days with average body weight (BW) 6.48 ± 0.14 kg were randomly assigned into a 2 (MOS) × 2 (ETEC) factorial experiment of four treatments (n = 8) that comprised CON (pigs were fed with a basal diet), CMOS (pigs were fed with a 3 g/kg MOS containing diet), ECON (pigs were fed with a basal diet and challenged by ETEC), EMOS (pigs were fed with 3 g/kg MOS containing diet and challenged by ETEC). MOS (with a purity of 98.5%) were purchased from Sichuan Junzheng Bio. Co., Ltd. The feeding trial lasted for 21 days. On 19 d, the challenge groups were orally dosed with 150 mL of a culture medium containing 1×10^{10} CFU/mL of ETEC culture by using an orogastric tube last for 3 d, whereas the non-challenge groups were orally equivalent amount of culture medium with the same method [22] (serotype O149:K91: K88ac; China Institute of Veterinary Drugs Control, Beijing, China). The corn-soybean based diets (Table 1) were formulated based on National Research Council 2012 [23]. Pigs were individually housed in 1.5×0.7 m^2 metabolism cage and were given ad libitum access to feed and fresh water with the room temperature controlled between 25–28 ℃, relative humidity 65% ± 5%.

Table 1
Experiment basal diet composition and nutrient level

Ingredients	%	nutrient level	contents
Corn	28.31	Digestible energy (calculated, MJ/kg)	14.78
Extruded corn	24.87	Crude Protein (%)	19.68
Soybean meal	8.50	Calcium (%)	0.81
Extruded full-fat soybean	10.30	Available phosphorus (%)	0.55
Fish meal	4.20	Lysine	1.35
Whey powder	7.00	Methionine	0.42
Soybean protein concentrate	8.00	Methionine + cysteine	0.60
Soybean oil	2.00	Threonine	0.79
Sucrose	4.00	Tryptophan	0.22
Limestone	0.90		
Dicalcium phosphate	0.50		
NaCl	0.30		
L -Lysine HCl (78%)	0.47		
DL-Methionine	0.15		
L -Threonine (98.5%)	0.13		
Tryptophan (98%)	0.03		
Chloride choline	0.10		
Vitamin premix [1]	0.04		
Mineral premix [2]	0.20		
Total	100		

[1]The vitamin premix provided the following per kg of diet: 9000 IU of VA, 3000 IU of VD 3, 20 IU of VE, 3 mg of VK 3, 1.5 mg of VB1, 4 mg of VB 2, 3 mg of VB6, 0.02 mg of VB12, 30 mg of niacin, 15 mg of pantothenic acid, 0.75 mg of folic acid, and 0.1 mg of biotin. [2] The mineral premix provided the following per kg of diet: 100 mg Fe, 6 mg Cu, 100 mg Zn, 4 mg Mn, 0.30 mg I, 0.3 mg Se.

Sample collection

On the d 22 morning of the experiment, after fasting for 12 h, all pigs were weighed and recorded. Subsequently, blood samples were obtained by jugular vein puncture and placed in 10 mL vacuum tubes. The serum was collected after centrifugation at 3,500 × g for 15 minutes (4 ℃) and stored at − 20 °C until the serum indexes analysis. Following the blood sampling, all pigs were killed with sodium pentobarbital

(200 mg kg^{-1} BW) to collect intestinal samples. Approximately 4 cm duodenal, jejunal and ileal middle segments were gently flushed with ice-cold phosphate-buffered saline (PBS), followed by fixation in PBS for flow cytometry or in 4% paraformaldehyde solution for immunofluorescence and immunohistochemical analyses. Besides, intestinal mucosa obtained from the residual intestinal segments with a scalpel blade and stored at -80 ℃ until analysis.

Growth performance evaluation

All pigs were individually weighed at the beginning of the trial and on d 22 prior to the morning feeding, respectively. Feed intake and waste feed were recorded every day throughout the experiment which could be provided to calculate the average daily feed intake (ADFI), average daily gain (ADG), the feed: gain ratio (F: G) and diarrhea ratios during challenge period (Diarrhea incidence (%) = (total number of pigs per pen with diarrhea)/(number of pigs per pen × 3 d) × 100) were measured [24] .

Serum parameter measurements

The level of D-lactic acid, and the activities of diammine oxidase (DAO), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in serum were measured by corresponding assay kits (Nanjing Jiancheng Institute of Bioengineering, Jiangsu, China) according to the manufacturer's instructions.

Analysis of intestinal antioxidant parameters

About 0.8 g duodenal, jejunal and ileal mucosa were homogenized (1:9, w:v) in glass homogenizer with chilled 0.9% normal saline. Homogenates were collected after centrifuging at 3500 g (4 °C) for 15 min. Glutathione peroxidase (GSH-Px), catalase (CAT), total antioxidant capacity (T-AOC), Malondialdehyde (MDA) and total superoxide dismutase (T-SOD) were measured by using corresponding diagnostic kits (Nanjing Jiancheng Institute of Bioengineering, Jiangsu, China) with UV-VIS Spectrophotometer (Leng Guang SFZ1606017568, Shanghai, China).

Flow cytometry assays

Jejunal epithelial cells were isolated to measure the proportion of apoptotic cells by flow cytometry with a PE Annexin V Apoptosis Detection Kit I (Becton, Dickinson and Company, BD Biosciences, San Jose, CA, USA) [25]. Briefly, the excised mucosal layer of jejunum was isolated, subsequently, ground and filtered to form a cell suspension. Cells were carefully washed twice with ice-cold PBS and suspended in cell culture medium at 1×10^6 cells/mL. After adding 5 μL of PE Annexin V and 5 μL of 7-aminoactinomycin D (7-AAD) to a 100-μL aliquot of the cell suspension, the mixture was incubated at room temperature for 15 min in a dark room. Then, adding 400 μL of Annexin V Binding Buffer (1×) into reaction liquid and mixed thoroughly. The apoptotic cells were examined by flow cytometry (CytoFlex, Beckman Coulter, Inc., Brea, CA, USA) within 1 h.

Immunofluorescence analysis

The localization of zonula occludins-1 (ZO-1) protein in jejunal tissues was determined by immunofluorescence followed the method as previously described [26]. Samples fixed with 4% paraformaldehyde were rinsed in PBS, and then incubated with ethylene diamine tetraacetic acid (EDTA,

1 mol/L, pH 9.0, Gooddbio Technology CO., LTD., Wuhan, China) for antigen retrieval. 3% bovine serum albumin (BSA) used to block Tissue sections prior to incubation with rabbit anti-ZO-1 polyclonal antibody (1 : 200; Abcam Plc., Cambridge, UK) overnight at 4 °C. Slides were then washed several times with PBS and incubated with goat anti-rabbit IgG-FITC secondary antibody (Gooddbio Technology Co., Ltd, Wuhan, China) at room temperature for 1 h in the dark. After rinsing in PBS, the slides were stained with 4'-6-diamidino-2-phenylindole (DAPI, Gooddbio Technology Co., Ltd, Wuhan, China) at room temperature for 10 min in the dark, to identify nuclei. The ZO-1 immunofluorescence images were captured using the NIKON DS-U3 software laser scanning confocal microscope (NIKON ECLIPSE C1).

Immunohistochemistry analysis of mucosal sIgA

To determine the abundance of sIgA in the intestinal epithelium, the 4% paraformaldehyde-fixed jejunal samples were embedded in paraffin wax blocks and sectioned into 2 μm thickness, then collected on glass slides. Briefly, after being deparaffinized and hydration, these sections were soaked with 3% H_2O_2 in methanol for 10 min at room temperature, to quench endogenous peroxidase activity, subsequently, heated in 10 mmol/L citrate buffer (pH 6.0) to retrieve the antigen. After several rinses in PBS, the sections were blocked with 10% goat serum for 20 min at room temperature, to eliminate non-specific antibody binding and then incubated overnight at 4 °C with 1:200 dilution of goat anti-pig secretory immunoglobulin A (sIgA) antibody (Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China). After rinsing with PBS several times, the sections were incubated with biotinylated goat anti-rabbit IgG secondary antibody (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) at 37 °C for 30 min. After rinsing several times in PBS and incubated with 3,3-diaminobenzidine (DAB) to visualize immune complexes. The sections were counterstained with haematoxylin and mounted with glycerol gelatin. For each section in the Motic BA210 digital microscope (Motic China Group Co., Ltd., Xiamen, China), five fields of vision were randomly selected, with a fixed window area. The integrated optical density of sIgA in the jejunal mucosa was detected by using Image-Pro Plus 6.0 image analysis system (Media Cybernetics, Inc), and the sIgA protein expression was reflected by the mean value of the integrated optical density.

RNA isolation, reverse transcription, and real-time quantitative PCR

The frozen intestinal mucosa samples(about 0.1 g)were ground in liquid nitrogen and homogenized in 1 mL of RNAiso Plus (Takara Biotechnology Co., Ltd., Dalian, China) to extract total RNA followed the manufacturer's instructions, and the purity and concentration of total RNA were detected by using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Inc., Waltham, MA, USA), samples which OD260/OD280 ratio ranged from 1.8 to 2.0 were deemed appropriate. Subsequently, a volume equivalent to 1 μg total RNA from each duodenal, jejunal, and ileal sample was used for reverse transcription into cDNA, which based on the protocol of PrimeScript™ RT reagent kit with gDNA Eraser (Takara Biotechnology Co., Ltd). This process consists of two steps: I: 37 °C for 15 min, II 85 °C for 5 s.

The expression level of the target gene (tumor necrosis factor, TNF-α; interleukin-1β, IL-1β; interleukin-6, IL-6; toll-like receptor 4, TLR4;, myeloid differentiation factor 88, MyD88; nuclear factor-κB, NF-κB; nuclear factor erythroid-derived 2-related factor 2, Nrf2; heme oxygenase-1, HO-1; B-cell lymphoma-2-associated X protein,

BAX; B-cell lymphoma-2, BCL-2; cysteinyl aspartate-specific proteinase-3 (caspase-3), caspase-8 and caspase-9, Porcine β-defensin (PBD)114 and PBD129) in intestinal mucosa was quantified using q-PCR, the oligonucleotide primers sequences used in q-PCR were presented in Table S1, qPCR was performed with the SYBR® Green PCR I PCR reagents (Takara Bio Inc., Dalian, China) using a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). All cDNA samples were detected in triplicate. The reaction mixture (10 μL) contained 5 μL SYBR Premix Ex Taq II (Tli RNaseH Plus), 0.5 μL forward primer, 0.5 μL reverse primer, 1 μL cDNA and 3 μL RNase-Free water. The protocol used in q-PCR was as followed: 95 °C for 30 s, followed by 40 cycles: at 95 °C for 5 s and 60 °C for 34 s. The generated Gene-specific amplification products were confirmed by melting curve analysis after each real-time quantitative PCR assay. The housekeeping gene β-actin was used to standardize the mRNA expression level of target genes, which calculated based on the $2^{-\Delta\Delta Ct}$ method [27].

