Animal Feed Science and Technology 248 (2019) 114–125

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Algae-derived β-glucan enhanced gut health and immune responses of weaned pigs experimentally infected with a pathogenic *E. coli*

Kwangwook Kim[a], Amy Ehrlich[b], Vivian Perng[a], Jennifer A. Chase[b], Helen Raybould[b], Xunde Li[b], Edward R. Atwill[b], Rose Whelan[c], Adebayo Sokale[d], Yanhong Liu[a,*]

[a] Department of Animal Science, University of California, Davis, CA 95616, United States
[b] School of Veterinary Medicine, University of California, Davis, CA 95616, United States
[c] Evonik Nutrition & Care GmbH, Hanau-Wolfgang, 63457, Germany
[d] Evonik Corporation, Kennesaw, GA 30144, United States

ARTICLE INFO

Keywords:
Algae-derived β-glucan
Gut barrier function
Gut immunity
Pathogenic *E. coli*
Weaned pigs

ABSTRACT

Most of the commercially available β-glucans are derived from yeast, while there are limited research on algae-derived β-glucan in pigs. Therefore, the objective of this experiment was to investigate the influence of dietary supplementation of algae-derived β-glucan on diarrhea, gut permeability, and immune responses of weaned pigs experimentally infected with a pathogenic *Escherichia coli* (*E. coli*). Thirty-six weaned pigs (7.69 ± 0.77 kg BW) were individually housed in disease containment rooms and randomly allotted to one of three dietary treatments (n = 12): control diet and 2 additional diets containing either 54 or 108 mg/kg of β-glucan. The experiment lasted 17 d [5 d before and 12 d post inoculation (PI)]. The inoculum used in this experiment was F18 *E. coli*, containing heat-labile toxin, heat-stable toxin b, and shiga-lie toxin 2. The inoculation doses were 10^{10} cfu/3 mL oral dose daily for 3 days. Diarrhea score (1, normal, to 5, watery diarrhea) was recorded for each pig daily to calculate frequency of diarrhea. Blood samples were collected on d 0 before *E. coli* challenge, and on d 2, 5, 8, and 12 PI to measure total and differential blood cell count in whole blood and several inflammatory markers in serum. Fresh jejunal tissues were collected from 4 pigs in the control group and high dose β-glucan group to analyze gut permeability on d 5 and d 12 PI with Ussing Chamber. Jejunal and ileal mucosa were also collected to measure the mRNA expression of several genes related to gut barrier function and immune responses. Results of this experiment revealed that inclusion of high dose β-glucan reduced ($P < 0.05$) frequency of diarrhea (29.01% vs. 17.28%) for the entire experimental period. This was likely due to the reduced ($P < 0.05$) gut permeability and increased ($P < 0.05$) mRNA expression of gut barrier function genes (*Claudin*, *Occludin*, and *MUC2*) in jejunal mucosa of *E. coli* challenged pigs as β-glucan supplemented. Supplementation of β-glucan also reduced ($P < 0.05$) white blood cells, neutrophils, serum tumor necrosis factor (TNF)-α, cortisol, and haptoglobin, and down-regulated ($P < 0.05$) the mRNA expression of several immune genes (*IL1B*, *IL6*, and *TNFA*) in ileal mucosa of *E. coli* challenged pigs, compared with the control diet. In conclusion, in feed supplementation of algae-derived β-glucan alleviated diarrhea

Abbreviations: E. coli, *Escherichia coli*; BW, body weight; COX2, cytochrome c oxidase subunit 2; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; IL, interleukin; mAb, monoclonal antibody; MUC2, mucin 2; PI, post-inoculation; TNF-α, tumor necrosis factor-α; ZO-1, zona occludens 1

* Corresponding author.
E-mail address: yahliu@ucdavis.edu (Y. Liu).

https://doi.org/10.1016/j.anifeedsci.2018.12.004
Received 14 May 2018; Received in revised form 2 August 2018; Accepted 22 December 2018

of F18 *E. coli* infected pigs by enhancing gut integrity. Feeding β-glucan also boosted host immune response against *E. coli* infection.

1. Introduction

Escherichia coli that express F18 fimbria are the predominant strains that cause post-weaning diarrhea in pigs (Nagy et al., 1997). This diarrhea *per se* accounts for 20–30% of cases of mortality in weanling pigs, therefore, is responsible for a huge economic losses in pig production (Fairbrother et al., 2005; Zhang et al., 2007). Many feed-based health technologies have been developed to either modulate gut microbiota and/or enhance immune responses of weaned pigs in order to improve disease resistance and production of weaned pigs (Pettigrew, 2006; Liu et al., 2018).

β-glucans are a heterogeneous group of polysaccharides naturally present in cereal grains, fungi, seaweed, and algae (Akramiene et al., 2007). The individual glucose in β-glucans are primarily linked by (1,3)-, (1,4)-, or (1,6)-β glycosidic bonds. It has been

Table 1
Ingredient compositions of experimental diets[1].

Ingredient, g/kg	Control diet
Corn	445.1
Dried whey	150.0
Soybean meal	140.0
Fish meal	100.0
Soy protein concentrate	70.0
Lactose	60.0
Soybean oil	20.0
Limestone	5.6
L-Lysine·HCl	1.5
DL-Methionine	0.6
L-Threonine	0.2
Salt	4.0
Vit-mineral prelim[2]	3.0
Total	1000.0
Calculated energy and nutrient	
Metabolizable energy, kcal/kg	3,487
Net energy, kcal/kg	2,615
Isoleucine,[3] g/kg	8.6
Leucine,[3] g/kg	16.8
Lysine,[3] g/kg	13.5
Methionine,[3] g/kg	4.4
Threonine,[3] g/kg	7.9
Tryptophan,[3] g/kg	2.3
Valine,[3] g/kg	9.5
Methionine + Cysteine,[3] g/kg	7.4
Analyzed dry matter and nutrients, g/kg	
Dry matter	902.0
Crude protein	223.7
Ash	63.6
Acid detergent fiber	22.6
Neutral detergent fiber	74.0
Calcium	10.6
Phosphorus	6.7

[1] Two additional diets were prepared by adding low dose (54 mg/kg) or high dose (108 mg/kg) of algae-derived β-glucan to the control diet, respectively.

[2] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3] Amino acids were indicated as standardized ileal digestible amino acids.

reported that the biological properties of β-glucans include anti-tumor and immunomodulatory effects *in vitro* (Smiderle et al., 2014; Choromanska et al., 2015; Choi et al., 2016). The *in vivo* immune-stimulatory effects of β-glucan have also been demonstrated in human, mice, and pigs (Gu et al., 2005; Li et al., 2006; Shen et al., 2009; Samuelsen et al., 2014). However, not all types of β-glucans exhibit similar immune-modulatory effects. For instance, cellulose, that is a β-(1,4)-glucan, does not have immuno-modulatory effects (Lange, 2000; Kumar et al., 2012). However, β-(1,3)-glucans derived from fungi and yeast are well known for their benefits to regulate immune system of both human and animals (Thompson et al., 2010; Samuelsen et al., 2014; Smiderle et al., 2014). This variation is due to differences in physiochemical properties, including purity, solubility, molecular mass, degree of branching, polymer charge, chemical structure, and tertiary structure between these β-glucans (Brown and Gordon, 2005). In addition, Dectin-1 highly recognizes β-(1,3)-glucans from a variety of sources, therefore, triggers the modulation of immune system (Thompson et al., 2010).

Currently, the majority of commercially available β-glucans are derived from yeast, whereas limited research have been conducted on algae-derived β-glucan in pigs. The β-glucans extracted from algae *Euglena gracilis* are linked by (1,3)-glycosidic bonds and are categorized as paramylon (Bacic et al., 2009). Sonck et al. (2010) has reported that β-glucan from algae *Euglena gracilis* strongly stimulated porcine leukocytes *in vitro*. Therefore, the hypothesis of this experiment was supplementation of algae *Euglena gracilis*-derived β-glucan could modulate immune responses and gut integrity, therefore, enhance disease resistance and health of weaned pigs. The objectives of this study were to investigate the effects of algae-derived β-glucan on diarrhea, gut permeability and immune responses of weaned pigs experimentally infected with F18 *E. coli*.

