IMMUNOLOGY

Effects of inulin supplementation on intestinal barrier function and immunity in specific pathogen-free chickens with *Salmonella* infection

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Abstract

We investigated the effects of inulin on intestinal barrier function and mucosal immunity in *Salmonella enterica* serovar Enteritidis (SE)-infected specific pathogen-free (SPF) chickens. SPF chickens ($n = 240$, 1-d-old) were divided into 4 groups (6 replicates per group, 10 chickens per replicate): a control group (CON) fed a basal diet without inulin supplementation and 3 SE-infected groups fed a basal diet supplemented with inulin 0% (SE group), 0.5% (0.5% InSE group), and 1% (1% InSE group), respectively. At 28 d of age, the chickens in SE-infected groups were orally infected with SE and in CON group were administrated with phosphated-buffered saline (PBS). Intestinal morphology, mucosal immunity, and intestinal barrier function-related gene expression were analyzed at 1- and 3-d post-infection (dpi). SE challenge significantly increased the mucosal gene expression, such as interleukin-1 β (IL-1 β), lipopolysaccharide-induced tumor necrosis factor factor (LITAF), interferon- γ (IFN- γ), and interleukin-6 (IL-6), and increased serum IFN- γ , secretory IgA (sIgA), and IgG concentration, and significantly decreased the gene expression levels of mucin 2 (MUC2) and claudin-1 at 3 dpi compared with the CON group ($P < 0.05$). Inulin supplementation improved the expression levels of these immunity- and intestinal barrier function-related genes, increased villus height (VH), and decreased crypt depth (CD) in the duodenum, jejunum, and ileum at 1 and 3 dpi within the SE-challenged groups ($P < 0.05$). SE challenge significantly increased ileal Toll-like receptor 4 (TLR4) mRNA at 1 and 3 dpi, suppressor of cytokine signaling 3 (SOCS3) mRNA at 1 dpi, and phospho-signal transducer and activator of transcription 3 (p-STAT3) and Janus kinase1 (JAK1) protein expression at 3 dpi compared with the CON group ($P < 0.05$). Inulin supplementation suppressed p-STAT3 and JAK1 protein expression and promoted ileal TLR4 and SOCS3 mRNA expression at 3 dpi compared with SE group ($P < 0.05$). In conclusion, inulin alleviated SE-induced gut injury by decreasing the proinflammatory response and enhancing mucosal immunity in chickens.

Key words: chicken, barrier function, gut morphology, immunity, inulin, *Salmonella*

Introduction

Salmonella enterica serovar Enteritidis (SE) is one of the most prominent food-borne microbial agents worldwide, with dangerous implications for food security and human health (Kirk et al., 2015). Traditional antibiotic therapy for *Salmonella* infection can lead to resistance and acute diarrhea and may cause chronic toxicity, which is a major concern in poultry production (Castillo et al., 2012). *Salmonella* are Gram-negative facultative anaerobes, and antibiotics normally increase epithelial oxygenation via decreased butyrate levels, resulting in aerobic post-antibiotic pathogen expansion. Antibiotic treatment may thus promote relapse of *Salmonella* gastroenteritis (Rivera-Chávez et al., 2016). In addition, although antibiotic therapy can clear *Salmonella* pathogen stool loads, it cannot effectively eliminate mucosal inflammation, partly because of the *Salmonella*-induced interferon γ (IFN- γ) response (Dolowschiak et al., 2016). Intestinal inflammation provides *Salmonella* with a competitive metabolic advantage over the resident microorganisms, thus delaying the resolution of mucosal pathology and gut barrier function. There is thus an urgent need for effective alternative treatments for combating *Salmonella* infections in poultry production.