Result

Effect of MOS on growth performance and diarrhea incidence in weaned pigs upon ETEC challenge

The growth performance was presented in Table 2. There were no differences in ADG, ADFI, and F: G among the four treatments throughout the 21 d experimental period ($P > 0.05$). ETEC challenge increased the diarrhea incidence of pigs; however, MOS supplementation significantly reduced the diarrhea incidence in the ETEC-challenged pigs ($P < 0.05$).

Table 2
Effect of MOS on growth performance of weaned pigs throughout the experiment

ITEM	Treatments				SEM	P-value		
	CON	CMOS	ECON	EMOS		MOS	ETEC	Interaction
1–21 d								
ADG(g/d)	316.67	321.24	295.90	314.57	10.17	0.60	0.54	0.75
ADFI(g/d)	440.88	447.81	428.55	442.13	16.27	0.78	0.80	0.93
F: G	1.39	1.39	1.45	1.40	0.03	0.69	0.59	0.68
Diarrhea ratios (%)	29.17[b]	16.67 [b]	58.33[a]	25.00 [b]	18.12	0.01	0.04	0.26

[1] Mean and total SEM are list in Separate columns (n = 8). [2]ADG, average daily gain; ADFI, average daily feed intake; F: G, feed: gain ratio. [3]CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet (3 g/kg) and challenged by ETEC. [4]a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Effect of MOS on intestinal permeability and distribution of ZO-1 protein

The serum D-lactate content and DAO activity were higher in the ECON group than in other groups ($P < 0.05$). However, MOS supplementation significantly decreased the D-lactate content and DAO activity in the serum upon ETEC challenge (Fig. 1A and B). MOS supplementation also decreased the serum concentrations of AST and ALT in the ETEC-challenged pigs (Fig. 1C and D). Moreover, we explored the distribution of ZO-1, the major tight-junction-related protein, by immunofluorescence analysis (Fig. 2). As compared to the CON group, ZO-1 staining in the duodenal and jejunal epithelium of the ECON group was diffuse with little staining at the intercellular tight junction region, indicating disruption of the tight junction upon ETEC challenge. In contrast, ZO-1 was highly expressed and localized to the apical intercellular region of the duodenal and jejunal epithelium after MOS supplementation. MOS had no influence on the localization of ZO-1 in the ileal epithelium.

Effect of MOS on apoptosis of intestinal epithelial cells

As shown in Fig. 3, the late and total apoptosis rate of the intestinal epithelial cells was higher in ECON group than in other groups; however, MOS supplementation significantly decreased the late and total apoptosis rate in the jejunal epithelium ($P < 0.05$). Moreover, MOS supplementation decreased the early apoptosis rate both in the non-challenged and ETEC-challenged pigs ($P < 0.05$).

Effect of MOS on mucosal immunity

The mucosal sIgA content was determined by immunohistochemistry analysis. As shown in Fig. 4, the abundance of sIgA positive cells in the jejunal epithelium was lower in the ECON group than in other groups ($P < 0.05$). However, MOS supplementation significantly elevated the abundance of sIgA positive cells in the ETEC-challenged pigs ($P < 0.05$). ETEC challenge elevated the expression levels of inflammatory cytokines such as the TNF-α and IL-1β in the duodenal and jejunal mucosa. However, MOS supplementation significantly decreased their expression levels in the intestinal mucosa (Fig. 5). Moreover, MOS significantly decreased the expression levels of TLR4 and NF-κB in the duodenal and jejunal mucosa of the ETEC-challenged pigs ($P < 0.05$).

Effect of MOS on antioxidant capacity of the intestinal mucosa

As shown in Table 3, the mucosal content of MDA was higher in the ECON group than in other groups ($P < 0.05$); however, MOS supplementation significantly reduced its content in the duodenal and ileal mucosa. ETEC challenge decreased the content of GSH-Px in the jejunal and ileal mucosa. But MOS supplementation significantly elevated the GSH-Px content in the mucosa ($P < 0.05$). Moreover, MOS also significantly elevated the content of SOD and CAT in the jejunal and ileal mucosa upon ETEC challenge, respectively ($P < 0.05$).

Table 3
Effect of MOS on Intestinal antioxidant of weaned pigs upon ETEC challenge

ITEM	Treatments				SEM	P-value		
	CON	CMOS	ECON	EMOS		MOS	ETEC	Interaction
Duodenum								
CAT(U/gprot)	32.69	34.53	27.41	31.89	3.58	0.69	0.61	0.87
MDA(nmol/mL)	0.66[a]	0.60[b]	1.46[a]	0.65 [b]	0.11	0.02	0.02	0.04
GSH-Px(mg/gprot)	285.71	312.92	247.48	307.81	15.19	0.17	0.49	0.59
T-AOC(U/mgprot)	0.75	0.84	0.52	0.72	0.08	0.36	0.28	0.72
T-SOD((U/mgprot))	25.52	27.38	24.79	28.21	1.12	0.37	0.98	0.75
Jejunum								
CAT(U/gprot)	19.02	24.46	15.86	20.47	1.65	0.14	0.29	0.90
MDA(nmol/mL)	1.10	0.87	1.26	1.10	0.13	0.49	0.48	0.92
GSH-Px(mg/gprot)	211.70[b]	236.01[a]	123.59[b]	219.66[a]	15.70	0.04	0.07	0.20
T-AOC(U/mgprot)	0.67	0.70	0.28	0.61	0.08	0.21	0.11	0.31
T-SOD((U/mgprot))	28.08[a]	31.84[a]	16.81[b]	26.84[a]	1.86	0.04	0.02	0.33
Ileum								
CAT(U/gprot)	41.96[a]	49.11[a]	20.14[b]	43.28[a]	3.30	0.01	0.02	0.14
MDA(nmol/mL)	1.39[b]	1.01[b]	2.51[a]	1.49[b]	0.22	0.01	0.04	0.19
GSH-Px(mg/gprot)	249.87[ab]	286.35[a]	209.92[b]	262.74[a]	10.01	0.02	0.08	0.64
T-AOC(U/mgprot)	0.43	0.48	0.26	0.45	0.04	0.13	0.18	0.40
T-SOD((U/mgprot))	22.88	25.63	22.39	23.15	0.56	0.15	0.50	0.79

[1] Mean and total SEM are list in Separate columns (n = 8). [2] CAT, catalase. MDA, malonic dialdehyde. SOD, superoxide dismutase. GSH-Px, glutathione peroxidase. T-AOC, total antioxidant capacity. [3]CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet (3 g/kg) and challenged by ETEC. [4]a, b, mean values within a row with unlike superscript letters were significantly different ($P < 0.05$)

Effect of MOS on critical genes related to apoptosis and intestinal barrier functions

The expression levels of critical genes related to apoptosis and intestinal barrier functions were determined. As shown in Fig. 6, the expression levels of caspase 3, caspase 9, and Bax in the jejunal mucosa were higher in the ECON group than in other groups ($P < 0.05$). However, MOS supplementation significantly down-regulated their expressions in the mucosa ($P < 0.05$). MOS also down-regulated the expression levels of caspase 3 and caspase 8 in the duodenal mucosa upon ETEC challenge ($P < 0.05$). In contrast, MOS significantly elevated the expression level of Bcl-2 in the duodenal and jejunal mucosa upon ETEC challenge ($P < 0.05$). Moreover, MOS supplementation elevated the expression levels of HO-1 and Nrf2 in the jejunal and ileal mucosa upon ETEC challenge ($P < 0.05$). Additionally, MOS supplementation significantly up-regulated the expression of PBD114 in the duodenal mucosa ($P < 0.05$).

Discussion

The intestinal epithelium is the first line of defense against various pathogens in the intestinal lumen [2]. For neonatal mammals including the pigs, disruption of the intestinal epithelium usually leads to altering intestinal permeability, facilitating the invasion of enteric pathogenic bacteria, and triggering intestinal inflammatory response [28–30]. MOS is a nondigestible oligosaccharide that has been reported to improve the growth performance and intestinal health due to its prebiotic effects [18, 20]. In the present study, we investigated the potential of MOS against inflammation and disruption of the intestinal epithelium in weaned pigs exposure to ETEC. We found that the growth performance was not affected by MOS supplementation; however, MOS significantly decreased the diarrhea incidence upon ETEC challenge. This is probably due to its specific structure, as the enteric pathogens can attach to the mannan compounds in the gut lumen instead of the epithelium, which significantly reduced their colonization [14].

The serum D-lactic acid and DAO concentrations are two critical indices of intestinal permeability. The D-lactic acid is a special endproduct of bacteria fermentation and will be released into the blood upon disruption of the intestinal mucosa [31]. Whereas, the DAO is an intracellular enzyme synthesized by intestinal epithelium and mainly distributed in cytoplasm [32]. In the present study, ETEC challenge significantly elevated the serum concentrations of D-lactic acid and DAO, indicating disruption of the intestinal epithelium. However, MOS supplementation decreased their concentrations in the serum. Moreover, MOS also decreased the serum concentrations of ALT and AST upon ETEC challenge, which indicated that MOS can alleviate various ETEC-induced tissue damages [33, 34]. To further explore its influence on the integrity of intestinal epitehlium, we investigated the distribution of the major tight-junction-related protein ZO-1 by immunofluorescence analysis. The tight junction (TJ) proteins such as claudin-1 and ZO-1 can binds to cytoskeletons, which not only act as major constituents of the intestinal epithelial epithelium but also act as critical regulators of paracellular permeability [35, 36]. We found that the ZO-1 protein was highly expressed and localized to the apical intercellular region of the duodenal and jejunal epithelium in the ETEC-challenged pigs fed with an MOS-containing diet. The result is consistent with the measurements of the blood indices (D-lactic acid and DAO).