2. Materials and methods

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of California, Davis (IACUC #19322). The experiment was conducted at the Teaching and Research Animal Care Services P building at the University of California, Davis.

2.1. Animals, housing, experimental design, and diet

A total of 36 weaning pigs (21 d old) with an equal number of gilts and barrows and an average initial body weight (BW) of 7.69 ± 0.77 kg were selected from the Swine Teaching and Research Center of the University of California, Davis. The sows and piglets used in this experiment did not receive *E. coli* vaccines, antibiotic injections, or antibiotics in creep feed. After weaning, all pigs were randomly assigned to one of three dietary treatments in a randomized complete block design with weight and sex within litter as the blocks and pig as the experimental unit. There were 12 replicate pigs per treatment. Pigs were individually housed (pen size: 0.61 × 1.22 m) in an environmental control unit for 17 d [5 d before and 12 d after the first *E. coli* challenge (d 0)]. The piglets had *ad libitum* access to feed and water. Environmental enrichment was provided for each pig. The light was on at 0700 and off at 1900 h daily in the environmental control unit. The room temperature was 25 to 27 °C throughout the experiment.

The 3 dietary treatments included: 1) a complex nursery basal diet as the control diet, 2) a complex nursery basal diet including 54 mg/kg (low dose) of algae-derived β-glucan, and 3) a complex nursery basal diet including 108 mg/kg (high dose) of algae-derived β-glucan (Table 1). All diets met the current estimates for nutrient requirements for nursery pigs (NRC, 2012). Spray-dried plasma, antibiotics, and zinc oxide were not included in the diets. The experimental diets were fed to pigs as a mash form throughout the experiment. The β-glucan used in this experiment was ProGlucan™ (dried algae *Euglena gracilis*) and provided by Algal Scientific, USA (Plymouth, MI). The product contained around 50% β-glucan, in which 95% of them was β-1,3-glucan.

After 5 days adaptation, all pigs were orally inoculated with 3 mL of F18 *E. coli* for 3 consecutive days from d 0 post-inoculation (PI). The F18 *E. coli* were isolated from a field disease outbreak by the University of Illinois Veterinary Diagnostic Lab (isolate number: U.IL-VDL # 05–27242), and expressed heat-labile, heat-stable b, and Shiga-like toxins. The inoculums were provided at 10^{10} cfu per 3 mL dose in phosphate buffer saline. This dose caused mild diarrhea in the current experiment, which is consistent with our previous published research (Song et al., 2012; Almeida et al., 2013; Liu et al., 2013).

2.2. Clinical observations and sample collection

The procedures for this experiment were adapted from previous research methods (Liu et al., 2013). Before weaning, feces were collected from sows and all their piglets destined for this experiment and plated on blood agars and MacConkey agars to verify the free of β-hemolytic *E. coli* (Liu et al., 2013). All pigs that were used in the current experiment were negative to the F18 *E. coli*. Clinical observations (diarrhea score and alertness score) were recorded twice daily throughout the experiment. The diarrhea score of each pig was assessed visually each day by 2 independent evaluators, with the score ranging from 1 to 5 (1 = normal feces, 2 = moist feces, 3 = mild diarrhea, 4 = severe diarrhea, and 5 = watery diarrhea). The frequency of diarrhea was calculated as the percentage of the pen days with diarrhea score 3 or greater. The alertness score of each pig was assessed visually with a score from 1 to 3 (1 = normal, 2 = slightly depressed or listless, and 3 = severely depressed or recumbent). All pigs had alertness score 1 throughout the experiment, so data were not reported. Rectal temperature was recorded daily throughout the experiment. Pigs were weighed on weaning day, d 0 before inoculation, and d 5 and 12 PI. Feed intake was recorded throughout the experiment. Average daily gain, average daily feed intake, and feed conversion ratio (gain:feed) was calculated for each interval from d -5 to 0, d 0 to 5 PI, and d 5 to 12 PI.

After inoculation, rectal swabs were collected from each pig using a cotton swab on d 2 PI to test β-hemolytic coliforms (Song

et al., 2012; Almeida et al., 2013), and all pigs were β-hemolytic positive. Questionable colonies were subcultured on new MacConkey and blood agars, verified as the β-hemolytic *E. coli* challenge stain by using triple sugar iron and lysine iron agars reactions and as F18+ *E. coli* by means of PCR (DebRoy and Maddox, 2001). All pigs were successfully infected with F18 *E. coli*. Blood samples were collected from the jugular vein of each pig with or without ethylenediaminetetraacetic acid to yield whole blood and serum, respectively, before *E. coli* challenge (d 0), and on d 2, 5, 8, and 12 PI. Fresh whole blood samples were used for measuring total and differential blood cell count and analyzing the population of T cells and B cells. Serum samples were collected and immediately stored at −80 °C before further analysis.

Eighteen pigs (3 barrows and 3 gilts from each treatment) were randomly selected and euthanized on d 5 PI near the peak of infection, and the remained pigs were euthanized at the end of the experiment (d 12 PI) that was the recovery period of *E. coli* infection. Before euthanization, pigs were anesthetized with 1 mL mixture of 100 mg telazol, 50 mg ketamine, and 50 mg xylazine (2:1:1) by intramuscular injection. After anesthesia, intracardiac injection with 78 mg sodium pentobarbital (Vortech Pharmaceuticals, Ltd., Dearborn, MI) per 1 kg of BW was used to euthanize each pig. Jejunal samples were freshly collected from pigs in the control group and high β-glucan group (4 replicates/group) and stored in Krebs solution for gut permeability analysis.

2.3. Determination of total and differential blood cell counts

Whole blood samples were used to measure total and differential blood cell counts by Comparative Pathology Laboratory at the University of California, Davis. A multiparameter, automated programmed hematology analyzer (Drew/ERBA Scientific 950 FS Hematological Analyzer, Drew Scientific Inc., Miami, FL) was used for the assay to optimally differentiate porcine blood.

Differentiation of lymphocytes was performed on whole blood by single-color flow cytometer analysis using CD4 ($CD4^+$ T cells), CD8 ($CD8^+$ T cells), and CD21 (B cells) monoclonal antibody (mAb) labeling, respectively. Briefly, 1.5 μL of anti-CD4-fluorescein isothiocyanate (FITC) (mAb 74-12-4; 0.5 mg/mL), 1 μL of anti-CD8α-Spectral Red (0.1 mg/mL) (mAb 76-2-11), and 1 μL of anti-CD21-phycoerythrins (mAb BB6-11C9.6; 0.1 mg/mL) were simultaneously added to 100 μL of whole blood. Antibodies for analysis were purchased from Southern Biotechnology Associate Inc, Birmingham, AL. After incubating 20 min in the dark at room temperature, erythrocytes were lysed by adding 1.7 mL of lysing solution and incubated for 10 min. After washing the cells twice with 1.7 mL of wash buffer, the cells were resuspended in 0.5 mL of 1% paraformaldehyde solution and analyzed on a flow cytometry analyzer (BD Biosciences LSR II; BD Biosciences, San Jose, CA). For each sample, 10,000 cells were analyzed using FlowJo software (FlowJo LLC, Ashland, OR). Cell populations were identified in dot plots of cells size forward scatter versus cell granularity side scatter and cell counts versus fluorescence (FITC, SPRD, or phycoerythrins).

2.4. Measurements of serum cytokines and acute phase proteins

Serum samples were analyzed for pro-inflammatory cytokines (Tumor necrosis factor-α (TNF-α) and IL-6) and anti-inflammatory cytokine (IL-10) with porcine-specific ELISA kits (R&D System Inc., Minneapolis, MN). All samples were analyzed in duplicate including standard and control. Briefly, standard, control and samples were plated to the wells with a coated monoclonal antibody specific for each measurement of cytokine. After 2 h of incubation, any unbound substances were washed away with diluted washing solution, and an enzyme-linked polyclonal antibody specific for the tested cytokine was added to the wells to sandwich the cytokine immobilized during the first incubation. Another 2 h of incubation was followed by a wash to remove any unbound antibody-enzyme reagent, and then a substrate solution was added to the wells and color developed in proportion to the amount of the cytokine bound in the initial step. A stop solution was added to all wells to stop development of color prior to measurement of color intensity at 450 nm with a correction wavelength set at 540 nm using a plate reader (BioTek Instruments, Inc., Winooski, VT). Concentrations of each cytokine in the tested samples were calculated based on a standard curve. The concentration of cortisol (R&D System Inc., Minneapolis, MN) and haptoglobin (GenWay Biotech Inc., San Diego, CA) in serum samples were also measured by commercial ELISA kits. Similar procedures have been used as those described above, except pre-treatment of samples and incubation times. The intra-assay coefficients of variation for TNF-α, IL-6, IL-10, cortisol, and haptoglobin were 3.6, 4.4, 2.6, 5.4, and 2.7%, respectively. The inter-assay coefficients of variation for TNF-α, IL-6, IL-10, cortisol, and haptoglobin were 9.2, 5.9, 4.5, 9.3, and 6.2% respectively. The results of cytokines, cortisol and haptoglobin were expressed in picograms, nanograms or micrograms per milliliter based on the standard curves.

