β-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica* serovar Typhimurium

Yujing Shao,*† Yuming Guo,† and Zhong Wang†¹

*State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, and †College of Biology, China Agricultural University, Beijing 100193, PR China

ABSTRACT This study investigated the protective effect of β-1,3/1,6-glucan on gut morphology, intestinal epithelial tight junctions, and bacterial translocation of broiler chickens challenged with *Salmonella enterica* serovar Typhimurium. Ninety *Salmonella*-free Arbor Acre male broiler chickens were randomly divided into 3 groups: negative control group (NC), *Salmonella* Typhimurium-infected positive group (PC), and the *Salmonella* Typhimurium-infected group with dietary 100 mg/kg of β-1,3/1,6-glucan supplementation (T) to determine the effect of β-1,3/1,6-glucan on intestinal barrier function. *Salmonella* Typhimurium challenge alone significantly decreased villus height (*P* < 0.001), villus height/crypt depth ratio (*P* < 0.05), and the number of goblet cells (*P* < 0.001) in the jejunum at 14 d post-infection (dpi), but significantly increased the number of intestinal secretory IgA (sIgA)-expressing cells at 14 dpi (*P* < 0.01) and total sIgA levels in the jejunum at 7 (*P* < 0.05) and 14 dpi (*P* < 0.01) compared with the unchallenged birds (NC). Dietary β-1,3/1,6-glucan supplementation not only significantly increased villus height, villus height/crypt depth ratio, and the number of goblet cells (*P* < 0.01), but also increased the number of sIgA-expressing cells (*P* < 0.05) and sIgA content in the jejunum at 14 dpi (*P* < 0.01) in birds challenged with *Salmonella* Typhimurium in comparison with *Salmonella* Typhimurium challenge alone. β-1,3/1,6-Glucan addition had significant inhibitory effects (*P* < 0.05) on cecal *Salmonella* colonization levels and liver *Salmonella* invasion of the *Salmonella* Typhimurium-infected birds compared with the PC group. Intestinal tight junction proteins claudin-1, claudin-4, and occludin mRNA expression in the jejunum at 14 dpi was significantly decreased by *Salmonella* Typhimurium challenge alone (*P* < 0.01) compared with that of the NC group, whereas β-1,3/1,6-glucan supplementation significantly increased claudin-1 and occludin mRNA expression (*P* < 0.01) at 14 dpi in the jejunum of the *Salmonella* Typhimurium-infected birds in comparison with the PC group. Our results indicate that dietary β-1,3/1,6-glucan can alleviate intestinal mucosal barrier impairment in broiler chickens challenged with *Salmonella* Typhimurium.