Previous study indicated that ETEC infection led to elevated apoptosis of the intestinal epithelial cells, which contributed to disruption of the intestinal epithelium [37, 38]. In this study, ETEC challenged significantly increased the total apoptosis rate in weaned pigs; however, MOS supplementation decreased the total apoptosis rate upon ETEC challenge. The decreased apoptosis may result from suppressing of the inflammatory response, as the MOS can prevent the attachment of ETEC to the intestinal epithelia cells [38]. Previous study indicated that simulation of inflammatory cytokines such as the TNF-α can promote distinct apoptotic pathways, including RIPK1-independent apoptosis, necroptosis, and RIPK1-dependent apoptosis (RDA) [39, 40]. In this study, MOS significantly decreased the expression levels of TNF-α and IL-1β (as well as two critical regulators of inflammation: TLR4 and NF-κB) in the jejunal mucosa, which may subsequently reduce the apoptosis of intestinal epithelial cells upon ETEC challenge.

sIgA is the most critical immunoglobulin in maintaining intestinal mucosal immunity and preventing various infections [41]. By using immunohistochemistry analysis, we found that ETEC challenge decreased the abundance of sIgA positive cells in the jejunal epithelium; however, MOS supplementation significantly elevated the abundance of sIgA positive cells in the upon ETEC challenge. Interestingly, MOS also elevated the GSH-Px and SOD content in the jejunal mucosa upon ETEC challenge, indicating an elevated antioxidant capacity by MOS. Previous study indicated that pathogenic infections usually leads to overproduction of radical oxygen species (ROS) such as the hydroxyl radicals, superoxide anions, and hydrogen peroxide, which subsequently results in oxidation of lipids, proteins, DNA, and other tissue components, causing tissue damages [12, 42, 43]. Antioxidant enzymes including the GSH-Px and SOD constitute the first line of defense against ROS [44], and elevation of their content is ,of great importance to prevent various ROS-induced tissue damages.

Finally, we explored the expression levels of critical genes related to apoptosis and intestinal barrier functions. Caspases (a family of cysteine proteases) are central regulators of apoptosis [45]. In this study, the expression levels of initiator caspase (caspase 9) and downstream effector caspases (caspase 3) were both elevated upon ETEC challenge. However, MOS significantly decreased their expressions in the jejunal mucosa. Bcl-2 is a negative regulator of apoptosis and is capable of suppressing apoptosis in a variety of cell systems [46–48]. Whereas, the Bax plays a role in the mitochondrial apoptotic process [46]. In this study, MOS enhanced the expression level of Bcl-2, but decreased the expression level of Bax in the jejunal mucosa upon ETEC challenge, which is in agreement well with the results obtained from the flow cytometry assays. Nrf2 and HO-1 are two critical genes involved in antioxidant signaling [49, 50]. Previous study indicated that various oligosaccharides can elevate the activities of antioxidant enzymes via upregulating their expressions [51–53]. In this study, MOS significantly elevated the expression levels of Nrf2 and HO-1 in the ETEC-challenged pigs, which may offer a molecular basis for its antioxidant properties. Additionally, MOS enhanced the expression level of PBD114 in the duodenal mucosa. PBD114 is a novel porcine β-defensin with antibacterial activity against *E. coli* K88 [54]. Importantly, PBD114 is a homologous protein of DEFB114, a human β-defensin with antimicrobial and anti-inflammatory activities [55, 56]. Elevated expression of PBD114 by MOS in the intestinal mucosa suggested an improved resistance to various pathogenic infections.

Conclusion

In conclusion, the present study indicated that dietary MOS supplementation can alleviate the diarrhea and disruption of the intestinal epithelium in weaned pigs exposure to ETEC. The mechanisms of action might be closely associated with improving the tight junction protein expression and distribution, suppressing cell apoptosis and intestinal inflammation, and elevating the antioxidant capacity in the intestinal epithelium. However, further researches will also be required to illustrate the exact mechanisms of action.

Declarations

Ethics approval

All experimental protocols used in the animal experiment were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (No.20181105)

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

This work was supported by the Key Research and Development Program of Sichuan Province (20ZDYF003), and the National Natural Science Foundation of China (31972599).

Authors' contributions

He Jun conceived and designed the experiments. Yu En performed the experiments and wrote the manuscript. Chen Daiwen, Yu Bing, Mao Xiangbing, Zheng Ping, Yu Jie, Huang Zhiqing, Luo Junqiu, Luo Yuheng and Yan Hui gave constructive comments for the results and discussion of the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

We thank Wang Huifen, Wu Fali, and Liu Lei for their help during the animal trial and sample collections.

References

1. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. Journal of animal science and biotechnology. J Anim Sci Biotechnol. 2013;4(1):1–4.
2. Smith F, Clark JE, Overman BL, Tozel CC, Huang JH, Rivier JEF, et al. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. Am J Physiol Gastrointest Liver

Physiol. 2010;298(3):G352–63.
3. Ewaschuk JB, Murdoch GK, Johnson IR, Madsen KL, Field CJ. Glutamine supplementation improves intestinal barrier function in a weaned piglet model of Escherichia coli infection. Br J Nutr. 2011;106(6):870–7.
4. Pié S, Lallès JP, Blazy F, Laffitte J, Sève B, Oswald IP. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. J Nutr. 2004;134(3):641–7.
5. Viswanathan VK, Hodges K, Hecht G. Enteric infection meets intestinal function: how bacterial pathogens cause diarrhoea. Nat Rev Microbiol. 2009;7(2):110–9.
6. Zhu LH, Zhao KL, Chen XL, Xu JX. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. Journal of animal science. 2012;90(8):2581–9.
7. Cutler SA, Lonergan SM, Cornick N, Johnson AK, Stahl CH. Dietary inclusion of colicin e1 is effective in preventing postweaning diarrhea caused by F18-positive Escherichia coli in pigs. Antimicrob Agents Chemother. 2007;51(11):3830–5.
8. Bach Knudsen KE. Development of antibiotic resistance and options to replace antimicrobials in animal diets. The Proceedings of the Nutrition Society. 2001;60(3):291–299.
9. Chen Y, Kwon OS, Min BJ, Son KS, Cho JH, Hong JW, Kim I-S. The Effects of Dietary Biotite V Supplementation as an Alternative Substance to Antibiotics in Growing Pigs. Asian-Australasian Journal of Animal Sciences. 2005;18(11):1642–5.
10. Thacker PA. Alternatives to antibiotics as growth promoters for use in swine production: a review. J Anim Sci Biotechnol. 2013;4(1):1–12.
11. Roberfroid M. Prebiotics: the concept revisited. J Nutr. 2007;137:830S–837S.
12. Wan J, Jiang F, Xu Q, Chen D, He J. Alginic acid oligosaccharide accelerates weaned pig growth through regulating antioxidant capacity, immunity and intestinal development. RSC Adv. 2016;6(90):87026–35.
13. Mussatto SI, Mancilha IM. Non-digestible oligosaccharides: A review. Carbohyd Polym. 2007;68(3):587–97.
14. Miguel JC, Rodriguez-Zas SL, Pettigrew JE. Efficacy of a mannan oligosaccharide (Bio-Mos) for improving nursery pig performance. J Swine Health Prod. 2004;12:296–307.
15. Gao X, Yu B, Yu J, Mao X, Huang Z, Luo Y, et al. Influences of dietary starch structure on intestinal morphology, barrier functions, and epithelium apoptosis in weaned pigs. Food function. 2020;11(5):4446–55.
16. Quintero-Villegas M. Non-digestible Oligosaccharides: Anti-adherence and Other Biological Properties. https://digitalcommons.unl.edu/foodscidiss/50. Accessed 07 Aug. 2014.
17. Zhao PY, Jung JH, Kim IH. Effect of mannan oligosaccharides and fructan on growth performance, nutrient digestibility, blood profile, and diarrhea score in weanling pigs. Journal of animal science. 2012;90(3):833–9.
18. Agazzi A, Perricone V, Omodei Zorini F, Sandrini S, Mariani E, Jiang X-R, et al. Dietary Mannan Oligosaccharides Modulate Gut Inflammatory Response and Improve Duodenal Villi Height in Post-

Weaning Piglets Improving Feed Efficiency. Animals: an open access journal from MDPI. 2020;10(8):1–14.

19. Uriel RE, Satoh S, Haga Y, Fushim H, Sweetman J. Effects of single and combined supplementation of Enterococcus faecalis, Mannan Oligosaccharide and polyhydroxybutyrate acid on growth performance and immune response of rainbow trout Oncorhynchus mykiss. Aquaculture Science. 2009;57:609–14.
20. Duan XD, Chen DW, Zheng P, Tian G, Wang JP, Mao XB, et al. Effects of dietary mannan oligosaccharide supplementation on performance and immune response of sows and their offspring. Anim Feed Sci Technol. 2016;218:17–25.
21. Jahanian R, Ashnagar M. Effect of dietary supplementation of mannan-oligosaccharides on performance, blood metabolites, ileal nutrient digestibility, and gut microflora in Escherichia coli-challenged laying hens. Poultry science. 2015;94(9):2165–72.
22. Liu P, Piao XS, Thacker PA, Zeng ZK, Li PF, Wang D, Kim SW. Chito-oligosaccharide reduces diarrhea incidence and attenuates the immune response of weaned pigs challenged with Escherichia coli K88. Journal of animal science. 2010;88(12):3871–9.
23. National Research Council. Nutrient requirements of swine: eleventh revised edition. Washington, D.C.: The National Academies Press; 2012.
24. Chen H, Mao X, He J, Yu B, Huang Z, Yu J, et al. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets. Br J Nutr. 2013;110(10):1837–48.
25. Yu Z, Wang F, Liang N, Wang C, Peng X, Fang J, et al. Effect of Selenium Supplementation on Apoptosis and Cell Cycle Blockage of Renal Cells in Broilers Fed a Diet Containing Aflatoxin B1. Biol Trace Elem Res. 2015;168(1):242–51.
26. Wang G, Yu Y, Tu Y, Tong J, Liu Y, Zhang C, et al. Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus Infection Induced Apoptosis and Autophagy in Thymi of Infected Piglets. PloS one. 2015;10(6):1–12.
27. Fleige S, Walf V, Huch S, Prgomet C, Sehm J, Pfaffl MW. Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. Biotechnol Lett. 2006;28(19):1601–13.
28. Wang H, Zhang C, Wu G, Sun Y, Wang B, He B, et al. Glutamine enhances tight junction protein expression and modulates corticotropin-releasing factor signaling in the jejunum of weanling piglets. J Nutr. 2015;145(1):25–31.
29. Bjarnason I, Macpherson A, Hollander D. Intestinal permeability: An overview. Gastroenterology. 1995;108(5):1566–81.
30. Francis D, Grange P, Zeman D, Baker D, Sun R, Erickson A. Expression of Mucin-Type Glycoprotein K88 Receptors Strongly Correlates with Piglet Susceptibility to K88 + Enterotoxigenic Escherichia coli, but Adhesion of This Bacterium to Brush Borders Does Not. Infect Immun. 1998;66:4050–5.
31. Chen J, Yu B, Chen D, Huang Z, Mao X, Zheng P, et al. Chlorogenic acid improves intestinal barrier functions by suppressing mucosa inflammation and improving antioxidant capacity in weaned pigs. J Nutr Biochem. 2018;59:84–92.