2.5. Gut permeability analysis with ussing chamber

The procedures for this measurement followed the previously published methods (Garas et al., 2016). Tissues were mounted in an Ussing Chamber (Physiological Instruments, San Diego, CA) after being stripped of longitudinal muscle and opened along the mesenteric border. In the chamber, tissue surface area (0.5 cm^2) was exposed to 2.5 ml of oxygenated Krebs-mannitol (10 mM) and Krebs-glucose (10 mM) at 37 °C on the luminal and serosal sides, respectively. After a 30 min equilibration, short circuit current and conductance were measured. Transcellular and paracellular permeability were determined by measuring the flux of horseradish peroxidase and FITC-dextran across jejunal mucosa, respectively. Horseradish peroxidase (0.5 mg) and FITC-dextran (1 mg) were added to the mucosal chamber and 200 μL of sample were collected from the serosal chamber every 30 min for 1 h. To maintain a constant volume within the chamber, an equivalent volume of Krebs-glucose solution was replaced at each sampling point. O-dianisidine peroxidase substrate was used to detect horseradish peroxidase at absorbance 450 nm. Concentration of FITC-dextran was measured via fluorescence at excitation 485 nm and emission 538 nm, respectively.

2.6. Quantitative real-time PCR

Total RNA were extracted from jejunal and ileal mucosa samples that were collected on d 5 and 12 PI as previously published methods (Liu et al., 2014). The RNA quality and quantity were assessed prior to reverse transcription to synthesize first-strand cDNA with SuperScript III First-Strand Synthesis SuperMix for quantitative real time-PCR (qRT-PCR) kit (Invitrogen; Carlsbad, CA). The mRNA expression of zona occludens 1 (*ZO-1*), *Claudin, Occludin*, *MUC2,* and *Dectin* in jejunal mucosa and *COX2*, *IL1B*, *IL6, TNFA, MUC2,* and *Dectin* in ileal mucosa were analyzed by qRT-PCR. Data normalization was accomplished using *β-actin* and *glyceraldehyde 3-phosphate dehydrogenase* as housekeeping genes. Primers were designed based on published literature and commercially synthesized by Applied Biosystems, Foster, CA. All primers were verified prior to qRT-PCR (Supplementary Table 1). The qRT-PCR reaction conditions followed the published research (Liu et al., 2014). The $2_T^{-\Delta\Delta C}$ method was used to analyze relative quantification of genes compared with the control diet (Livak and Schmittgen, 2001).

2.7. Statistical analysis

Normality of data were verified and outliers were identified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified and removed as values that deviated from the treatment mean by more than 3 times the interquartile range. All data were analyzed by ANOVA using PROC MIXED of SAS in a randomized complete block design with pig as the experimental unit. The statistical model included diet as the main effect and blocks (gender and pig) as the random effects. Treatment means were separated by using the LSMEANS statement and the PDIFF option of PROC MIXED. The chi-squared test was used for analyzing frequency of diarrhea. Statistical significance and tendency was considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

3. Results

3.1. Growth performance, rectal temperature, and diarrhea score

No difference was observed in the initial BW of pigs among dietary treatments (7.77, 7.51, and 7.81 kg in control, low-dose, and high-dose β-glucan treatment, respectively). Before and after *E. coli* infection, dietary β-glucan did not affect pig BW compared with the control group. No differences were observed in average daily gain, average daily feed intake, and feed efficiency of pigs throughout the experiment among dietary treatments (Data not shown).

Pigs fed the diet containing a low dose of β-glucan had lower ($P < 0.05$) rectal temperature on d 5 and 7 PI, compared with pigs fed the control or high dose β-glucan diet (Fig. 1). Supplementation of high dose β-glucan reduced ($P < 0.05$) diarrhea score of *E. coli* infected-pigs on d 3 and 5 PI (Fig. 2). Inclusion of high dose β-glucan reduced ($P < 0.05$) the frequency of diarrhea (17.28%) for the entire experimental period, compared with pigs in the control (29.01%) and low dose β-glucan (27.10%) treatments.

3.2. Total and differential blood cell count

No treatment effects were observed in the total and differential white blood cells among dietary treatments on d 0 before inoculation, and d 5 and 12 PI (Table 2). Supplementation of low dose β-glucan reduced ($P < 0.05$) the number of white blood cells and neutrophils compared with the control diet on d 8 PI. No differences were observed in the percentage of $CD4^+$ T cells, $CD8^+$ T

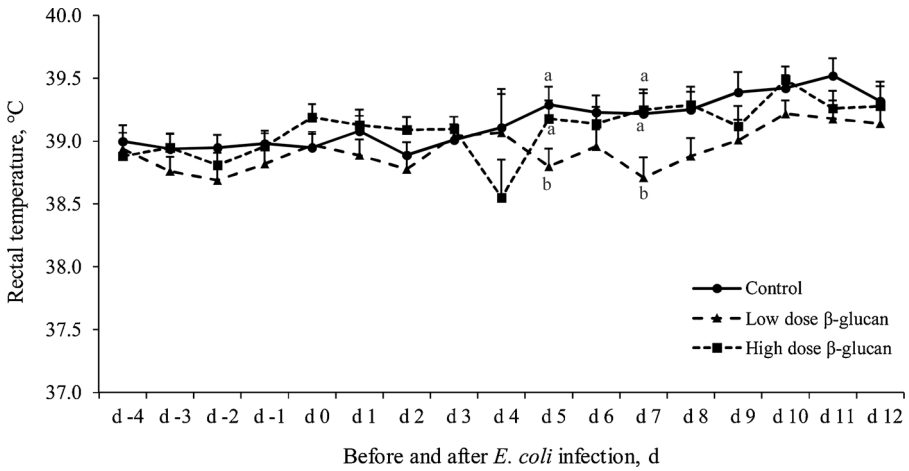

Fig. 1. Rectal temperature of weaned pigs fed diets supplemented with algae-derived β-glucan. The low-dose β-glucan diet contained 54 mg/kg β-glucan, whereas the high dose β-glucan diet contained 108 mg/kg β-glucan. Data were least squares mean of 12 observations per treatment before d 5 PI (post-inoculation), and 6 observations after d 5 PI. ^{a,b}within d 5 or d 7 PI, means without a common superscript are different ($P < 0.05$).

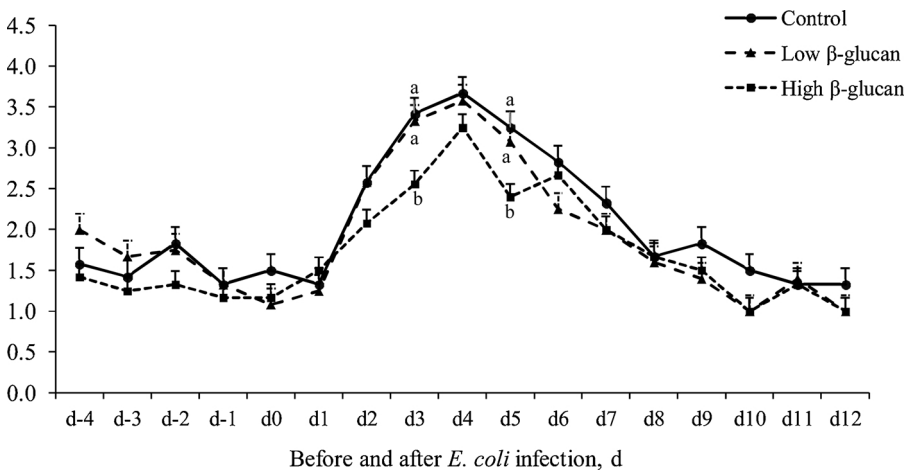

Fig. 2. Daily diarrhea score of weaned pigs fed diets supplemented with low or high dose algae-derived β-glucan. The low-dose β-glucan diet contained 54 mg/kg β-glucan, whereas the high dose β-glucan diet contained 108 mg/kg β-glucan. Diarrhea score = 1, normal feces, 2, moist feces, 3, mild diarrhea, 4, severe diarrhea, 5, watery diarrhea. Low or high dose β-glucan diet contained 54 or 108 mg/kg of algae-derived β-glucan in the diets, respectively. [a,b]within d 3 or d 5 PI, means without a common superscript are different ($P < 0.05$).