Key words: β-1,3/1,6-glucan, *Salmonella*, intestinal barrier, chicken

INTRODUCTION

Intestinal mucosa is not only the major site for digestion and absorption of nutrients, but also the innate defense barrier against most intestinal pathogens (Turner, 2009). The gut mucosal barriers are mainly composed of mechanical, biological, chemical, and immunological barriers, in which the mechanical and immunological barriers are the chief components of intestinal mucosal immunity (Reynolds et al., 1996). The intestinal mechanical barrier formed by the epithelial cells and the junctional complex, consisting of tight junctions (TJ) (such as occludin and claudins), adheren junctions, gap junctions, and desmosomes, plays an important role in the absorption of nutrients, electrolytes, and water, the maintenance of intestinal barrier function, and protecting the gut from enteric pathogen invasion (Tsukita et al., 2001; Schneeberger and Lynch, 2004; Catalioto et al., 2011). Intestinal TJ disruption results not only in increasing permeability to luminal antigens and bacteria translocation, which leads to endogenous infection and endotoxemia, but it also lowers the absorption of both exogenous and endogenous nutrients (Berkes et al., 2003; Ulluwishewa et al., 2011). Numerous studies have shown that several factors, such as alterations in dietary components, probiotics, or intestinal pathogens, can regulate intestinal barrier structure and function (Berkes et al., 2003; Groschwitz and Hogan, 2009; Ohland and Macnaughton, 2010; Ulluwishewa et al., 2011).
**Salmonella enterica** serovar Typhimurium is a leading cause of food-borne gastroenteritis in humans (Liljebjelke et al., 2005) and is commonly associated with gastroenteritis caused by the consumption of *Salmonella*-contaminated broiler poultry meat and eggs (Zhang et al., 2003). *Salmonella* Typhimurium can colonize or invade the gastrointestinal tract and damage intestinal barrier function, which causes acute gastroenteritis and bacteremia in poultry and humans (Jepson et al., 2000; McSorley, 2011). Furthermore, in the poultry industry, *Salmonella* infections can cause lost productivity, increased mortality, and the associated contamination of poultry products for human consumption (Fasina et al., 2008). It has been shown in vivo that *Salmonella* Typhimurium invasion of intestinal epithelia is accompanied by a loss of epithelial integrity and consequent loss of epithelial function (Clark et al., 1998; Sears, 2000). Furthermore, in vitro models of *Salmonella* Typhimurium infection can cause a progressive decrease in transepithelial electrical resistance, regulate intestinal epithelial cell TJ proteins, damage intestinal barrier function, and facilitate bacterial translocation (Jepson et al., 2000; Kohler et al., 2007). However, the direct interaction of *Salmonella* Typhimurium with intestinal TJ proteins has not been investigated in broilers in vivo.

Reduction of *Salmonella* colonization in the intestinal tract of poultry protects intestinal barrier integrity and decreases the risk of potential carcass contamination during slaughter. Various prophylactic approaches have been used to control *Salmonella* infection in poultry production, including increased standards of hygiene, antibiotics, competitive exclusion products and probiotics, genetic selection of chicken lines for improved immune responses, and development of *Salmonella* vaccines (Vandeplas et al., 2010). Although antibiotics are one of the effective measures to control *Salmonella* Typhimurium infection, the development of antibiotic resistance by pathogenic bacteria has led to a worldwide consideration of limiting the usage of antibiotics in animal agriculture. Therefore, it is essential to develop alternative ways to deal with bacterial challenges in commercial poultry production.

The β-glucans are polymers of glucose that can be derived from the cell wall of bacteria, fungi, and yeast, as well as cereal grains such as oats and barley. β-Glucans from yeast and fungal sources comprising 1,3-linked glucopyranosyl residues with small numbers of 1,6-linked branches (Manners et al., 1973) have been widely studied and shown to be most effective in enhancing host protective immunity against infectious agents (Soltanian et al., 2009; Volman et al., 2008). In chickens, in vitro and in vivo, numerous studies have shown that β-1,3/1,6-glucans from *Saccharomyces cerevisiae* have beneficial effects on both the innate and acquired immune systems in either nonchallenged or challenged settings (Guo et al., 2003; Lowry et al., 2005; Chae et al., 2006; Huff et al., 2006, 2010; Chen et al., 2008; Cox et al., 2010a,b). Furthermore, dietary inclusion of β-1,3/1,6-glucan was able to modulate intestinal mucosal immune response and reduce tissue lesion severity in broiler chickens during coccidiosis (Cox et al., 2010a,b). β-1,3/1,6-Glucan addition results in increased clearance of several important pathogens such as *Salmonella* and *Escherichia coli* in broiler chickens (Lowry et al., 2005; Huff et al., 2010).

These previous studies mainly focused on either the effect β-1,3/1,6-glucan on chicken systemic immune response, or in vitro experiments using chicken immune cells to investigate the function of β-1,3/1,6-glucan without evaluating the effect of β-1,3/1,6-glucan on intestinal epithelial TJ and bacterial translocation of broilers challenged with *Salmonella enteritidis*. Therefore, the objective of the present study was to assess the effects of β-1,3/1,6-glucan on intestinal morphology, the number of intestinal major cell {goblet cells and secretory IgA (sIgA)-expressing cells}, total sIgA, bacteria liver translocation, and gene expression of TJ proteins (claudin-1,4 and occludin) of broiler chickens challenged with *Salmonella* Typhimurium.