32. Thompson JS, Vaughan WP, Forst CF, Jacobs DL, Weekly JS, Rikkers LF. The effect of the route of nutrient delivery on gut structure and diamine oxidase levels. JPEN J Parenter Enteral Nutr. 1987;11:28–32.
33. Chowdhury MSI, Rahman ABMZ, Haque M, Nahar N. Taher A. Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Levels in Different Grades of Protein Energy Malnutrition. Journal of Bangladesh Society of Physiologist. 2007;2:17–9.
34. Bastos RKG, Meneses AMC, Almeida VT, Pereira LHC, Souza NF, Moraes CCG, et al. Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Determination in Dogs From Belem, Pará State, Brazil. https://www.vin.com/apputil/content/defaultadv1.aspx?pId=11290&id=4252859. Accessed 21–24 July. 2009.
35. Vermette D, Hu P, Canarie MF, Funaro M, Glover J, Pierce RW. Tight junction structure, function, and assessment in the critically ill: a systematic review. Intensive Care Med Exp. 2018;6(1):1–18.
36. Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. J Nutr. 2011;141(5):769–76.
37. Wan J, Zhang J, Chen D, Yu B, Mao X, Zheng P, et al. Alginate oligosaccharide alleviates enterotoxigenic Escherichia coli-induced intestinal mucosal disruption in weaned pigs. Food function. 2018;9(12):6401–13.
38. Deng Y, Han X, Tang S, Li C, Xiao W, Tan Z. Magnolol and Honokiol Attenuate Apoptosis of Enterotoxigenic Escherichia Coli-Induced Intestinal Epithelium by Maintaining Secretion and Absorption Homeostasis and Protecting Mucosal Integrity. Med Sci Monit. 2018;24:3348–56.
39. Herbein G, O'brien WA. Tumor Necrosis Factor (TNF)-α and TNF Receptors in Viral Pathogenesis. Proceedings of the Society for Experimental Biology and Medicine. 2000;223(3):241–257.
40. Slotta C, Storm J, Pfisterer N, Henkel E, Kleinwächter S, Pieper M, et al. IKK1/2 protect human cells from TNF-mediated RIPK1-dependent apoptosis in an NF-κB-independent manner. Biochim Biophys Acta Mol Cell Res. 2018;1865(8):1025–33.
41. Williams RC, Gibbons RJ. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science. 1972;177:697–9.
42. Zhao R, Shen G. Functional modulation of antioxidant enzymes in vascular endothelial cells by glycated LDL. Atherosclerosis. 2005;179:277–84.
43. Zhang Q, Piao X-L, Piao X-S, Lu T, Wang D, Kim S. Preventive effect of Coptis chinensis and berberine on intestinal injury in rats challenged with lipopolysaccharides. Food Chem Toxicol. 2011;49:61–9.
44. Rodriguez C, Mayo J, Sainz R, Antolín I, Herrera F, Martín V, Reiter R. Regulation of antioxidant enzymes: A significant role for melatonin. Journal of pineal research. 2004;36:1–9.
45. Nuñez G, Benedict MA, Hu Y, Inohara N. Caspases: the proteases of the apoptotic pathway. Oncogene. 1998;17(25):3237–45.
46. Chauhan AK, Mittra N, Kumar V, Patel DK, Singh C. Inflammation and B-cell Lymphoma-2 Associated X Protein Regulate Zinc-Induced Apoptotic Degeneration of Rat Nigrostriatal Dopaminergic Neurons. Mol Neurobiol. 2016;53(8):5782–95.

47. Wang Y, Sun Z, Zhang K, Xu G, Li G. Bcl-2 in suppressing neuronal apoptosis after spinal cord injury. World J Emerg Med. 2011;2:38–44.
48. Hockenbery D, Nuñez G, Milliman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature. 1990;348(6299):334–6.
49. Cheng L, Jin Z, Zhao R, Ren K, Deng C, Yu S. Resveratrol attenuates inflammation and oxidative stress induced by myocardial ischemia-reperfusion injury: Role of Nrf2/ARE pathway. Int J Clin Exp Med. 2015;8:10420–8.
50. Liu X, Gu X, Yu M, Zi Y, Yu H, Wang Y, et al. Effects of ginsenoside Rb1 on oxidative stress injury in rat spinal cords by regulating the eNOS/Nrf2/HO-1 signaling pathway. Experimental therapeutic medicine. 2018;16(2):1079–86.
51. Zhang H, Wang J, Liu Y, Sun B. Wheat bran feruloyl oligosaccharides modulate the phase II detoxifying/antioxidant enzymes via Nrf2 signaling. Int J Biol Macromol. 2015;74:150–4.
52. Xiang X-W, Zhang Z-S, Zhou Y-F, Zhou T-Y, Sun P-L, Zheng B. Protective effect of seleno-amino-oligosaccharide on oxidative damage of IPEC-1 cells by activating Keap1/Nrf2 signaling pathway. Int J Biol Macromol. 2020;155:972–8.
53. Varasteh S, Braber S, Garssen J, Fink-Gremmels J. Galacto-oligosaccharides exert a protective effect against heat stress in a Caco-2 cell model. Journal of Functional Foods. 2015;16:265–77.
54. Su G, Xie K, Chen D, Yu B, Huang Z, Luo Y, et al. Differential expression, molecular cloning, and characterization of porcine beta defensin 114. J Anim Sci Biotechnol. 2019;10:60.
55. Chen J, Qi S, Guo R, Yu B, Chen D. Different messenger RNA expression for the antimicrobial peptides beta-defensins between Meishan and crossbred pigs. Mol Biol Rep. 2010;37:1633–9.
56. Yu H, Dong J, Gu Y, Liu H, Xin A, Shi H, et al. The novel human β-defensin 114 regulates lipopolysaccharide (LPS)-mediated inflammation and protects sperm from motility loss. J Biol Chem. 2013;288:12270–82.

Figures

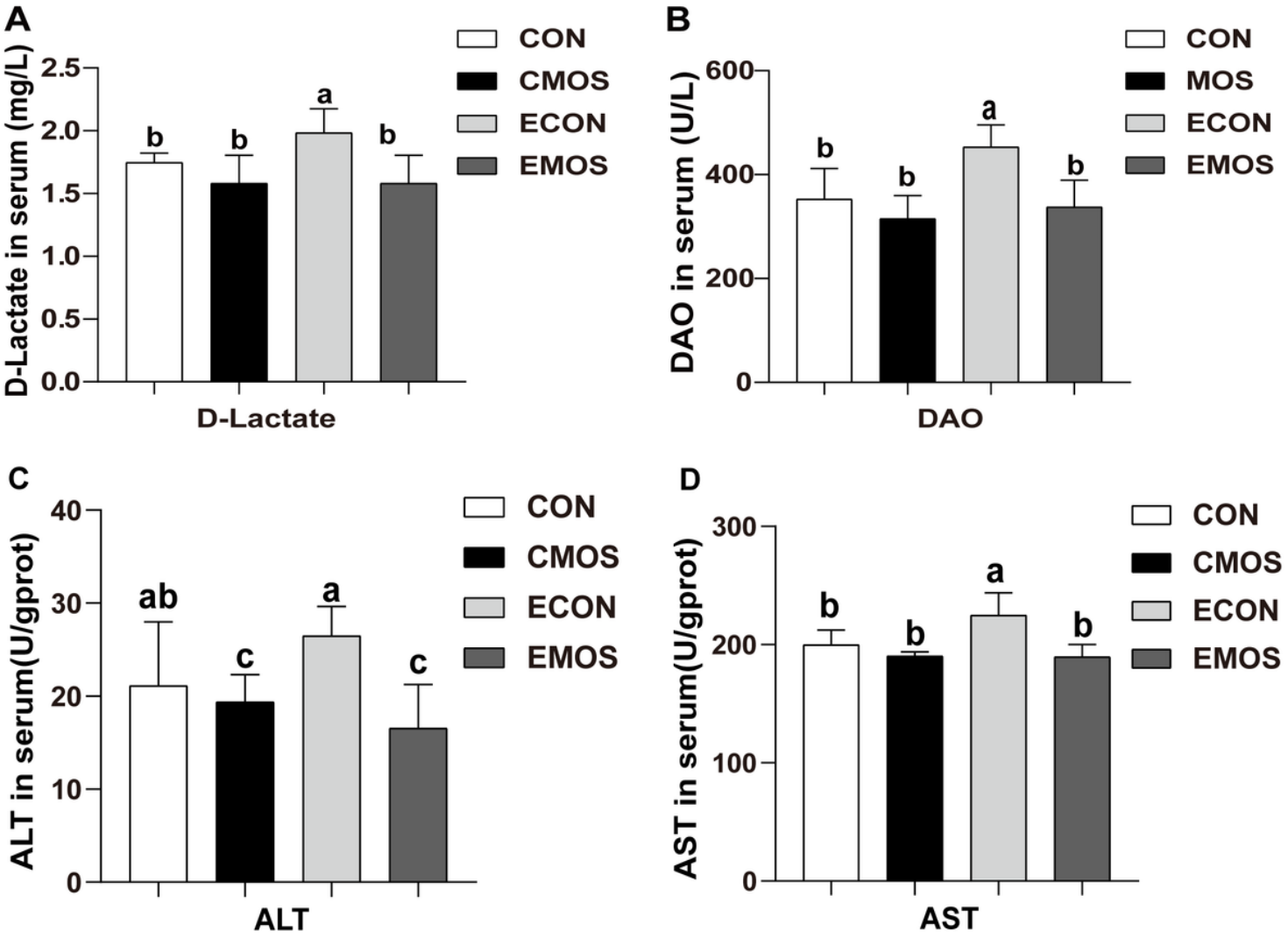

Figure 1

Effect of MOS supplementation on maintaining weaned pigs intestinal integrity after challenge with ETEC. D-lactic acid, Aspartate Aminotransferase, AST; Alanine Aminotransferase, ALT iammine oxidase, DAO. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC.