Table 2
Total and differential white blood cells in weaned pigs fed diets supplemented with algae-derived β-glucan[1].

Item[2]	Control	Low dose β-glucan	High dose β-glucan	SEM	*P*-value
d 0 before inoculation					
WBC, 10^3/μL	11.6	10.0	11.3	1.35	0.68
Neu, 10^3/μL	6.00	4.50	5.24	0.92	0.58
Lym, 10^3/μL	4.04	4.35	4.76	0.64	0.53
$CD4^+$ T, % of Lym	46.4	51.2	42.9	3.61	0.31
$CD8^+$ T, % of Lym	47.1	46.0	51.2	4.76	0.76
B cells, % of Lym	9.6	12.6	12.5	2.02	0.52
d 2 PI					
WBC, 10^3/μL	11.6	11.1	10.9	1.39	0.93
Neu, 10^3/μL	5.91	4.93	5.11	0.95	0.69
Lym, 10^3/μL	4.22	4.82	4.55	0.64	0.74
$CD4^+$ T, % of Lym	30.4^b	38.5^a	36.5^{ab}	2.82	< 0.05
$CD8^+$ T, % of Lym	61.0	61.1	65.4	3.04	0.51
B cells, % of Lym	18.3	18.9	19.3	2.45	0.96
d 5 PI					
WBC, 10^3/μL	14.2	12.3	12.5	0.95	0.55
Neu, 10^3/μL	7.11	5.84	5.25	0.92	0.49
Lym, 10^3/μL	5.26	5.88	5.89	0.66	0.65
$CD4^+$ T, % of Lym	28.2^b	35.4^a	32.7^{ab}	2.55	< 0.05
$CD8^+$ T, % of Lym	56.7^b	57.2^b	69.4^a	3.56	< 0.05
B cells, % of Lym	16.9	15.6	12.1	2.45	0.46
d 8 PI					
WBC, 10^3/μL	19.3^a	15.6^b	17.7^{ab}	1.16	< 0.05
Neu, 10^3/μL	8.75^a	5.85^b	7.75^{ab}	0.91	< 0.05
Lym, 10^3/μL	7.49	6.37	7.32	0.69	0.89
$CD4^+$ T, % of Lym	30.1	32.6	32.2	2.17	0.75
$CD8^+$ T, % of Lym	65.6	68.1	66.9	3.41	0.89
B cells, % of Lym	17.6	16.8	17.4	2.16	0.94
d 12 PI					
WBC, 10^3/μL	16.7	15.5	16.4	1.35	0.75
Neu, 10^3/μL	7.24	6.50	7.48	0.92	0.55
Lym, 10^3/μL	7.92	7.73	7.57	0.69	0.94
$CD4^+$ T, % of Lym	30.5	34.8	29.7	2.67	0.45
$CD8^+$ T, % of Lym	59.3^a	58.2^{ab}	47.8^b	3.60	< 0.05
B cells, % of Lym	15.8	13.1	17.9	2.32	0.39

[1] Data were least squares mean of 12 observations per treatment before d 5 PI, and 6 observations after d 5 PI. The low-dose β-glucan diet contained 54 mg/kg β-glucan, whereas the high dose β-glucan diet contained 108 mg/kg β-glucan.

[2] WBC = white blood cell; Neu = neutrophil; Lym = lymphocyte; Mono = monocyte; Eos = eosinophil; Baso = basophil; PI = post-inoculation.

cells, and B cells in lymphocytes among dietary treatments on d 0 before *E. coli* inoculation. Supplementation of low dose β-glucan increased ($P < 0.05$) the percentage of $CD4^+$ T cells in lymphocytes on d 2 and 5 PI compared with the control diet. Inclusion of high dose β-glucan increased ($P < 0.05$) the percentage of $CD8^+$ T cells in lymphocytes on d 5 PI, but reduced ($P < 0.05$) the percentage of $CD8^+$ T cells in lymphocytes on d 12 PI compared with the control diet.

3.3. Serum cytokines, cortisol, and haptoglobin

No differences were observed in the serum concentrations of TNF-α and IL-6, IL-10, cortisol, and haptoglobin among dietary treatments on d 0 before *E. coli* inoculation (Table 3). No differences were observed in serum IL-6 among dietary treatments throughout the experiment. Inclusion of either low or high dose of β-glucan reduced ($P < 0.05$) serum haptoglobin on d 2 and 5 PI, reduced ($P < 0.05$) serum cortisol on d 5, 8, and d 12 PI compared with the control diet. Inclusion of a low dose of β-glucan also reduced ($P < 0.05$) serum haptoglobin on d 12 PI compared with the control diet. Pigs fed the diet supplemented with a high dose of β-glucan had lower ($P < 0.05$) serum TNF-α concentration on d 5 PI, but higher ($P < 0.05$) serum IL-10 on d 2 PI than pigs fed the control diet.

3.4. Gut permeability

Supplementation of high dose β-glucan reduced ($P < 0.05$) jejunal transcellular permeability of *E. coli* infected-pigs on d 12 PI compared with pigs fed the control diet (Fig. 3). No difference was observed in transcellular permeability on d 5 PI and paracellular permeability on d 5 and 12 PI.

3.5. Gene expression

Supplementation of high dose β-glucan up-regulated ($P < 0.05$) the mRNA expression of *Dectin* in jejunal mucosa on d 5 and 12 PI, compared with the control diet (Fig. 4). Supplementation of low dose β-glucan enhanced ($P < 0.05$) the gene expression of *Claudin*, *Occludin*, and *MUC2* on d 12 PI, compared with the control diet. However, no differences were observed in the gene expression of *ZO-1* among dietary treatments.

Table 3
Serum inflammatory mediators in weaned pigs fed diets supplemented with algae-derived β-glucan[1].

Item	Control	Low dose β-glucan	High dose β-glucan	SEM	*P*-value
d 0 before inoculation					
TNF-a, pg/mL	118.3	123.4	143.8	28.6	0.48
IL-6, pg/mL	34.5	29.3	32.4	4.8	0.47
IL-10, pg/mL	21.5	18.4	20.7	2.6	0.35
Cortisol, ng/mL	74.2	91.3	74.4	9.3	0.37
Haptoglobin, μg/mL	1,355	1,167	1,273	212.8	0.77
d 2 PI[2]					
TNF-a, pg/mL	172.4	163.5	182.4	26.4	0.76
IL-6, pg/mL	34.1	34.7	40.	3.2	0.16
IL-10, pg/mL	16.1^{b}	17.1^{ab}	23.0^{a}	2.5	< 0.05
Cortisol, ng/mL	98.7	78.1	101.1	8.6	0.15
Haptoglobin, μg/mL	2,336^{a}	1,750^{b}	1,426^{b}	195.3	< 0.01
d 5 PI					
TNF-a, pg/mL	243.3^{a}	215.3^{ab}	180.1^{b}	21.1	< 0.05
IL-6, pg/mL	41.6	39.3	41.2	3.5	0.62
IL-10, pg/mL	28.1	27.0	24.3	1.7	0.33
Cortisol, ng/mL	138.1^{a}	97.1^{b}	106.1^{b}	9.2	< 0.01
Haptoglobin, μg/mL	2,316^{a}	1,705^{b}	1,374^{b}	159.6	< 0.01
d 8 PI					
TNF-a, pg/mL	192.6	162.8	139.2	21.7	0.27
IL-6, pg/mL	38.8	33.3	45.7	4.9	0.23
IL-10, pg/mL	17.5	19.3	18.5	2.6	0.59
Cortisol, ng/mL	148.6^{a}	101.5^{b}	106.3^{b}	13.5	< 0.05
Haptoglobin, μg/mL	1404	1129	1195	161.9	0.21
d 12 PI					
TNF-a, pg/mL	164.4	157.2	144.6	29.5	0.81
IL-6, pg/mL	46.3	50.3	53.3	5.2	0.39
IL-10, pg/mL	22.3	24.4	26.0	2.6	0.25
Cortisol, ng/mL	160.7^{a}	107.7^{b}	121.7^{b}	8.0	< 0.01
Haptoglobin, μg/mL	1,458^{a}	703^{b}	1,094^{ab}	218.6	< 0.05

[1] Data were least squares mean of 12 observations per treatment before d 5 PI, and 6 observations after d 5 PI. The low-dose β-glucan diet contained 54 mg/kg β-glucan, whereas the high dose β-glucan diet contained 108 mg/kg β-glucan.
[2] PI = post-inoculation.