### MATERIALS AND METHODS

#### β-1,3/1,6-Glucan Preparation

β-1,3/1,6-Glucan was extracted from *Saccharomyces cerevisiae* according to the method of Wang et al. (2008). The chemical content of the β-1,3/1,6-glucan product was 91.5% glucan, 1.15% CP, 0.43% crude fat, and some remaining unspecified components. Its average molecular weight was determined by light scattering to be ~800 kDa.

#### Birds, Diets, and Treatment

This project was approved and conducted under the guidelines of China Agricultural University Animal Care and Use Committee. A total of 90 one-day-old Arbor Acre male broiler chickens (Hua Du Broiler Company, Beijing, China) were randomly assigned to 3 treatments with 30 birds per group and housed in 3 stainless steel negative pressure experimental animal isolators (Jinhang Pure Air Conditioner Engineering Corp., Tianjin, China). The groups were the unchallenged negative control treatment (NC), *Salmonella* Typhimurium-challenged positive control treatment (PC), and *Salmonella* Typhimurium-challenged group with dietary 100 mg/kg of β-1,3/1,6-glucan supplementation (T). On arrival of the broiler chickens, cloacal swabs and fecal samples were collected and tested for *Salmonella*. Samples were preenriched with tetrathionate broth (02–057, Beijing AoBoxing Biotech Co. Ltd., Beijing, China) and selenite-cystine broth (SCB; 02–026, Beijing AoBoxing Biotech Co. Ltd.), and streaked on xylose–lysine–deoxycholate (XLD) agar (CM219,
Beijing Land Bridge Technology Co. Ltd., Beijing, China) and phenolphthalein brilliant green agar (CM 231, Beijing Land Bridge Technology Co. Ltd.) to confirm that the chickens were free of *Salmonella*. An unmediated corn-soybean meal diet was formulated to meet or exceed the nutrient requirements for broilers as recommended by the NRC (1994). A standard management procedure was used throughout the experiment. Chickens had ad libitum access to clean water and feed.

**Challenge Bacteria**

The *Salmonella* Typhimurium strain CMCC50115 was obtained from the China Institute of Veterinary Drug Control (Beijing, China). The frozen culture was thawed and 10 μL was inoculated into sterile tubes containing 10 mL of sterile tryptone soy broth. The inoculated broth was incubated at 37°C with orbital shaking for 24 h (THZ-C incubator; Su Zhou Pui Ying Experimental Equipment Co. Ltd., Jiang Su, China). Subsequently, 5 mL of *Salmonella* Typhimurium preculture was transferred to 100 mL of tryptone soy broth and incubated with orbital shaking at 37°C for 16 to 18 h. To determine the concentration of viable *Salmonella* Typhimurium in the culture, the inoculum was diluted with sterile PBS (pH 7.2) and plated on XLD agar for 24 h, and black colonies were counted after incubation for 24 h at 37°C. The stock culture was transferred to 100 mL of tryptone soy broth containing 10 mL of sterile tryptone soy broth. The frozen culture was thawed and 10^10 cfu/mL of *Salmonella* Typhimurium was obtained from the China Institute of Veterinary Drug Control (Beijing, China). The frozen culture was thawed and 10 μL was inoculated into sterile tubes containing 10 mL of sterile tryptone soy broth. The inoculated broth was incubated at 37°C with orbital shaking for 24 h (THZ-C incubator; Su Zhou Pui Ying Experimental Equipment Co. Ltd., Jiang Su, China). Subsequently, 5 mL of *Salmonella* Typhimurium preculture was transferred to 100 mL of tryptone soy broth and incubated with orbital shaking at 37°C for 16 to 18 h. To determine the concentration of viable *Salmonella* Typhimurium in the culture, the inoculum was diluted with sterile PBS (pH 7.2) and plated on XLD agar for 24 h, and black colonies were counted after incubation for 24 h at 37°C. The stock culture was prepared in sterile PBS and adjusted to 1 × 10^10 cfu/mL of *Salmonella* Typhimurium as the inoculum. At 7 d of age, birds in the PC and T groups were orally gavaged with 1.0 mL of 10^10 cfu/mL of *Salmonella* Typhimurium suspended in sterile PBS. Chickens in the NC group received 1.0 mL of sterile PBS on the same date and served as the control.