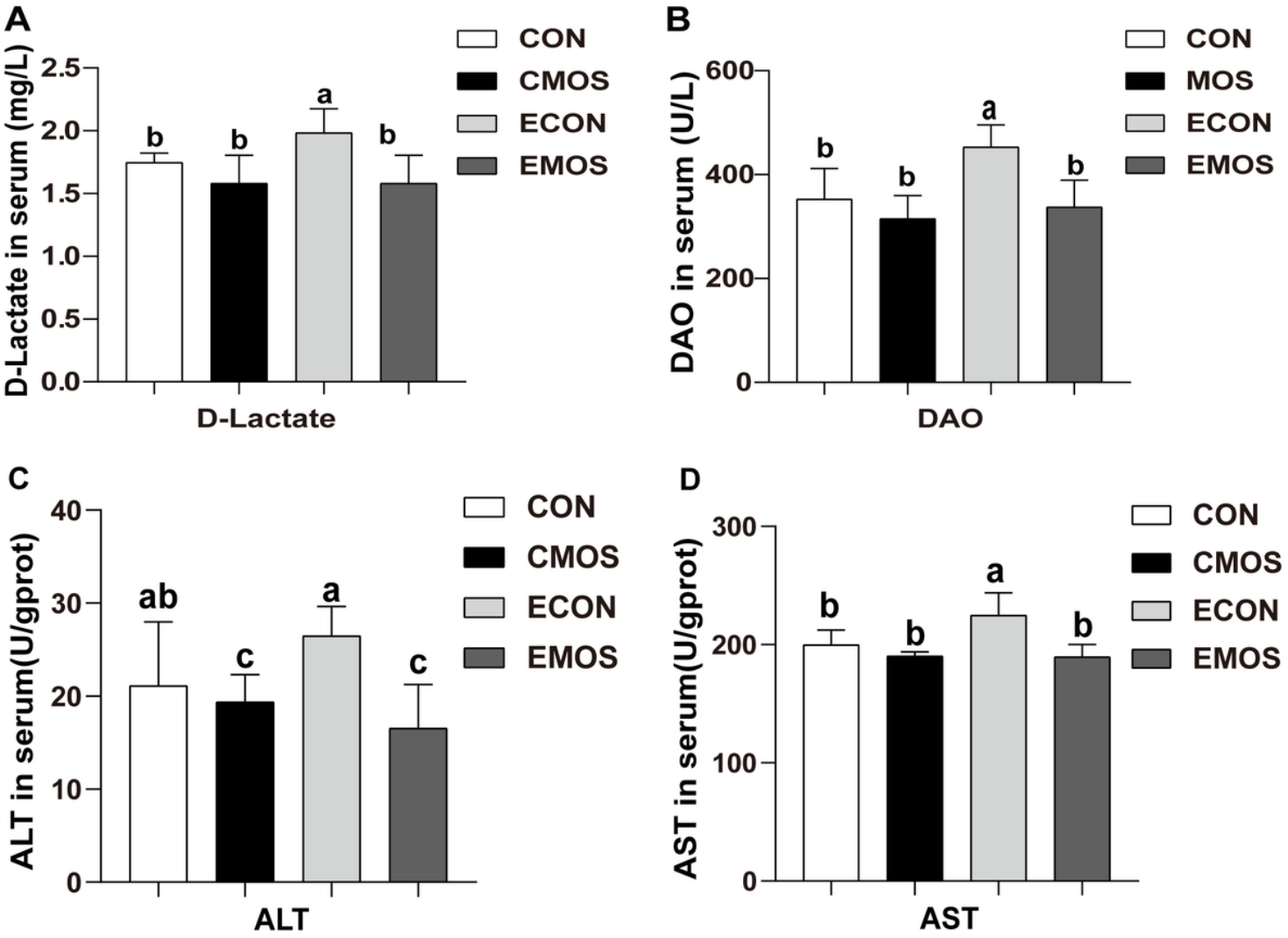

Figure 1

Effect of MOS supplementation on maintaining weaned pigs intestinal integrity after challenge with ETEC. D-lactic acid, Aspartate Aminotransferase, AST; Alanine Aminotransferase, ALT iammine oxidase, DAO. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC.

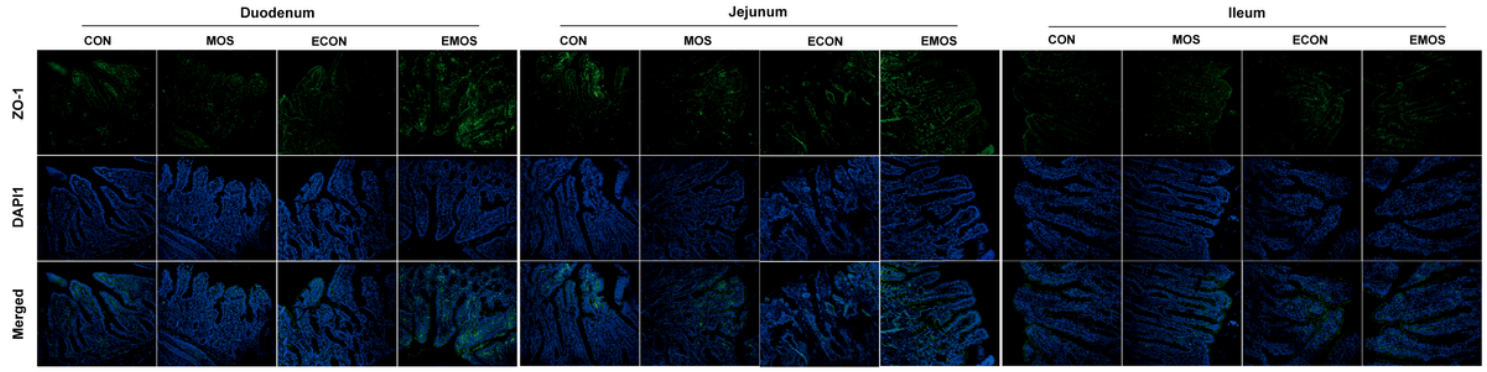

Figure 2

Effect of MOS supplementation on ZO-1 and and DAPI (DNA) distribution in intestine of weaned pigs after challenge with ETEC. zonula occludens 1, ZO-1; 4′-6-diamidino-2-phenylindole, DAPI. ZO-1 protein (green), DAPI stain (blue), and merged ZO-1 protein and DAPI are shown. CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC.

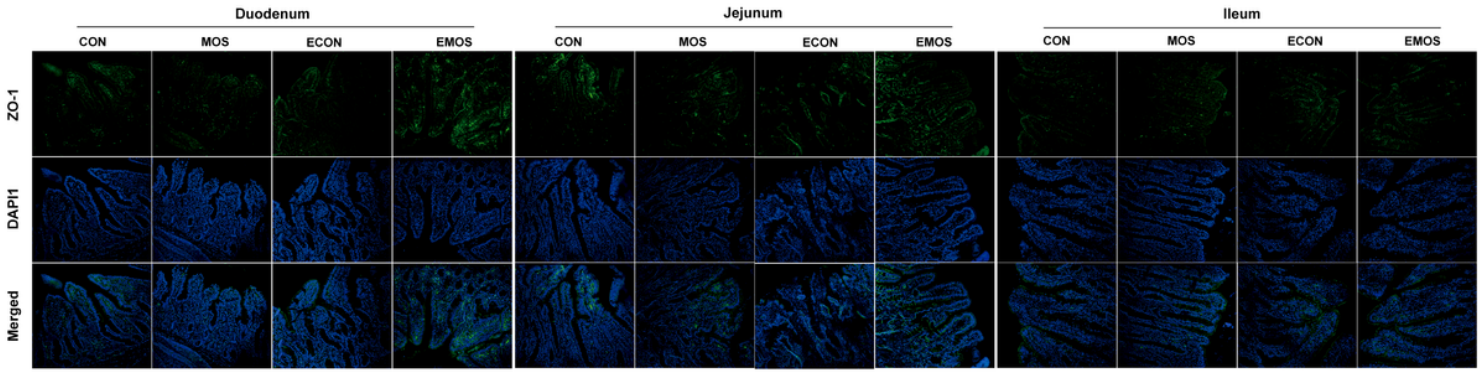

Figure 2

Effect of MOS supplementation on ZO-1 and and DAPI (DNA) distribution in intestine of weaned pigs after challenge with ETEC. zonula occludens 1, ZO-1; 4′-6-diamidino-2-phenylindole, DAPI. ZO-1 protein (green), DAPI stain (blue), and merged ZO-1 protein and DAPI are shown. CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC.

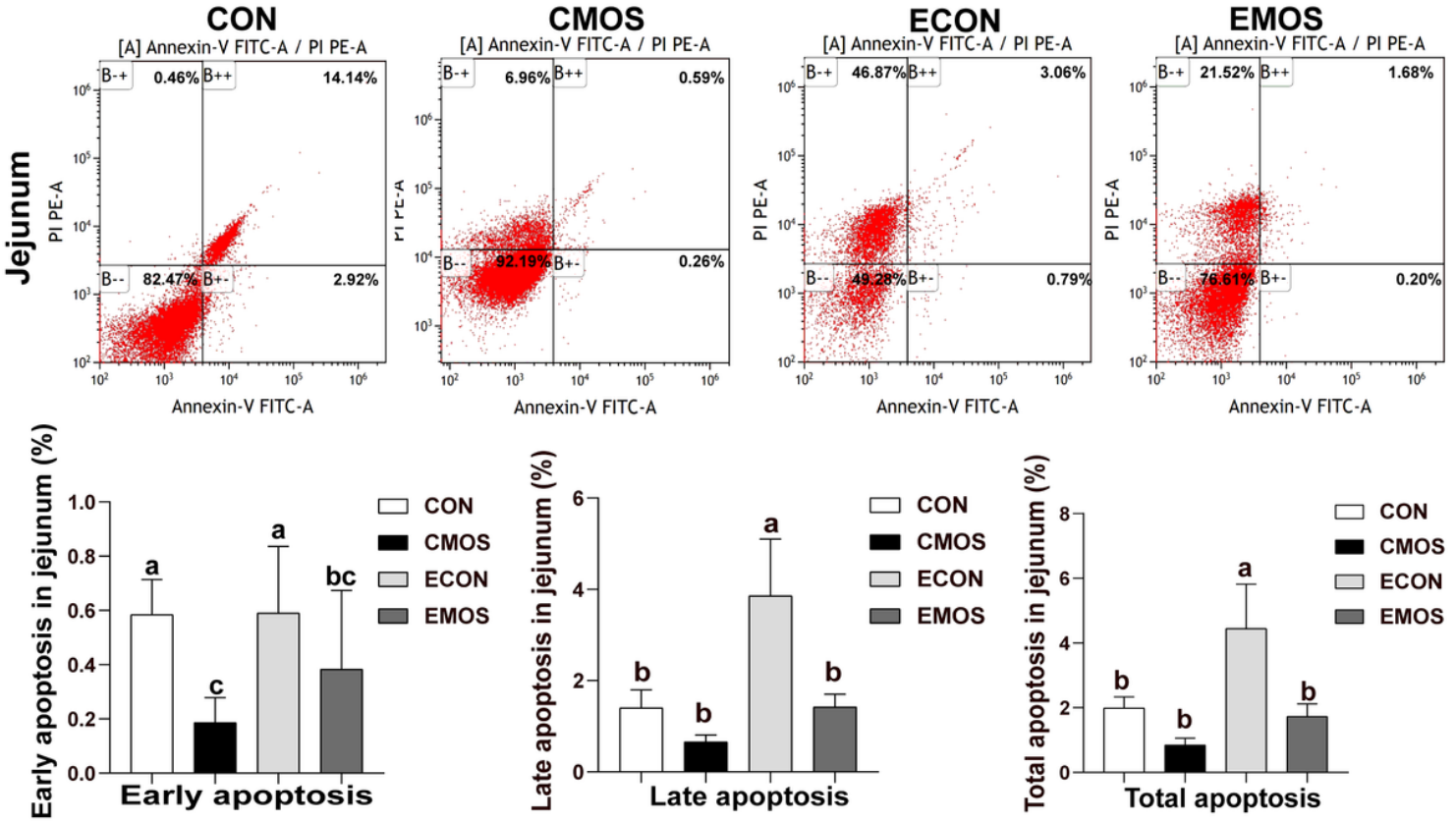

Figure 3

Effects of MOS on the percentage of apoptotic cells in the small intestine of weaned pigs after ETEC challenge a, b, c mean values within a row with unlike superscript letters were significantly different (P < 0.05). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