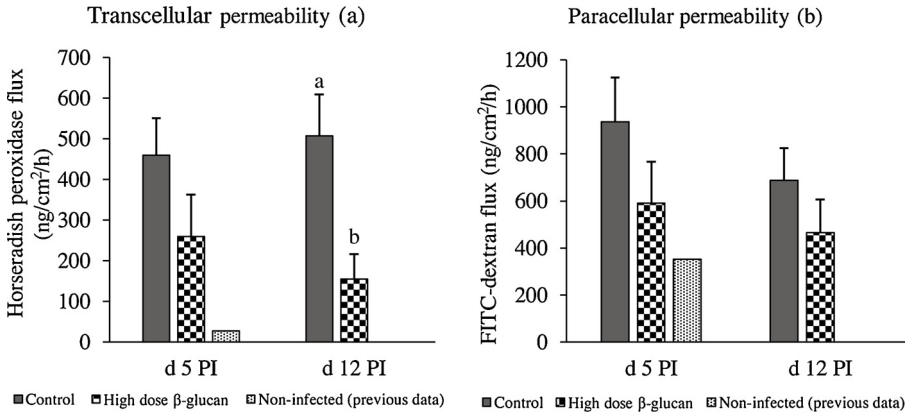

Fig. 3. Gut permeability of weaned pigs fed diets supplemented high dose β-glucan on d 5 and 12 post-inoculation. (a) Transcellular permeability, (b) Paracellular permeability. The high dose β-glucan diet contained 108 mg/kg of algae-derived β-glucan. The data for non-infected pigs were from our previous unpublished experiment. Data were least squares means of 4 observations per treatment. The flux values for horseradish peroxidase and FITC-dextran were analyzed from 0 to 60 min. [a,b]Means without a common superscript are different ($P < 0.05$).

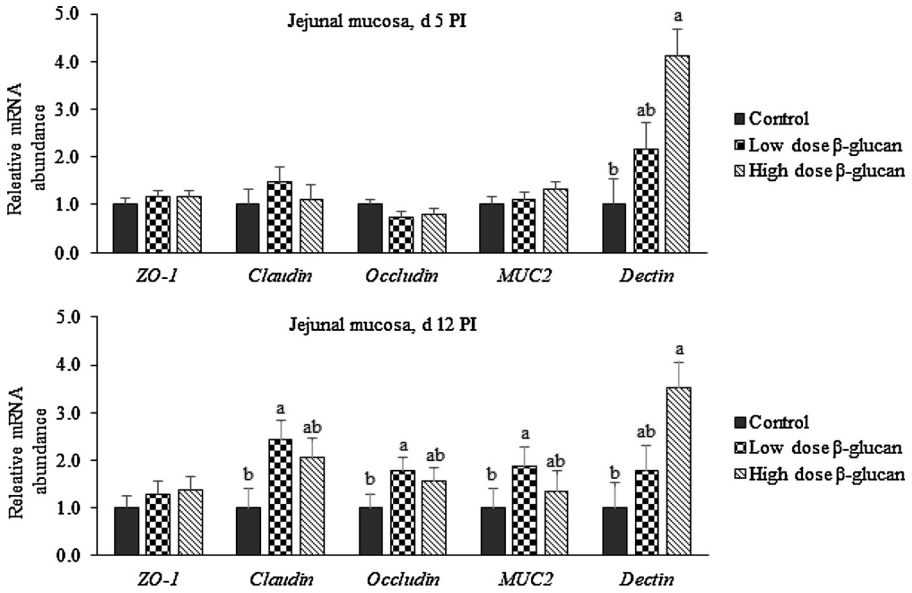

Fig. 4. Relative mRNA abundance of *ZO-1, Claudin*, *Occludin, MUC2*, and *Dectin* in jejunal mucosa of *E. coli*-challenged pigs fed diets supplemented with algae-derived β-glucan on d 5 and 12 PI (post-inoculation). The low-dose β-glucan diet contained 54 mg/kg β-glucan, whereas the high dose β-glucan diet contained 108 mg/kg β-glucan. Data were least squares means of 6 observations per treatment. [a,b]Means without a common superscript are different ($P < 0.05$).

On d 5 PI, supplementation of high dose β-glucan down-regulated ($P < 0.05$) the gene expression of *IL6*, but up-regulated ($P < 0.05$) the expression of *MUC2* in ileal mucosa of *E. coli* challenged pigs, compared with the control diet (Fig. 5). Supplementation of low dose β-glucan up-regulated ($P < 0.05$) the mRNA expression of *Dectin* in ileal mucosa of pigs, compared with the control diet. However, no differences were observed in the expression of *COX2*, *IL1B* and *TNFA* in ileal mucosa among dietary treatments.

On d 12 PI, addition of high dose β-glucan reduced ($P < 0.05$) the expression of *IL6* but enhanced ($P < 0.05$) the expression of *Dectin* in ileal mucosa of *E. coli* challenged pigs, compared with the control diet. Supplementation of low dose β-glucan down-regulated ($P < 0.05$) *IL1B* and *TNFA* but up-regulated ($P < 0.05$) *MUC2* expression in ileal mucosa of pigs compared with the control. No difference was observed in the expression of any gene between high and low dose β-glucan treatments.

4. Discussion

The F18 *E. coli*-induced post weaning diarrhea is a common cause of morbidity and mortality in weaned pigs (Verdonck et al., 2002). The results reported in this experiment indicate that supplementation of about 108 mg/kg of β-glucan could alleviate frequency of diarrhea and inflammation of weaned pigs caused by F18 *E. coli* infection. These findings are in agreement with previous

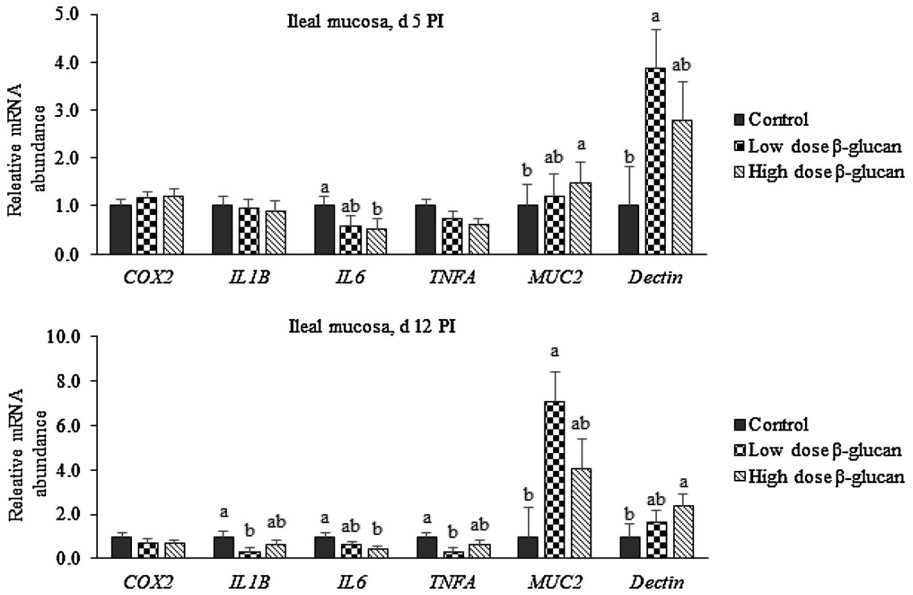

Fig. 5. Relative mRNA abundance of *COX2*, *IL1B*, *IL6*, *TNFA*, *MUC2*, and *Dectin* in ileal mucosa of *E. coli*-challenged pigs fed diets supplemented with algae-derived β-glucan on d 5 and 12 PI (post-inoculation). The low-dose β-glucan diet contained 54 mg/kg β-glucan, whereas the high dose β-glucan diet contained 108 mg/kg β-glucan. Data were least squares means of 6 observations per treatment. [a,b]Means without a common superscript are different ($P < 0.05$).