Inulin-type fructans are effective potential prebiotics that could be used to control *Salmonella* colonization and improve meat safety in poultry production (Dankowiakowska et al., 2013). Inulin-type fructans exert modulatory effects on the gut microbiota, intestinal mucosal barrier, and systemic immune response via indirect metabolite fermentation by the gut microbiota or by direct stimulation of the host mucosal immune response (Yang et al., 2016; Chen et al., 2017). Inulin prebiotics can promote the proliferation of beneficial microbiota in the gastrointestinal tract (such as *Bifidobacterium* and *Lactobacillus*) (Johnson et al., 2015; Wilson et al., 2017) and inhibit the growth of pathogenic bacteria (such as *Salmonella* and *Escherichia coli*) in poultry (Samanta et al., 2013; Buclaw et al., 2016; Micciche et al., 2018). Evidence also indicates that inulin-type fructans could modulate the immune response by activating the expression of genes and cytokines implicated in immune processes (Sevane et al., 2014). In general, inulin depresses the expression of pro-inflammatory cytokines, such as lipopolysaccharide-induced tumor necrosis factor (LITAF), interferon- γ (IFN- γ), interleukin-6 (IL-6), and inducible nitric oxide synthase, which in turn modifies the intestinal barrier and immune function (Whelan, 2011; Vogt et al., 2015; Ferenczi et al., 2016; Kareem et al., 2017). However, the ability of inulin to combat *Salmonella* infection via modulation of the intestinal inflammatory and immune responses is unclear. The current study thus aimed to clarify the mucosal immunoregulatory effects of inulin in SE-infected chickens, identify its restorative effects on the SE-damaged gut barrier, and identify the immune regulation signaling pathways associated with enhanced resistance to SE infection.

Materials and methods

All experimental procedures involving animals were approved by the Animal Management Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (IAS-CAAS, Beijing, China). Ethical approval regarding animal survival was given by the animal ethics committee of IAS-CAAS (approval number: IASCAAS-AE20140615).

Animal feeding and management

A total of 240 1-d-old Arbor Acres specific pathogen-free (SPF) chickens (Beijing Merial Vital Laboratory Animal Technology

Co., Ltd, Beijing, China) were randomly assigned to 4 groups (6 replicates of 10 birds each) and raised in a sterile IPQ-Type 3 negative pressure isolator (China Agricultural University, Beijing, China). The groups were treated as follows: a control group (CON group) was fed a corn/soybean-based sterile diet, and the other 3 groups were treated with inulin (Sigma-Aldrich, St. Louis, MO) at 0%, 0.5%, and 1% until 28 d and then were orally infected with 1-mL phosphated-buffered saline (PBS) containing 10^8 cfu SE (CMCC50041, China Institute of Veterinary Drugs Control, Beijing) respectively (SE, 0.5% InSE, and 1% InSE groups) at 28 d of age. Meanwhile, the CON group chickens were orally administrated with 1-mL PBS without SE. Detailed calculated nutrient contents of the basal diet ingredients are shown in Table 1. The temperature in the isolator was 35 °C for the first week and gradually reduced by 2 °C each week until the end of the experiment. All chickens had free access to feed and water (sterilized at 121 °C for 15 min).

Sample collection

One chicken from each replicate was randomly selected at 1- and 3-d post-infection (dpi) and slaughtered for sample collection. Blood was also sampled from 1 chicken chosen at random from each replicate, incubated at 37 °C for 10 min (GRP-9050; Shanghai Senxin Test Instrument Co., Ltd, Shanghai, China), and then serum were separated at 1000 g/min for 15 min and stored at -20 °C to analyze IFN- γ and antibody contents. Ileum segments (3–4 cm, near the cecum) were dissected aseptically and rinsed with sterile phosphate-buffered saline. Mucosa samples were then collected by opening the segments

Table 1. Composition and nutrient levels of basal diets (as-fed basis) (g/kg)

Item	Treatment		
	CON	0.5% Inulin	1% Inulin
Ingredients			
Corn	680.1	669.8	660.2
Choline chloride	0.3	0.3	0.3
Soybean meal	275.6	277.8	279.6
Corn oil	0	3	5.8
Salt	2	2	2
Limestone powder	4.8	4.8	4.8
Calcium dihydrogen phosphate	16	16	16
Cystine	3	3.1	3.1
Methionine	0.2	0.2	0.2
Vitamin premix ¹	10	10	10
Microelement premix ²	5	5	5
Feed grade silicodioxide/titanium	3	3	3
Inulin	0	5	10
Calculated nutrient level			
ME, MJ/kg	11.98	11.98	11.98
CP	181.0	181.0	181.0
Available phosphorus	4.0	4.0	4.0
Calcium	9.0	9.0	9.0
Lysine	9.1	9.0	9.1
Methionine	3.1	3.1	3.1
Cystine	6.2	6.2	6.2