**Sample Collection**

On d 7 and 14 postchallenge, 15 birds from each group were randomly selected, euthanized, and sampled. Sampled chickens were killed by cervical dislocation, and livers and cecal contents were aseptically removed and assessed for *Salmonella* Typhimurium as soon as possible. On d 14 postinfection, tissue samples (2 cm) of jejunum were washed in PBS (flushed with 0.05 M PBS, pH7.2) and fixed in 2.5% (vol/vol) polyoxymethylene-glutaraldehyde solution for histological and immunohistochemistry examination. Samples of jejunum (1 cm) were aseptically excised, snap-frozen in liquid nitrogen, and stored at −80°C for mRNA determination. Jejunal mucosa from each group was collected in 5 mL of protease inhibitor cocktail containing EDTA, 0.3 mg/mL; phenylmethylsulfonyl fluoride, 75 μg/mL; pepstatin, 0.7 μg/mL; and leupeptin, 0.5 μg/mL (Sigma-Aldrich, St. Louis, MO) in ice-cold PBS (pH 7.2). After centrifugation (10,000 × g, 10 min, 4°C), the supernatant was separated and stored at −20°C until required for total sIgA analysis.

**Cecal Colonization and Organ Invasion by Salmonella**

To determine cecal colonization levels, the cecal contents were removed aseptically and placed into preweighed 15-mL sterile plastic tubes, weighed, and diluted 1:10 with sterile buffered peptone water (BPW; 02–030, Beijing Aoboxing Biotech) and then centrifuged at 500 × g for 1 min at 4°C. The supernatant was serially diluted (10^-2, 10^-3, 10^-4, 10^-5, 10^-6, and 10^-7) with sterile PBS to appropriate levels for isolation on XLD agar plates. Black colonies were counted on XLD agar plates after incubation for 24 h at 37°C. *Salmonella* enumeration results were expressed as log_{10} cfu/g of feces.

To determine organ invasion after oral *Salmonella* Typhimurium inoculation, samples of livers from each treatment were aseptically removed, weighed, and homogenized separately. The homogenates of each organ were diluted 1:10 with a sterile solution of BPW and preenriched for 24 h at 37°C in SCB (CM 201–01, Land Bridge Technology Ltd.). After this incubation, 100 μL of suspension was spread on XLD agar. Organ invasion rate was calculated by the ratio of *Salmonella*-positive liver samples to the total number of liver samples. Samples from the NC group were diluted 1:10 with a sterile solution of BPW and preenriched for 24 h at 37°C in SCB, and then streaked on XLD agar.

**Measurement of Villus Morphology**

Tissue samples were dehydrated and embedded in paraffin by routine methods. The serial paraffin sections (5 μm) were prepared with the laboratory protocol. Some sections were processed with common hematoxylin-eosin staining for villus morphology measurement according to previous reports (Wang et al., 2009a). Villus height and crypt depth were measured on the stained sections under a microscope with 40× combined magnification and an ocular micrometer. At least 15 well-oriented, intact villi were measured in triplicate portions of the slide for each broiler chicken within each treatment group. The length of the villi was measured from the crypt mouth to the villus tip, and all measurements were made (villus height and crypt depth) in 10-μm increments.