published report, showing an improved immune responses by feeding different sources of β-glucan (Li et al., 2006; Akramiene et al., 2007; Shen et al., 2009; Vetvicka and Oliveira, 2014). There are two potential mechanisms related to those benefits based on the observations in the current experiment. First, inclusion of β-glucan improved gut integrity by enhancing the mRNA expression of tight junction proteins and reducing gut permeability of the jejunum. Second, supplementation of β-glucan enhanced immune responses of *E. coli* infected pigs, which would help pigs to recover sooner from diarrhea and *E. coli* infection.

The F18 *E. coli* infection was successfully achieved in the current experiment and mild diarrhea was observed for all pigs, which is consistent with our previous research using a F18 *E. coli* challenge model in pigs (Song et al., 2012; Almeida et al., 2013; Liu et al., 2013). The pathogenesis of F18 *E. coli* mainly relies on two major virulent factors: F18 fimbria and toxins (Nagy et al., 1997). Fimbriae help bacteria adhere to the small intestinal epithelial cells. Then the colonized *E. coli* produce one or more enterotoxins, such as heat-labile toxins, heat-stable toxins, or shiga toxins. In summary, heat-labile toxins could enhance gut permeability by increasing chloride and water secretion and inhibiting of sodium and chloride absorption, heat-stable toxin b is mainly involved in histological damage, whereas shiga toxins are highly corrected with systemic inflammation and remarkably increased immune cytokines (Nagy and Fekete, 1999; Hasegawa and Shimonishi, 2005; Tarr et al., 2005). Inclusion of 108 mg/kg of β-glucan reduced the incidence of diarrhea in *E. coli* infected pigs. This observation is also in agreement with a previous published reports by Stuyven et al. (2009), which indicated the antidiarrheal activity of yeast originated β-glucans in piglets. The reduced diarrhea is likely due to the enhanced gut barrier function in pigs fed high dose β-glucan, as indicated by the improved mRNA expression of *Claudin*, *Occludin*, and *MUC2* in jejunal and ileal mucosa of *E. coli* infected pigs fed high dose β-glucan. Similar observations were reported in a chicken study, in which yeast-derived β-glucan also increased the gene expression of tight junction proteins in chicken challenged with *Salmonella Typhimurium* (Shao et al., 2013). Both mucins and tight junction proteins are critically important in maintaining gut barrier function. Mucin 2 is the major core polypeptide of mucins interfacing with luminal contents, and it also plays an important role in defense against inflammation (Hollingsworth and Swanson, 2004; Hansson, 2012). Our previous experiment has indicated that F18 *E. coli* infection could reduce the mRNA expression of *MUC2* in ileal mucosa of weaned pigs (Liu et al., 2014). Tight junctions are important junctional complexes in the epithelial cellular sheets, which are highly involved in the regulation of intermembrane diffusion and paracellular diffusion of small molecules (Tsukita and Furuse, 1999). Several unique proteins, including ZO-1, occludin, and claudin are critical in the maintenance of integrity and barrier function of tight junction (Furuse et al., 1993). Previous studies reported that the reduction of tight junction protein expression is highly correlated with the increased gut permeability (Bruewer et al., 2003), which further induce diarrhea in human and animals during a pathogenic bacterial infection (Hecht, 1995; Sears and Kaper, 1996; Sawada et al., 2003). In the present experiment, the reduced gut permeability was also observed in *E. coli* challenged pigs that were fed 108 mg/kg of β-glucan. Therefore, results in the present study indicate that supplementation of β-glucan could enhance gut barrier function, delay diarrhea of *E. coli* infected pigs, and then reduce diarrhea incidence of weaned pigs under disease conditions. The detailed modes of action for the enhanced gut barrier function have to be explored in future research.

Total white blood cell counts are commonly used to estimate the severity of bacterial infection, and rapid increase in the number of white blood cells led by presence of systemic inflammation (Gordon-Smith, 2009). Our previous research has confirmed that F18 *E. coli* infection dramatically enhanced white blood cells and neutrophils on d 5 and 11 post-infection (Liu et al., 2013). In the current

experiment, we observed that supplementation of β-glucan to *E. coli* infected pigs decreased white blood cells and neutrophils on d 8 PI, which indicates that β-glucan may alleviate the systemic inflammation caused by *E. coli* infection.

The ideal situation when a disease challenge arises would be a vigorous immune response, followed by a prompt reversion to normal growth. In the current experiment, we investigated the two major subsets of T lymphocytes, $CD4^+$ and $CD8^+$ T cells due to their important roles in cell-mediated immunity of weaned pigs (Ellmeier et al., 1999; Germain, 2002). Helper cells, $CD4^+$ T cells, assist to enhance adaptive immune system by activating cytotoxic T cells, macrophages, and other immune cells. Cytotoxic T cells, $CD8^+$ T cells, play a key role in identifying and destroying infected cells (Scott and Kaufmann, 1991; Miceli and Parnes, 1993). The flow cytometry results in the current experiment show that inclusion of β-glucan to *E. coli* infected pigs increased both $CD4^+$ and $CD8^+$ T cells, and the response of $CD4^+$ T cells (at d 2 and 5 PI) appeared earlier than $CD8^+$ T cells (d 5 and 12 PI). This observation suggests that supplementation of β-glucan to *E. coli*-infected pigs enhanced adaptive immunity likely mediated by T cell activation, which is consistent with previous published research from Wang et al. (2010) and Qi et al. (2011). These results suggest that supplementation of β-glucan could activate T cell-mediated immunity and accelerate the recovery of weaned pigs from bacterial infection.

Serum cortisol level is commonly used as an indicator of stress from various responses such as weaning and inflammation (Widowski et al., 1989; Möstl and Palme, 2002). Haptoglobin is an acute phase protein that binds to free hemoglobin and inhibits oxidative activities. Concentration of serum haptoglobin is also widely used as an indicator of diverse acute phase responses such as viral or bacterial infection, inflammation, and other pathological reactions (Asai et al., 1999; Petersen et al., 2002). In the present study, supplementation of β-glucan decreased serum cortisol on d 5, 8, and 12 PI and haptoglobin level on d 2 and 5 PI in *E. coli* infected pigs. Those observations are in agreement with previous published researches, in which feeding β-glucans from different origins reduced serum cortisol level in lipopolysaccharide challenged pigs (Mao et al., 2005; Vetvicka and Oliveira, 2014), broiler chickens (Haldar et al., 2011), and fish (Cain et al., 2003). Inclusion of β-glucans was also reported to reduce serum haptoglobin concentration in pigs challenged with *Staphylococcus aureus* (Dritz et al., 1995). The results of differential blood cell count and serum inflammatory parameters indicate that supplementation of algae-derived β-glucan could enhance immune responses and alleviate inflammation caused by *E. coli* infection.

Tumor necrosis factor-α, mainly produced by macrophages and neutrophils, is one of the most important pro-inflammatory cytokines in response to bacterial infection. Serum TNF-α levels have been commonly used as a systemic inflammatory indicator for humans and animals (Dinarello, 2000; Sommer and Kress, 2004; Grivennikov et al., 2005). Consistent with the results of white blood cell counts, serum TNF-α level was also decreased by feeding β-glucan to *E. coli* infected pigs. It has been also reported that β-glucan of different origins reduced serum TNF-α level in lipopolysaccharide challenged pigs (Li et al., 2006), mice (Soltys and Quinn, 1999), and *in vitro* cell culture assays (Wakshull et al., 1999). Serum IL-10 is an anti-inflammatory cytokine that can regulate immune responses against inflammation (Ouyang et al., 2011). In the current study, serum IL-10 was increased by feeding β-glucan to *E. coli* infected pigs. These findings support that supplementation of β-glucan to *E. coli* infected pigs might alleviate inflammation caused by *E. coli* infection.