¹Vitamin premix provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3500 IU; vitamin E, 25 IU; nicotinic acid, thiamin 60 mg; vitamin B12, 0.014 mg; calcium pantothenate, 20 mg; vitamin K3, 2.0 mg; thiamin, 2.0 mg; riboflavin, 8.0 mg; vitamin B6, 7.0 mg; folic acid, 0.8 mg; biotin, 0.2 mg.

²Microelement premix provided the following per kilogram of diet: Fe, 100 mg; Cu, 8 mg; Mn, 120 mg; Zn, 100mg; I, 0.7 mg; Se, 0.3 mg.

lengthwise and scraping with a glass slide. The samples were snap-frozen in liquid nitrogen and then stored at -80°C for gene and protein expression analyses.

Intestinal morphological analysis

The chickens were infected with SE at 3 dpi and the intestinal morphology was analyzed according to Song et al (2018). Briefly, duodenum, jejunum, and ileum segments (2 cm long) were cut and flushed three times with phosphate-buffered saline to wipe off the intestinal contents. The contents from each sampling day were fixed in 2-mL sterile tubes with 4% paraformaldehyde for histological examination and stain with hematoxylin and eosin (performed by Beijing Xuebang Technology Co., Ltd., Beijing, China). For each staining section, 10 representative intact villi and their related crypt were selected to measure villus height (VH) and crypt depth (CD) using a Leica DMI6000B (Wetzlar, Germany) light microscope equipped with image-processing software (Leica application suite V4.2). Then, the ratio of the VH to CD (V/C) was calculated.

Enzyme-linked immunosorbent assay

Serum levels of immunoglobulin (Ig) A, IgM, IgG, secretory IgA (sIgA), and IFN- γ were measured using enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Biotech Co., Ltd., Wuhan, China) following the manufacturer's procedures. Absorbance was measured at 450 nm within 5 min using an automated microplate reader (Multiskan GO; Thermo Fisher Scientific Oy, Van-taa, Finland).

Quantitative real-time polymerase chain reaction

Total RNA was extracted from the ileum using a MiniBEST Universal RNA Extraction Kit (TaKaRa, Dalian, China). Extracted RNA was dissolved in RNase-free water and quantified using a Nanodrop spectrophotometer (ND-2000 UV-Vis; Thermo Fisher Scientific, Waltham, MA). RNA purity was verified by determining the 260:280 nm absorbance ratio. RNA integrity was evaluated based on the spectral curve. cDNA samples were obtained by reverse transcription of total RNA using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham) and stored at -20°C until analysis. Intestinal gene expression levels were measured by real-time polymerase chain reaction (PCR) using SYBR1 Premix Ex Taq (Tli RNaseH Plus; Takara Biotechnology Inc., Osaka, Japan) and an ABI 7500 Real Time PCR Systems (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression levels were used as an internal control to normalize the amount of initial RNA for each sample. Primer sequences for the target and reference genes are shown in Table 2. The mRNA expression levels of intestinal genes were calculated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001).

Western blotting analysis of JAK-STAT pathway proteins

The ileal mucosal tissues of SE-infected chickens at 3 dpi were analyzed to western blotting and carried out according to previous studies (Xue et al., 2017). Briefly, ileal mucosal tissue (5 mg) was treated with 300- μL RIPA lysis buffer and homogenized using an electrical homogenizer (SCIENZT-48, Ningbo, China) and the lysate was then kept on ice for 30 min and cell debris was removed by centrifugation at $16,000 \times g$ for 30 min at 4°C . Protein concentrations were determined by Bradford assay. Equal amounts (30 μg) of protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a