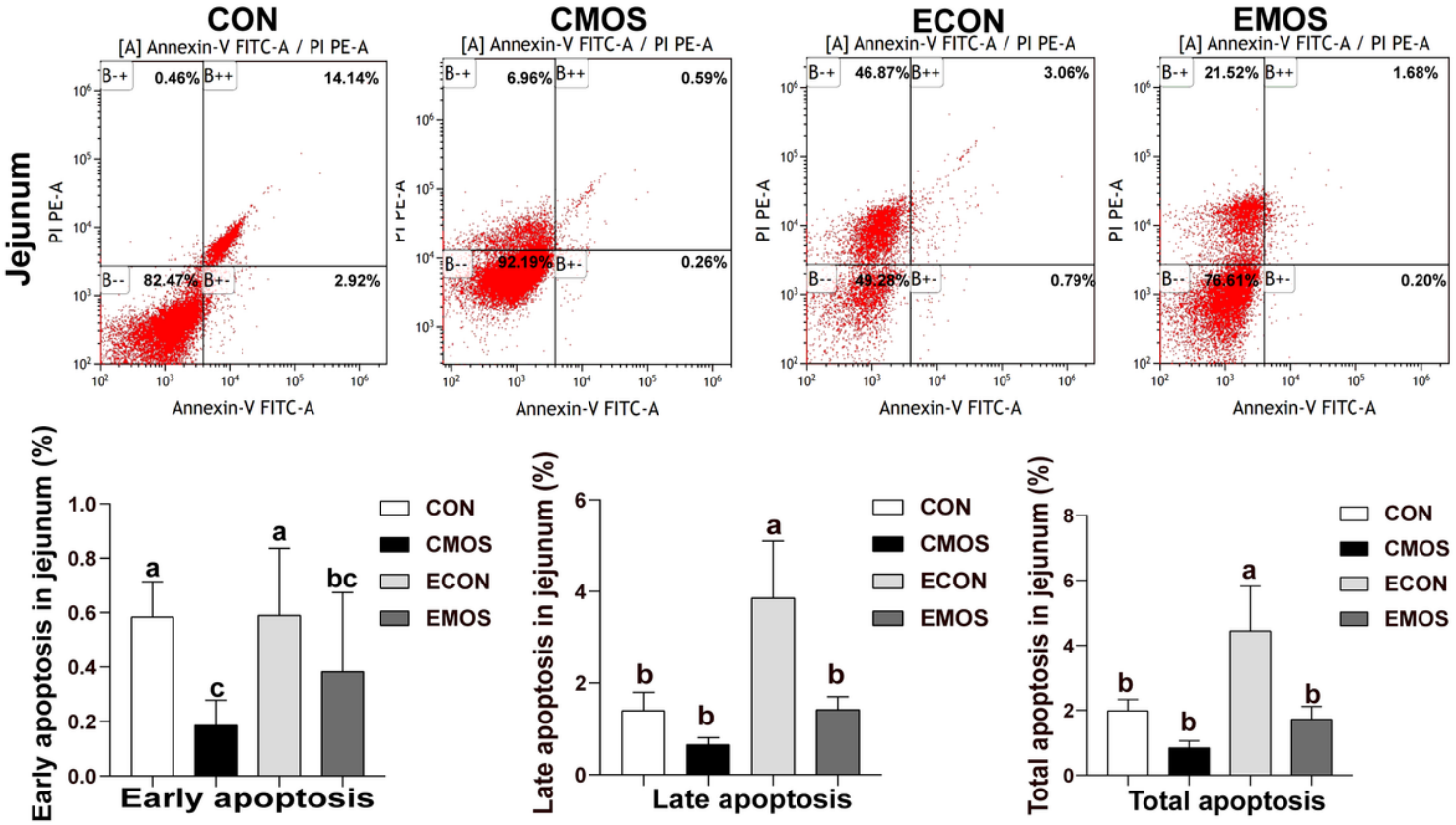

Figure 3

Effects of MOS on the percentage of apoptotic cells in the small intestine of weaned pigs after ETEC challenge a, b, c mean values within a row with unlike superscript letters were significantly different (P < 0.05). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

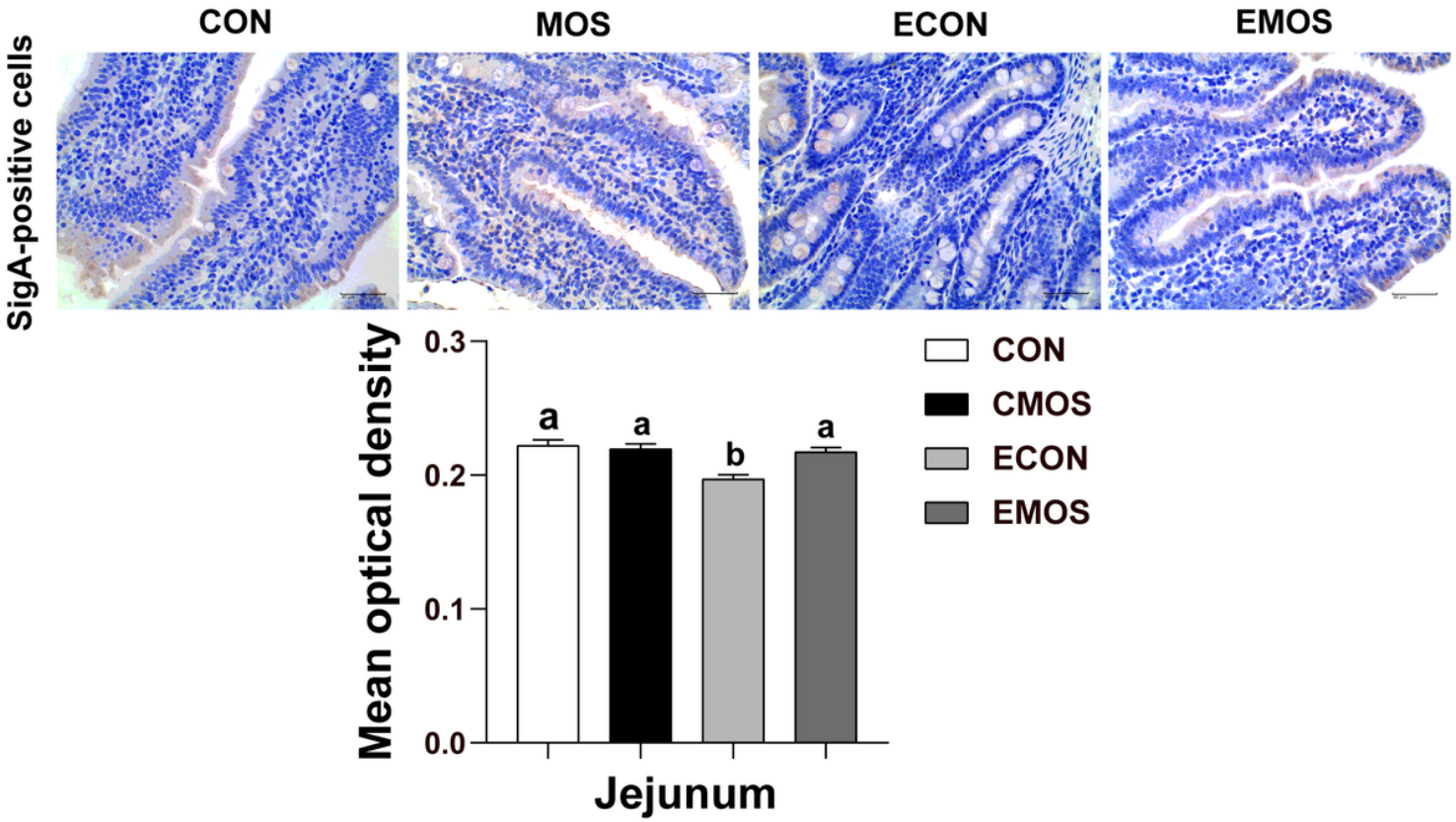

Figure 4

Effects of MOS on the sIgA content in the jejunum of weaned pigs after ETEC challenge. (immunohistochemistry; × 400). secretory immunoglobulin A, sIgA. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