The potential modes of action for immune modulation effects of β-glucan are still not fully understood, but many studies have reported the involvement of Dectin receptor. Dectin, a major β-glucan receptor on several immune cells (e.g. macrophages), could recognize and bind β-glucan from a variety of sources (Goodridge et al., 2009). This recognition plays important roles in intracellular signaling, activating adaptive immune response, activating anti-microbial and anti-fungal activities, and maybe therefore enhancing disease resistance (Brown et al., 2003; Taylor et al., 2007; Goodridge et al., 2009). In the present experiment, β-glucan supplements up-regulated the mRNA expression of *Dectin* in both jejunal and ileal mucosa of *E. coli* infected pigs. It has also been observed that the addition of β-glucan down-regulated the expression of several genes associated with inflammatory responses in ileal mucosa of weaned pigs. These results indicate that the Dectin receptor may be involved in the reduced gut inflammation in *E. coli*-challenged pigs fed with β-glucan.

Results of this experiment indicate that in feed supplementation of approximately 108 mg/kg of algae-derived β-glucan alleviated diarrhea of F18 *E. coli* infected pigs by enhancing gut integrity. Feeding algae-derived β-glucan also boosted host immune response against *E. coli* infection. The β-glucan product stimulated T cell activation and reduced inflammation and, therefore, may accelerate the recovery of pigs from *E. coli* infection. The results of this study indicate that supplementation of around 108 mg/kg of β-glucan in animal feed could improve gut barrier function and immunity of weaned pigs and reduce post-weaning diarrhea, which will promote weaned pig health and increase profitability of pork producers as the use of antibiotics in feed is restricted.

Conflict of interest

All of the authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anifeedsci.2018.12.004.

References

Akramiene, D., Kondrotas, A., Didziapetriene, J., Kevelaitis, E., 2007. Effects of beta-glucans on the immune system. Medicina Kaunas (Kaunas) 43, 597–606.

Almeida, J.A.S., Liu, Y., Song, M., Lee, J.J., Gaskins, H.R., Maddox, C.W., Osuna, O., Pettigrew, J.E., 2013. Escherichia coli challenge and one type of smectite alter intestinal barrier of pigs. J. Anim. Sci. and Biotech. 4 52-52.
Asai, T., Mori, M., Okada, M., Uruno, K., Yazawa, S., Shibata, I., 1999. Elevated serum haptoglobin in pigs infected with porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 70, 143–148.
Bacic, A., Fincher, G.B., Stone, B.A., 2009. Chemistry, Biochemistry, and Biology of 1-3 Beta Glucans and Related Polysaccharides. Academic Press.
Brown, G.D., Gordon, S., 2005. Immune recognition of fungal β-glucans. Cell. Microbiol. 7, 471–479.
Brown, G.D., Herre, J., Williams, D.L., Willment, J.A., Marshall, A.S.J., Gordon, S., 2003. Dectin-1 mediates the biological effects of β-Glucans. J. Exp. Med. 197, 1119–1124.
Bruewer, M., Luegering, A., Kucharzik, T., Parkos, C.A., Madara, J.L., Hopkins, A.M., Nusrat, A., 2003. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. J. Immunol. 171, 6164–6172.
Cain, K., Grabowski, L., Reilly, J., Lytwyn, M., 2003. Immunomodulatory effects of a bacterial-derived β-1,3 glucan administered to tilapia (Oreochromis nilotocus L.) in a Spirulina-based diet. J. Aquac. Res. Dev. 34, 1241–1244.
Choi, E., Lee, S., Hyeon, J., Choe, S., Keum, B., Lim, J., Park, D., Choi, I., Cho, K., 2016. Effects of β-glucan on the release of nitric oxide by macrophages stimulated with lipopolysaccharide. Asian-australas. J. Anim. Sci. 29, 1664–1674.
Choromanska, A., Kulbacka, J., Rembialkowska, N., Pilat, J., Oledzki, R., Harasym, J., Saczko, J., 2015. Anticancer properties of low molecular weight oat beta-glucan–an in vitro study. Int. J. Bio. Macromol. 80, 23–28.
DebRoy, C., Maddox, C.L., 2001. Identification of virulence attributes of gastrointestinal *Escherichia coli* isolates of veterinary significance. Anim. Health Res. Rev. 2, 129–140.
Dinarello, C.A., 2000. Proinflammatory cytokines. Chest. 118, 503–508.
Dritz, S.S., Shi, J., Kielian, T.L., Goodband, R.D., Nelssen, J.L., Tokach, M.D., Chengappa, M.M., Smith, J.E., Blecha, F., 1995. Influence of dietary beta-glucan on growth performance, nonspecific immunity, and resistance to Streptococcus suis infection in weanling pigs. J. Anim. Sci. 73, 3341–3350.
Ellmeier, W., Sawada, S., Littman, D.R., 1999. The regulation of CD4 and CD8 coreceptor gene expression during T cell development. Annu. Rev. Immunol. 17, 523–554.
Fairbrother, J.M., Nadeau, E., Gyles, C.L., 2005. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6, 17–39.
Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S., Tsukita, S., 1993. Occludin: a novel integral membrane protein localizing at tight junctions. J. Cell Biol. 123, 1777–1788.
Garas, L.C., Feltrin, C., Hamilton, M.K., Hagey, J.V., Murray, J.D., Bertolini, L.R., Bertolini, M., Raybould, H.E., Maga, E.A., 2016. Milk with and without lactoferrin can influence intestinal damage in a pig model of malnutrition. Food Funct. 7, 665–678.
Germain, R.N., 2002. T-cell development and the CD4-CD8 lineage decision. Nat. Rev. Immunol. 2, 309–322.
Goodridge, H.S., Wolf, A.J., Underhill, D.M., 2009. Beta-glucan recognition by the innate immune system. Immunol. Rev. 230, 38–50.
Gordon-Smith, T., 2009. Structure and function of red and white blood cells. Medicine 37, 119–124.
Grivennikov, S.I., Tumanov, A.V., Liepinsh, D.J., Kruglov, A.A., Marakusha, B.I., Shakhov, A.N., Murakami, T., Drutskaya, L.N., Förster, I., Clausen, B.E., Tessarollo, L., Ryffel, B., Kuprash, D.V., Nedospasov, S.A., 2005. Distinct and nonredundant in vivo functions of TNF produced by T cells and macrophages/neutrophils. Immunity 22, 93–104.
Gu, Y.-h., Takagi, Y., Nakamura, T., Hasegawa, T., Suzuki, I., Oshima, M., Tawaraya, H., Niwano, Y., 2005. Enhancement of radioprotection and anti-tumor immunity by yeast-derived β-glucan in mice. J. Med. Food 8, 154–158.
Haldar, S., Ghosh, T.K., Toshiwati, Bedford, M.R., 2011. Effects of yeast (Saccharomyces cerevisiae) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with Salmonella enteritidis. Anim. Feed Sci. Technol. 168, 61–71.
Hansson, G.C., 2012. Role of mucus layers in gut infection and inflammation. Curr. Opin. Microbiol. 15, 57–62.
Hasegawa, M., Shimonishi, Y., 2005. Recognition and signal transduction mechanism of *Escherichia coli* heat-stable enterotoxin and its receptor, guanylate cyclase C. J. Peptide Res. 65, 261–271.
Hecht, G., 1995. Bugs and barriers: enteric pathogens exploit yet another epithelial function. Physiol. 10, 160–166.
Hollingsworth, M.A., Swanson, B.J., 2004. Mucins in cancer: protection and control of the cell surface. Nat. Rev. Cancer 4, 45–60.
Kumar, V., Sinha, A.K., Makkar, H.P.S., de Boeck, G., Becker, K., 2012. Dietary roles of non-starch polysaccharides in human nutrition: a review. Critical Rev. Food Sci. Nutr. 52, 899–935.
Lange, C.F.M., 2000. Characterizations of Non-starch Polysaccharides. Feed Evaluation: Principles and Pactices. pp. 77–92.
Li, J., Li, D.F., Xing, J.J., Cheng, Z.B., Lai, C.H., 2006. Effects of [beta]-glucan extracted from Saccharomyces cerevisiae on growth performance, and immunological and somatotropic responses of pigs challenged with Escherichia coli lipopolysaccharide1. J. Anim. Sci. 84, 2374–2381.
Liu, Y., Song, M., Che, T.M., Almeida, J.A., Lee, J.J., Bravo, D., Maddox, C.W., Pettigrew, J.E., 2013. Dietary plant extracts alleviate diarrhea and alter immune responses of weaned pigs experimentally infected with a pathogenic Escherichia coli. J. Anim. Sci. 91, 5294–5306.
Liu, Song, M., Che, T., Lee, J., Bravo, D., Maddox, C., Pettigrew, J., 2014. Dietary plant extracts modulate gene expression profiles in ileal mucosa of weaned pigs after an Escherichia coli infection. J. Anim. Sci. 92, 2050–2062.
Liu, Y., Espinosa, C.D., Abelilla, J.J., Casas, G.A., Lagos, L.V., Lee, S.A., Kwon, W.B., Mathai, J.K., Navarro, D.M.D.L., Jaworski, N.W., Stein, H.H., 2018. Non-antibiotic feed additives in diets for pigs: a review. Anim. Nutr. 4, 113–125.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25, 402–408.
Mao, X.F., Piao, X.S., Lai, C.H., Li, D.F., Xing, J.J., Shi, B.L., 2005. Effects of β-glucan obtained from the Chinese herb Astragalus membranaceus and lipopolysaccharide challenge on performance, immunological, adrenal, and somatotropic responses of weanling pigs1. J. Anim. Sci. 83, 2775–2782.
Miceli, M.C., Parnes, J.R., 1993. Role of CD4 and CD8 in T cell activation and differentiation. Adv. Immunol. 53, 59–122.
Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23, 67–74.
Nagy, B., Fekete, P.Z., 1999. Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. Vet. Res. 30, 259–284.
Nagy, B., Whipp, S.C., Imberechts, H., Bertschinger, H.U., Dean-Nystrom, E.A., Casey, T.A., Salajka, E., 1997. Biological relationship between F18ab and F18ac fimbriae of enterotoxigenic and verotoxigenic Escherichia coli from weaned pigs with oedema disease or diarrhoea. Microb. Pathog. 22, 1–11.
NRC, 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. National Academies Press, Washington, DC.
Ouyang, W., Rutz, S., Crellin, N.K., Valdez, P.A., Hymowitz, S.G., 2011. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu. Rev. Immunol. 29, 71–109.
Petersen, H.H., Ersbøll, A.K., Jensen, C.S., Nielsen, J.P., 2002. Serum-haptoglobin concentration in Danish slaughter pigs of different health status. Prev. Vet. Med. 54, 325–335.
Pettigrew, J.E., 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 1. Anim. Biotechnol. Bull. 17, 207–215.
Qi, C., Cai, Y., Gunn, L., Ding, C., Li, B., Kloecker, G., Qian, K., Vasilakos, J., Saijo, S., Iwakura, Y., Yannelli, J.R., Yan, J., 2011. Differential pathways regulating innate and adaptive antitumor immune responses by particulate and soluble yeast-derived β-glucans. Blood 117, 6825–6836.
Samuelsen, A.B.C., Schrezenmeir, J., Knutsen, S.H., 2014. Effects of orally administered yeast-derived beta-glucans: a review. Mol. Nutr. Food Res. 58, 183–193.
Sawada, N., Murata, M., Kikuchi, K., Osanai, M., Tobioka, H., Kojima, T., Chiba, H., 2003. Tight junctions and human diseases. Med. Electron Microsc. 36, 147–156.
Scott, P., Kaufmann, S.H.E., 1991. The role of T-cell subsets and cytokines in the regulation of infection. Immunol. Today 12, 346–348.
Sears, C.L., Kaper, J.B., 1996. Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. Microbiol. Rev. 60, 167.
Shen, Y.B., Piao, X.S., Kim, S.W., Wang, L., Liu, P., Yoon, I., Zhen, Y.G., 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs1. J. Anim. Sci. 87, 2614–2624.
Smiderle, F.R., Baggio, C.H., Borato, D.G., Santana-Filho, A.P., Sassaki, G.L., Iacomini, M., Van Griensven, L.J., 2014. Anti-inflammatory properties of the medicinal mushroom Cordyceps militaris might be related to its linear (1→ 3)-β-D-glucan. PLoS One 9, e110266.