Table 2. Primers used in PCR analysis

Target genes ¹	Primer sequence, 5'-3'	Gene Bank ID
MUC2	F: ACTCCTCCTTTGTATGCGTGA R: GTTAACGCTGCATTCAACCTT	NM_001318434.1
Claudin-1	F: GGTGTACGACTCGCTGCTTA R: CGAGCCACTCTGTTGCCATA	NM_001013611.2
IL-6	F: AATCCCTCCTCGCCAATCT R: TCACGGTCTTCTCCATAAACG	NM_204628.1
LITAF	F: TGTGTATGTGCAGCAACCGTAGT R: GGCATTGCAATTTGGACAGAAGT	NM_205134.1
IL-1 β	F: TGGGCATCAAGGGCTACAAG R: CCAGGCGGTAGAAGAAGATGAAG	HQ739080.1
IFN- γ	F: ATGACTTGCCAGACTTACAAC R: GCTACATCTGAATGACTTGAG	NM_205149.1
TLR4	F: ACGGCATTTTCAAGACGGACT R: ACAGCTTCTCAGCAGGCAAT	NM_001030693.1
SOCS3	F: TGGCCTCAAGACGTTCA R: GTACTCGCTCTTAGAGCT	NM_204600.1
GAPDH	F: GGAGAAACCAGCCAAGTAT R: CCATTGAAGTCACAGGAGA	NM_204305.1

¹MUC2, mucin 2; IL-6, interleukin-6; LITAF, lipopolysaccharide-induced tumor necrosis factor factor; IL-1 β , interleukin-1 β ; IFN- γ , interferon- γ ; TLR4, Toll-like receptor 4; SOCS3, suppressor of cytokine signaling 3.

BioRad mini protein electrophoresis separation unit and then transferred onto a nitrocellulose membrane. After blocking in 5% nonfat milk with Tween 20 buffer and 3% bovine serum albumin at room temperature for 1 h, the membranes were incubated with primary antibodies against signal transducer and activator of transcription 3 (STAT3) (Ab68153, 1:5000), phospho (p)-STAT3 (ab76315, 1:500), and Janus kinase 1 (JAK1) (Ab133666, 1:500), respectively, followed by appropriate horseradish peroxidase-conjugated secondary antibodies. The chemiluminescent substrate (Immobilon Western HRP substrate, Millipore, MA) was applied to the blot according to manufacturer's recommendations. Protein signals were detected using the Image Quant Tanon-5200 system (Tanon Technology Inc., Shanghai, China). Protein expression levels were normalized to β -actin (YM3028, 1:5000) using image analysis software (GIS 1D, ver.4.00).

Statistical analysis

All statistical analyses were performed using SAS V8. Means were compared by one-way analysis of variance followed by Duncan's multiple tests. Statistical significance was considered at $P < 0.05$.

Results

Villus morphology in the small intestine

As shown in Figure 1, inulin supplementation alleviated the villus atrophy and shedding in the duodenum, jejunum, and ileum induced by SE-infection. SE infection significantly increased the CD and decreased the V/C ratio in the duodenum, jejunum, and ileum at 1 and 3 dpi compared with the CON group ($P < 0.05$) (Table 3). Although the VHs in the duodenum, jejunum, and ileum were greater in the CON group compared with the SE group at 1 and 3 dpi, the difference was only significant in the ileum at 1 dpi ($P < 0.05$). Compared with the SE group, VH and V/C ratio (except ileum at 1 dpi) were significantly increased and the CD were significantly decreased ($P < 0.05$) in the duodenum, jejunum, and ileum at 1 and 3 dpi by inulin supplementation at all concentrations (Table 3).

Figure 1. Effects of inulin on gut morphology at 3 d post-infection with *Salmonella*.