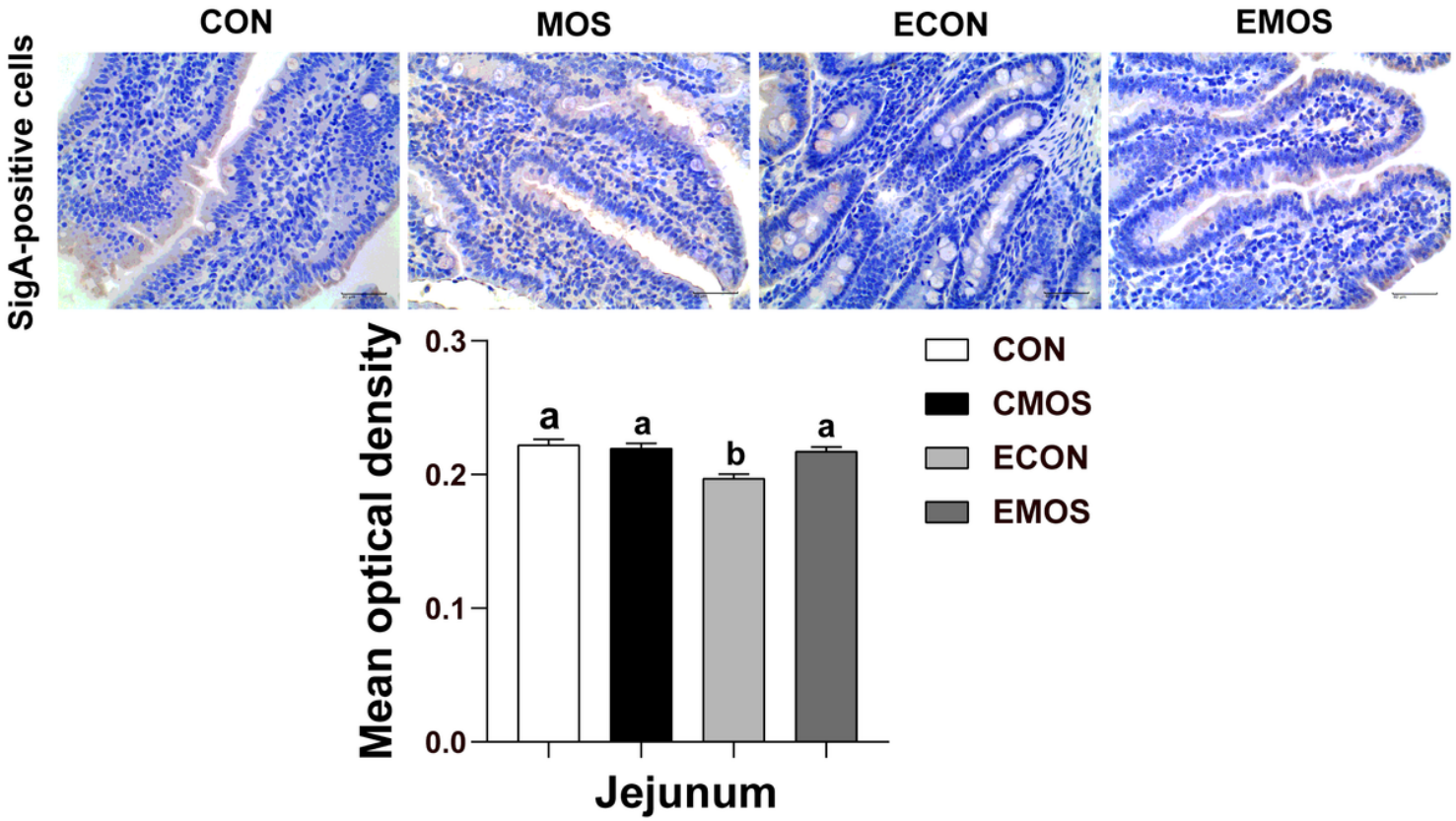

Figure 4

Effects of MOS on the sIgA content in the jejunum of weaned pigs after ETEC challenge. (immunohistochemistry; × 400). secretory immunoglobulin A, sIgA. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

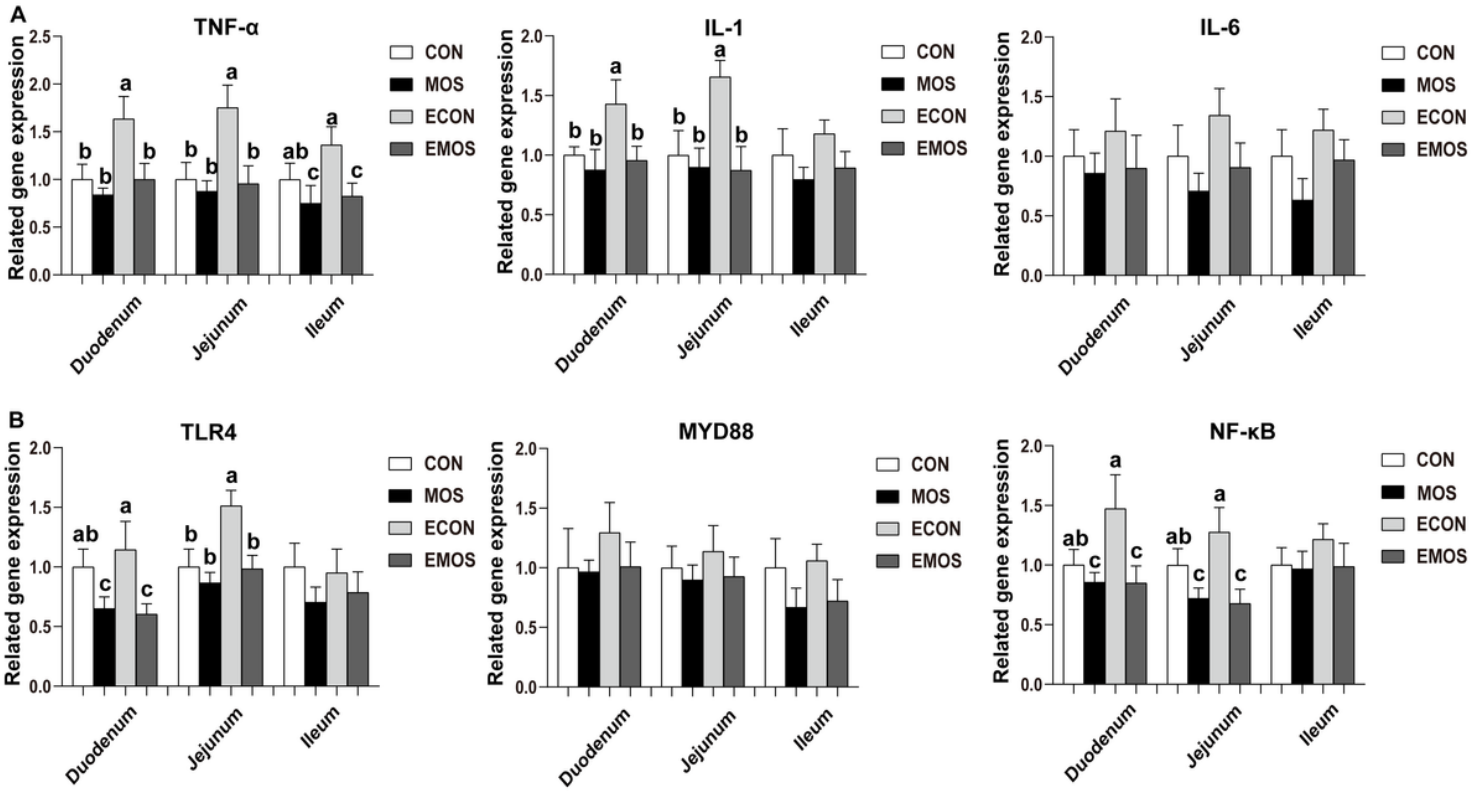

Figure 5

Effect of MOS supplementation on intestinal mucosal related gene expressions in weaned pigs upon ETEC challenge tumor necrosis factor, TNF-α; interleukin-1β, IL-1β; interleukin-6, IL-6; toll-like receptor 4, TLR4;, myeloid differentiation factor 88, MyD88; nuclear factor-κB, NF-κB; nuclear factor erythroid-derived 2-related factor 2, Nrf2; heme oxygenase-1, HO-1; B-cell lymphoma-2-associated X protein, BAX; B-cell lymphoma-2, BCL-2; cysteinyl aspartate-specific proteinase-3 (caspase-3), caspase-8 and caspase-9, Porcine β-defensin (PBD)114 and PBD129. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

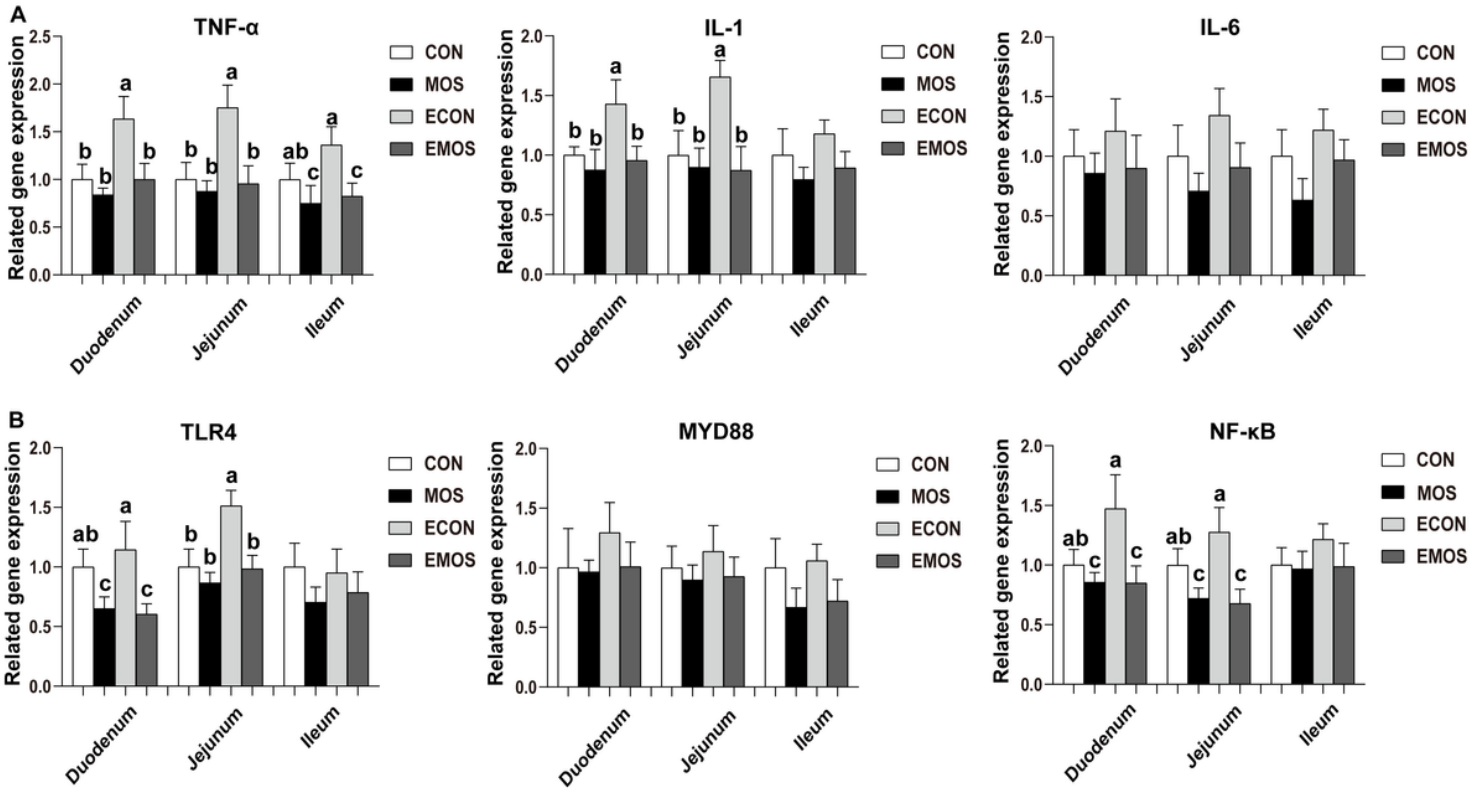

Figure 5

Effect of MOS supplementation on intestinal mucosal related gene expressions in weaned pigs upon ETEC challenge tumor necrosis factor, TNF-α; interleukin-1β, IL-1β; interleukin-6, IL-6; toll-like receptor 4, TLR4;, myeloid differentiation factor 88, MyD88; nuclear factor-κB, NF-κB; nuclear factor erythroid-derived 2-related factor 2, Nrf2; heme oxygenase-1, HO-1; B-cell lymphoma-2-associated X protein, BAX; B-cell lymphoma-2, BCL-2; cysteinyl aspartate-specific proteinase-3 (caspase-3), caspase-8 and caspase-9, Porcine β-defensin (PBD)114 and PBD129. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