Soltys, J., Quinn, M.T., 1999. Modulation of endotoxin- and enterotoxin-induced cytokine release by in vivo treatment with beta-(1,6)-branched beta-(1,3)-glucan. Infect. Immun. 67, 244–252.
Sommer, C., Kress, M., 2004. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci. Lett. 361, 184–187.
Sonck, E., Stuyven, E., Goddeeris, B., Cox, E., 2010. The effect of β-glucans on porcine leukocytes. Vet. Immunol. Immunopathol. 135, 199–207.
Song, M., Liu, Y., Soares, J.A., Che, T.M., Osuna, O., Maddox, C.W., Pettigrew, J.E., 2012. Dietary clays alleviate diarrhea of weaned pigs. J. Anim. Sci. 90, 345–360.
Stuyven, E., Cox, E., Vancaeneghem, S., Arnouts, S., Deprez, P., Goddeeris, B.M., 2009. Effect of β-glucans on an ETEC infection in piglets. Vet. Immunol. Immunopathol. 128, 60–66.
Tarr, P.I., Gordon, C.A., Chandler, W.L., 2005. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet 365, 1073–1086.
Taylor, P.R., Tsoni, S.V., Willment, J.A., Dennehy, K.M., Rosas, M., Findon, H., Haynes, K., Steele, C., Botto, M., Gordon, S., Brown, G.D., 2007. Dectin-1 is required for β-glucan recognition and control of fungal infection. Nat. Immunol. 8, 31–38.
Thompson, I.J., Oyston, P.C., Williamson, D.E., 2010. Potential of the β-glucans to enhance innate resistance to biological agents. Expert Rev. Anti. Infect. Ther. 8, 339–352.
Tsukita, S., Furuse, M., 1999. Occludin and claudins in tight-junction strands: leading or supporting players? Trends Cell Biol. 9, 268–273.
Verdonck, F., Cox, E., van Gog, K., Van der Stede, Y., Duchateau, L., Deprez, P., Goddeeris, B.M., 2002. Different kinetic of antibody responses following infection of newly weaned pigs with an F4 enterotoxigenic Escherichia coli strain or an F18 verotoxigenic Escherichia coli strain. Vaccine 20, 2995–3004.
Vetvicka, V., Oliveira, C., 2014. B(1-3)(1-6)-D-glucans modulate immune status in pigs: potential importance for efficiency of commercial farming. Ann. Transl. Med. 2, 16.
Wakshull, E., Brunke-Reese, D., Lindermuth, J., Fisette, L., Nathans, R.S., Crowley, J.J., Tufts, J.C., Zimmerman, J., Mackin, W., Adams, D.S., 1999. PGG-Glucan, a soluble β-(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-κB-like factor in human PMN: evidence for a glycosphingolipid β-(1,3)-glucan receptor. Immunopharmacology 41, 89–107.
Wang, J., Dong, S., Liu, C., Wang, W., Sun, S., Gu, J., Wang, Y., Boraschi, D., Qu, D., 2010. β-glucan oligosaccharide enhances CD8+ T cells immune response induced by a DNA vaccine encoding hepatitis B virus core antigen. J. Biomed. Biotechnol. 2010, 645213.
Widowski, T.M., Curtis, S.E., Graves, C.N., 1989. The neutrophil:lymphocyte ratio in pigs fed cortisol. Can. J. Anim. Sci. 69, 501–504.
Zhang, W., Zhao, M., Ruesch, L., Omot, A., Francis, D., 2007. Prevalence of virulence genes in Escherichia coli strains recently isolated from young pigs with diarrhea in the US. Vet. Microbiol. 123, 145–152.