Table 3. Effects of inulin on intestinal morphology of SE-infected chickens¹

		Treatment ²					
Item		CON	SE	0.5% InSE	1% InSE	SEM	P-value
VH ³ , μm							
Duodenum	1 dpi ⁴	1006 ^{b,c}	975 ^c	1080 ^a	1054 ^{a,b}	40	0.003
	3 dpi	1010 ^{a,b}	939 ^b	1050 ^a	1072 ^a	56	0.019
Jejunum	1 dpi	999 ^{a,b}	905 ^b	1057 ^a	1079 ^a	81	0.023
	3 dpi	938 ^{b,c}	903 ^c	1092 ^a	1002 ^b	54	0.001
Ileum	1 dpi	620 ^a	510 ^{b,c}	537 ^b	478 ^c	32	0.001
	3 dpi	452 ^{b,c}	425 ^c	540 ^a	499 ^{a,b}	37	0.005
CD ³ , μm							
Duodenum	1 dpi	101 ^b	134 ^a	110 ^b	98 ^b	11	0.001
	3 dpi	90 ^b	119 ^a	93 ^b	98 ^b	8	0.001
Jejunum	1 dpi	98 ^b	119 ^a	95 ^b	97 ^b	7	0.001
	3 dpi	94 ^b	119 ^a	85 ^b	92 ^b	9	0.001
Ileum	1 dpi	86 ^b	99 ^a	83 ^b	85 ^b	7	0.011
	3 dpi	85 ^b	106 ^a	90 ^b	93 ^b	6	0.001
V/C ³							
Duodenum	1 dpi	9.99 ^a	7.38 ^b	9.84 ^a	10.84 ^a	0.85	0.001
	3 dpi	11.32 ^a	7.89 ^b	11.28 ^a	10.94 ^a	0.98	0.001
Jejunum	1 dpi	10.22 ^a	7.60 ^b	11.10 ^a	11.23 ^a	0.89	0.001
	3 dpi	10.10 ^b	7.64 ^c	12.98 ^a	10.95 ^b	1.25	0.001
Ileum	1 dpi	7.24 ^a	5.19 ^b	6.51 ^a	5.63 ^b	0.55	0.001
	3 dpi	5.19 ^a	4.01 ^b	6.06 ^a	5.41 ^a	0.49	0.001

¹Means were calculated on $n = 6$ replicates per treatment.

²CON, basal diet without inulin supplementation; SE, *Salmonella*-infected chicken fed with basal diet; 0.5% InSE, *Salmonella*-infected chicken fed with 0.5% inulin; 1% InSE, *Salmonella*-infected chickens fed with 1% inulin.

³VH, villus height (μm); CD, crypt depth (μm); V/C, VH/CD.

⁴dpi: days post-infection.

^{a-c}Means within rows with different superscript letters were significantly different ($P < 0.05$).

Figure 2. Ileal mRNA expression levels of genes related to mucosal immune and barrier functions in SE-infected chickens at 1- and 3-dpi. Different superscripts above bars indicate significant differences ($P < 0.05$). All data expressed as mean $\pm$ standard error ($n = 6$). MUC2, mucin 2; IL-6, interleukin-6; LITAF, lipopolysaccharide-induced tumor necrosis factor; IL-1 β , interleukin-1 β ; IFN- γ , interferon- γ ; SOCS3, suppressor of cytokine signaling 3; TLR4, Toll-like receptor 4; dpi, days post-infection.

Gene expression related to ileal mucosal immune and barrier functions

The results of inulin effecting on mucosal immune and barrier functions in ileum are shown in Figure 2. SE challenge significantly decreased ($P < 0.05$) expression levels of MUC2 and claudin-1 at 1 and 3 dpi and SOCS3 at 3 dpi and significantly increased ($P < 0.05$) levels of IL-1 β , LITAF, IL-6, TLR4, and IFN- γ level at 1 and 3 dpi and SOCS3 at 1 dpi in the ileum compared with the CON group.

Supplementation with 0.5% and 1% inulin after SE challenge significantly increased the expression levels of MUC2, claudin-1, and TLR4 at 1 and 3 dpi and SOCS3 at 3 dpi ($P < 0.05$), and significantly decreased expression levels of IL-1 β , LITAF, IL-6, and

IFN- γ at 1 and 3 dpi and SOCS3 at 1 dpi ($P < 0.05$) compared with the SE group.

Serum parameters

Serum levels of IgA, sIgA, IgG, IgM, and IFN- γ at 1 and 3 dpi are shown in Table 4. SE challenge significantly increased the levels of IgA at 3 dpi, sIgA at 1 and 3 dpi, IgM at 1 dpi, IgG at 3 dpi, and IFN- γ at 1 and 3 dpi compared with the CON group (all P 's < 0.05). Compared with the SE group, 0.5% and 1% inulin supplements significantly increased the levels of IgA, sIgA, and IgG (except at 1 dpi) and decreased IFN- γ levels (all P 's < 0.05). However, SE challenge and inulin supplementation had no significant effects on serum IgM at 3 dpi and IgG at 1 dpi ($P > 0.05$).