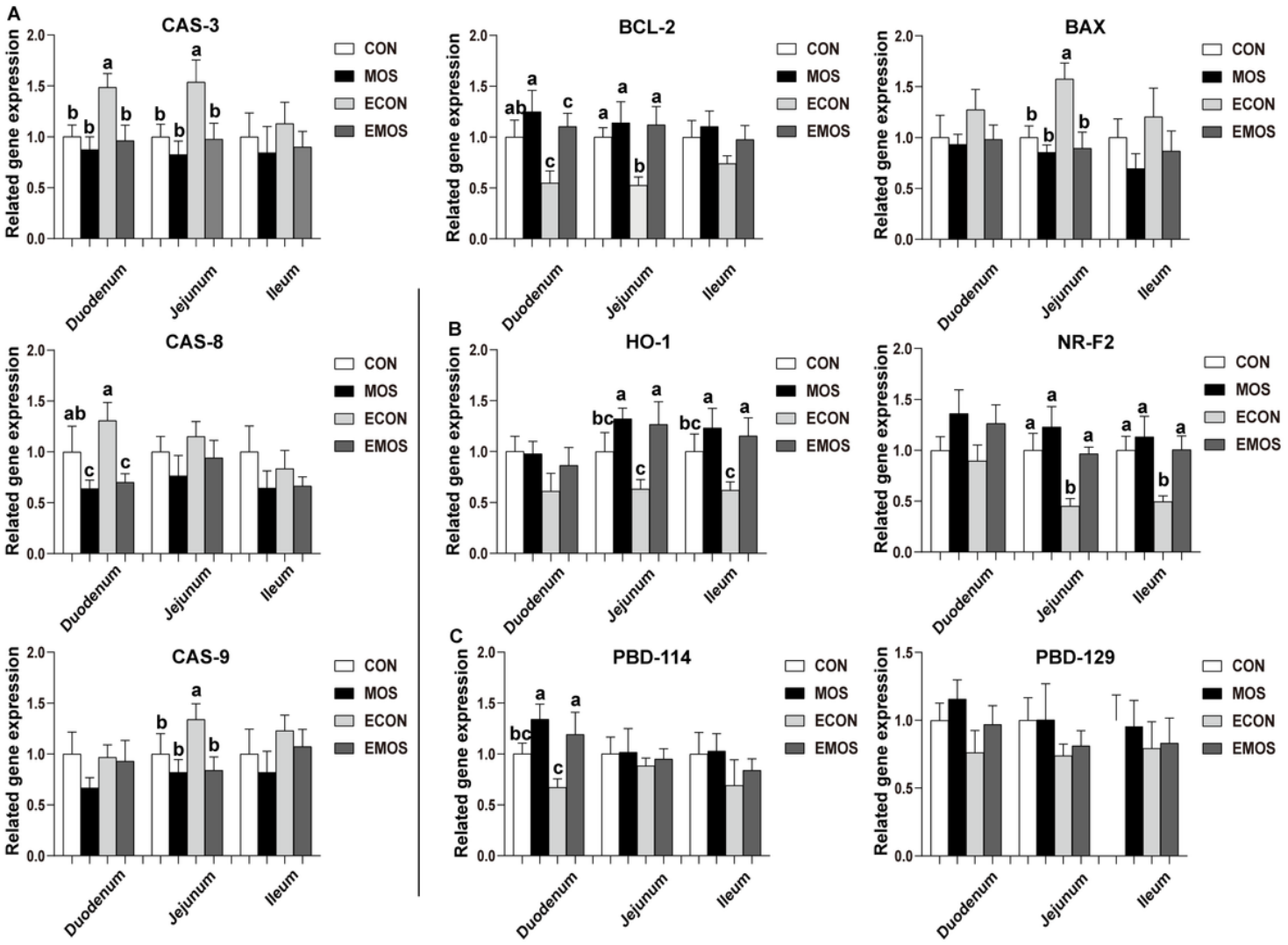

Figure 6

Effect of MOS supplementation on intestinal mucosal related gene expressions in weaned pigs upon ETEC challenge tumor necrosis factor, TNF-α; interleukin-1β, IL-1β; interleukin-6, IL-6; toll-like receptor 4, TLR4;, myeloid differentiation factor 88, MyD88; nuclear factor-κB, NF-κB; nuclear factor erythroid-derived 2-related factor 2, Nrf2; heme oxygenase-1, HO-1; B-cell lymphoma-2-associated X protein, BAX; B-cell lymphoma-2, BCL-2; cysteinyl aspartate-specific proteinase-3 (caspase-3), caspase-8 and caspase-9, Porcine β-defensin (PBD)114 and PBD129. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

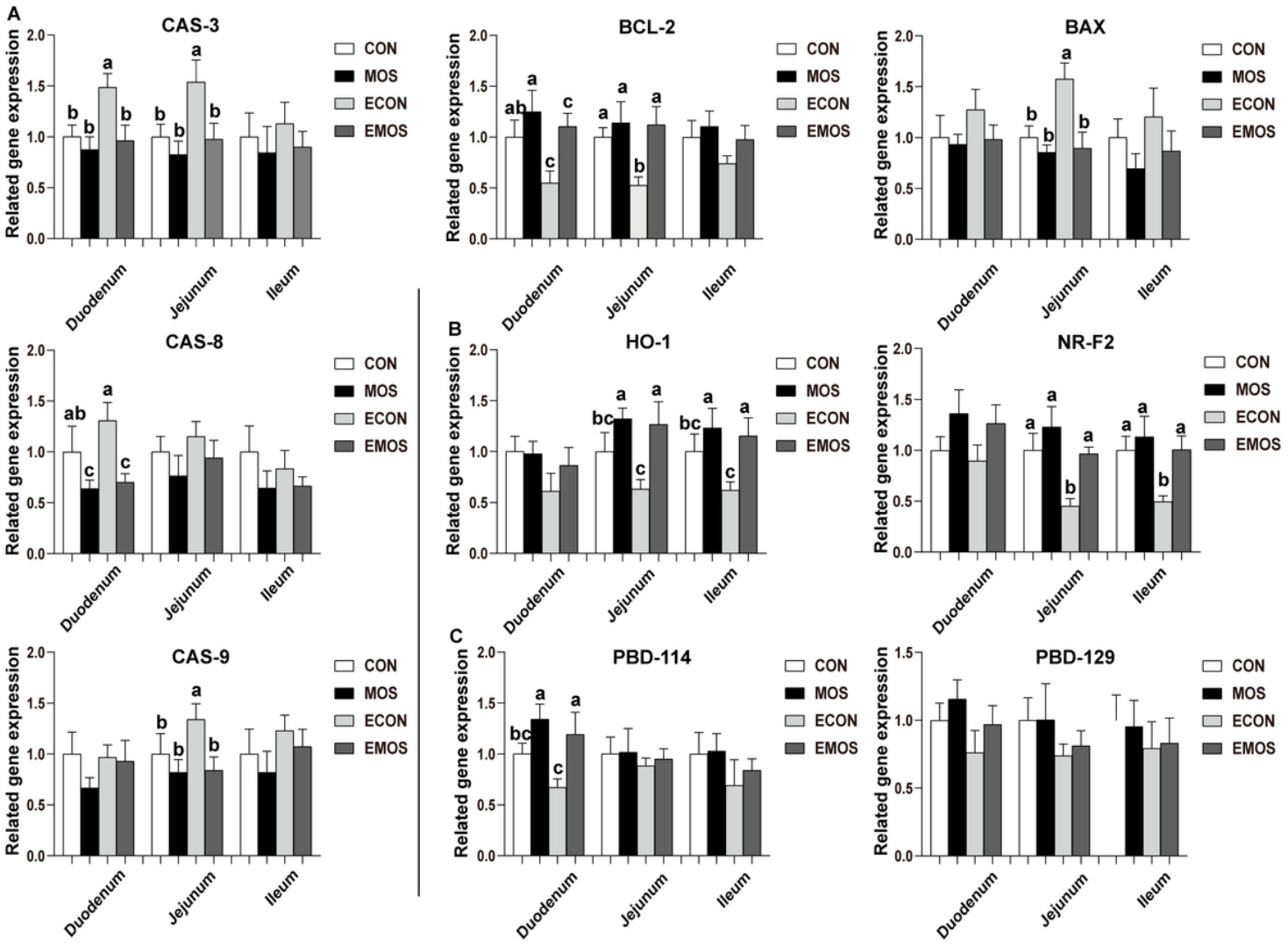

Figure 6

Effect of MOS supplementation on intestinal mucosal related gene expressions in weaned pigs upon ETEC challenge tumor necrosis factor, TNF-α; interleukin-1β, IL-1β; interleukin-6, IL-6; toll-like receptor 4, TLR4;, myeloid differentiation factor 88, MyD88; nuclear factor-κB, NF-κB; nuclear factor erythroid-derived 2-related factor 2, Nrf2; heme oxygenase-1, HO-1; B-cell lymphoma-2-associated X protein, BAX; B-cell lymphoma-2, BCL-2; cysteinyl aspartate-specific proteinase-3 (caspase-3), caspase-8 and caspase-9, Porcine β-defensin (PBD)114 and PBD129. a, b, c mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CMOS, pigs were fed with a MOS containing diet (3 g/kg); ECON, pigs were fed with a basal diet and challenged by ETEC; EMOS, pigs were fed with a MOS containing diet and challenged by ETEC

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx

- TableS1.docx