Table 4. Effects of inulin on serum Ig and IFN- γ levels in SE-infected chickens¹

Item	Treatment ²				SEM	P-value
	CON	SE	0.5% InSE	1% InSE		
IgA ³ , mg/mL						
1 dpi ⁴	7.07 ^c	7.33 ^c	7.83 ^b	9.84 ^a	0.14	0.001
3 dpi	6.40 ^c	8.68 ^b	9.51 ^a	9.80 ^a	0.15	0.001
SIgA ³ , mg/mL						
1 dpi	6.41 ^d	7.44 ^c	8.40 ^b	10.53 ^a	0.15	0.001
3 dpi	3.78 ^d	5.63 ^c	6.51 ^b	8.81 ^a	0.10	0.001
IgM ³ , mg/mL						
1 dpi	0.93 ^b	1.04 ^a	1.00 ^a	1.00 ^a	0.02	0.001
3 dpi	0.98	1.03	1.01	1.00	0.03	0.597
IgG ³ , mg/mL						
1 dpi	0.28	0.29	0.28	0.31	0.02	0.377
3 dpi	0.24 ^d	0.34 ^c	0.40 ^b	0.46 ^a	0.01	0.001
IFN- γ ³ , pg/mL						
1 dpi	56.63 ^c	74.42 ^a	61.29 ^b	46.96 ^d	0.99	0.001
3 dpi	58.81 ^b	68.32 ^a	55.04 ^c	37.07 ^d	0.87	0.001

¹Means were calculated on $n = 6$ replicates per treatment.²CON, basal diet without inulin supplementation; SE, *Salmonella*-infected chicken fed with basal diet; 0.5% InSE, *Salmonella*-infected chicken fed with 0.5% inulin; 1% InSE, *Salmonella*-infected chicken fed with 1% inulin.³IgA, immunoglobulin A; SIgA, secretory immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G; IFN- γ , interferon- γ .⁴dpi, days post-infection.^{a-d}Means within rows with different superscript letters were significantly different ($P < 0.05$).

Effects of inulin on signaling pathway proteins

Levels of JAK1/STAT3 signaling pathway proteins in the ileum were determined by western blotting analysis (Figure 3). SE infection with 3 dpi significantly increased p-STAT3 and JAK1 protein expression levels compared with the CON group ($P < 0.05$), and these increases were significantly suppressed by inulin (0.5% and 1%) ($P < 0.05$).

Discussion

The modern poultry industry aims to provide high-quality and efficient animal protein for human consumption. However, *Salmonella* infections pose a major challenge to poultry production worldwide, with important implications for food security and human health, including via the production of subclinical infections in the avian intestine (Micciche et al., 2018). Even in the absence of clinical signs of infection, chronic, persistent *Salmonella* infection may damage the integrity of the gut mucosal barrier and disrupt intestinal homeostasis (Schultz et al., 2018). *Salmonella* can be recognized by pattern recognition receptors in the epithelial cells, resulting in the production of inflammatory cytokines such as ILs, tumor necrosis factor (TNF)- α , and IFN- γ , which disrupt the gut mucosal homeostasis among the microbial community, epithelial cells, and the gut immune system (Ibrahim et al., 2018). *Salmonella* thus triggers an inflammatory immune response, which in turn affects gut mucosal barrier integrity. Many reports showed that SE-challenged chickens exhibited higher IFN- γ expression in spleen after 18-h infection (Cheeseman et al., 2007) and higher TLR4, IL-8 expression in cecum after 48-h infection (Tohidi et al., 2013). In the present study, we showed that *Salmonella* challenge at 1- and 3-dpi significantly upregulated inflammatory cytokine genes such as IL-1 β , IL-6, LITAF, and IFN- γ and downregulated Claudin-1 and MUC2. IFN- γ has been shown to play a key role in the resolution of intestinal *Salmonella* infections (Dolowschiak et al., 2016). Moreover, chronic intestinal inflammation supports *Salmonella* colonization and helps it to compete against the

resident microbiota, leading to the persistence of *Salmonella* in the intestine. Although traditional antibiotic therapies can effectively eliminate the pathogen, they cannot eliminate the mucosal inflammation and may lead to immune tolerance and chronic toxicity. Therefore, the present study demonstrated that the inulin could effectively eliminate the susceptibility to intestinal inflammation and enhance the immune response against *Salmonella* in intestinal tissues, which provides a new strategy to prevent SE-infection in poultry production (Micciche et al., 2018; Tran et al., 2018).

Inulins have been shown to be effective in preventing SE infection by modulating the intestinal inflammation that allows SE to colonize and propagate (Teng et al., 2018; Tran et al., 2018). In general, intestinal inflammation provides *Salmonella* with a metabolic advantage over the resident microorganisms. Inulin could thus be used to prevent SE infection by reducing the expression of pro-inflammatory cytokines. The present results showed that inulin had the potential to reduce the gene expression levels of pro-inflammatory cytokines such as LITAF, IFN- γ , IL-6, and IL-1 β , which play essential roles in controlling intestinal inflammation (Whelan, 2011; Vogt et al., 2015; Ferenczi et al., 2016; Kareem et al., 2017). Moreover, inulin-type fructans have been shown to possess anti-inflammatory properties and to increase the activity of goblet cells and the thicker mucus layer (Bhatia et al., 2015). Inulin might thus improve the intestinal inflammatory environment to make it unfavorable for *Salmonella* colonization.

Furthermore, many studies have shown that inulin strengthens the intestinal barrier function to limit invasion and colonization by SE pathogens by activating the expression of MUC2, Claudin-1, and sIgA (Awad et al., 2017; Genda et al., 2017). sIgA acts as a first-line barrier to protect the intestinal mucosal surface against invading pathogens by inhibiting their attachment to the gut epithelium (Adhikari et al. 2018). The present study showed that serum sIgA and IgG, which play important roles in protecting against SE infection, were increased in chickens fed with inulin (Shao et al., 2016). This

Figure 3. Protein expression levels of p-STAT3 and JAK in ileum of SE-infected chickens at 3 d post-infection. Different superscripts above bars indicate significant differences ($P < 0.05$). All data expressed as mean $\pm$ standard error ($n = 3$). JAK, Janus kinase; STAT3, signal transducer and activator of transcription 3; p-STAT3, phospho-STAT3.

also confirmed that inulin enhanced sIgA levels to ameliorate SE-induced damage to the gut barrier by downregulation of pro-inflammatory cytokines such as TNF- α , IFN- γ , nuclear factor- κ B, and IL-6 (Janardhana, et al., 2009; Mantis et al., 2011; Adhikari et al. 2018). MUC2 and Claudin-1 are also important intestinal epithelial barrier components that prevent harmful substances from reaching the surface of the epithelium. The function of inulin modification of epithelial tight junction integrity is attributed to the expression of tight junction proteins and secretion of mucus protein (Wu et al., 2017a). The immunomodulatory effect of inulin on the gene expression levels of MUC2 and claudin-1 may be associated with the secretion of the pro-inflammatory cytokines LITAF, IL-6, which in turn effects the expression of tight junction proteins (Poritz et al., 2011; Garcia-Hernandez et al., 2017). Therefore, inulin exerted beneficial effects on the integrity of the gut barrier, increased the height of microvilli, and alleviated the damage to the mucosal morphology caused by SE (Teng et al., 2018), which is consistent with the present results. Therefore, inulin thus appears to repair SE-induced damage to

the mucosal morphology via its effects on pro-inflammatory cytokine expression.

Inulin may directly regulate host mucosal immune signaling to modulate the effects of SE infection. Several reports showed that inulin protected the intestinal epithelial barrier and dampened bacterial infection by modulating protein kinase C and mitogen-activated protein kinase signaling in the intestinal epithelium (Wu et al., 2017a, 2017b). Inulin-type fructans could be directly recognized by TLRs, NODs, and CLRs on the surface of immune cells (B cells, T cells, and NK cells) and thus elicit an immune regulation response by increasing the levels of serum IgA, IgG, and IgM antibodies (Seifert et al., 2007; Janardhana, et al., 2009; Adhikari et al., 2018). The present study also showed that inulin (0.5% and 1%) increased serum IgA and IgG levels compared with control groups. We previously demonstrated that TLR4 signaling plays a key role in resistance to *Salmonella* infection in poultry (Li et al., 2017). We also showed that SE challenge increased TLR4 mRNA expression levels, which play an important role in regulating

mucosal immune responses, mucosal barrier function, and inflammatory cytokine expression in the gut (de Kivit et al., 2014; Adhikari et al., 2019). Inulin may directly modulate the proinflammatory cytokine production and epithelial cell kinase signaling via upregulation of TLR-4 expression (Capitán-Cañadas et al., 2014; de Kivit et al., 2014; Ortega-González et al., 2014; Lehmann et al., 2015; Adhikari et al., 2018). These results suggest that prebiotics may directly activate TLR signaling to modulate cytokine expression and epithelial barrier integrity to regulate the effects of SE infection (Capitán-Cañadas et al., 2014; Wu et al., 2017a).

But some researcher also showed that inulin modulates the host gut inflammatory response and intestinal health via beneficial intestinal microbes including *Bifidobacteria* and *Lactobacilli* and the end-products of fermentation of inulins, such as SCFAs, mainly acetate, propionate, and butyrate (Bhatia et al., 2015; Wu et al., 2017a), which exert important regulatory functions in intestinal epithelium cell proliferation and anti-inflammatory properties in the intestinal mucosa (Ferreira et al., 2014). Song et al (2018) also showed that inulin significantly increased serum butyrate and acetate levels. Butyrate is the main fuel source for the growth of intestinal epithelium cells, which exert anti-inflammatory effects by decreasing the expression of pro-inflammatory cytokines (Ferreira et al., 2014). Therefore, inulin exerted beneficial effects on the integrity of the gut barrier, increased the height of microvilli, and alleviated the damage to the mucosal morphology caused by SE (Teng et al., 2018). Several reports have shown that SCFAs, especially butyrate, stimulate the growth of *Bifidobacteria* and *Lactobacilli*, which in turn promote an increase in SCFAs and enhance the mucosal immune function (Ricke, 2015).

The JAK/STAT signaling pathway not only plays an important role in the expression of cytokine genes, but can also be activated by many cytokines (Murray et al., 2007). The induction of proinflammatory cytokines (such as IL6 and IFN- γ) by SE infection may thus activate the JAK/STAT signaling pathway and increase p-STAT protein expression (Hotson et al., 2016; Kogut et al., 2016; Ibrahim et al., 2018a), consistent with the present results. Aberrant activation of the JAK/STAT signaling pathway disrupts the immune and inflammatory responses (Murray et al., 2007; Villarino et al., 2015). Inulin alleviated the inflammatory response in SE-infected chickens by inhibiting JAK1/STAT3 signaling pathways, which in turn regulated the intestinal mucosal immune and inflammatory responses to mitigate SE infection (Liu et al., 2010). SOCS3 has been demonstrated to be involved in the negative feedback regulation of the JAK/STAT signaling pathway (Liu et al., 2017). Accordingly, increased SOCS3 gene expression levels in the inulin groups were associated with inhibition of JAK1/STAT3 expression. JAK1/STAT3 inhibition also has the potential to increase TLR-mediated production of pro- and anti-inflammatory cytokines (Wang et al., 2013), which may also help us to explain the role of inulins in controlling SE-infection in chickens intestines.

In conclusion, our results indicated that *Salmonella* challenge destroyed the mucosal barrier function and caused gut inflammation, which in turn supported *Salmonella* colonization in the gut in chickens. Inulin alleviated SE-induced gut injury by directly decreasing the proinflammatory response and enhancing mucosal immunity in chickens. In the following research, we will study the effects of metabolites (such as SCFA) or microbiota on the immunity and gut barrier of SE-infected chickens. This study also indicated that inulins, as important prebiotics, may represent promising new therapeutic candidates

for manipulating the mucosal immune responses and gut SE infection in chickens.

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