**Measurement of Goblet Cells in Jejunum with Periodic Acid–Schiff Staining**

Goblet cells are the major source of mucin and are usually displayed with periodic acid–Schiff staining. The serial histological sections (5 μm) were prepared using the same protocol as described previously (Wang et al., 2009a). After dewaxing and immediately washing with distilled water for 1 min, the specimens were immersed in 0.5% periodate solution (Sigma) for 5 min at room temperature in the dark. Afterward, sections
were immediately washed (30 s \times 2) and soaked in Schiff’s reagent (Sigma) at 37°C. After 1 h, sections were washed twice with a sulfuric acid solution then quickly rinsed with distilled water. The slides were dehydrated and mounted. The area of the goblet cells in different intestinal sections was counted from the length and width of the goblet cell “cup” in cross sections of the villi under an Olympus light microscope (Olympus Optical Co., Beijing, China). Glycogen appeared as a prune color and glycoprotein as pink. The preparations of the villi under an Olympus light microscope. The results were expressed as the number of goblet cells per unit surface area (mm²).

**Measurement of IgA-Expressing Cells Using Immunohistochemistry**

The histological sections (5 μm) were prepared using the same protocol as described above. The mouse anti-chicken IgA monoclonal antibody was obtained from Bethyl Laboratories Inc. (Montgomery, TX) and used at a dilution of 1:100 in PBS (0.01 M, pH 7.4). The number of cells secreting IgA was revealed using the avidin-biotin complex immunohistochemical method. The detailed staining procedure was based on the methods of Wang et al. (2009b). The number of positive cells in the intestinal lamina propria was counted using an Olympus light microscope. The results were expressed as the number of cells/mm².

**ELISA Detection of Total IgA in Jejunal Flushes**

The levels of IgA in intestinal fluid were quantified using commercial ELISA assay kits (Bethyl Laboratories Inc.) according to the manufacturer’s instructions.

**Quantitative Real-Time PCR**

Total RNA was isolated from snap-frozen jejunal tissue samples (50 mg) according to the RNaseasy mini kit following the animal tissue protocol (Qiagen, Germantown, MD). The purity and concentration of total RNA were measured by a spectrophotometer (NanoDrop-2000, Thermo Fisher Scientific, Waltham, MA) at 260 and 280 nm. Ratios of absorption (260:280 nm) of all samples were between 1.8 and 2.0. First-strand cDNA was synthesized from 2 μg of total RNA using Oligo dT primers and Superscript II reverse transcriptase (Invitrogen Life Technologies, Shanghai, China) according to the manufacturer’s instructions. Synthesized cDNA was stored at -20°C until processed. Primers were designed (Table 1) based on known chicken sequences using the Primer Express 3.0 software (Applied Biosystems, Foster City, CA) and synthesized by Invitrogen.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>GenBank accession number</th>
<th>Forward sequence (5’ to 3’)</th>
<th>Reverse sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin-1</td>
<td>NM_001013611.2</td>
<td>TGAGGGATGACGAGATTGAAAGA</td>
<td>CGGCCACCTCTGTTGCCATA</td>
</tr>
<tr>
<td>Claudin-4</td>
<td>AY435420.1</td>
<td>GAAGCGCTGAACCGATACCA</td>
<td>TGCTTCTGTGCCATTGTTCC</td>
</tr>
<tr>
<td>Occludin</td>
<td>NM_205128.1</td>
<td>TCTTCTGCGCGTCTTCTGG</td>
<td>TCTTACGTGGCGTCTTCTGG</td>
</tr>
<tr>
<td>β-Actin</td>
<td>NM_205518.1</td>
<td>ACTGCGTGTAGGTTGTTAC</td>
<td>GGGTGGATCTCTTCTG</td>
</tr>
</tbody>
</table>

1Primers designed by Primer Express software (Invitrogen, Shanghai, China).

### Statistical Analysis

Data were analyzed by one-way ANOVA using the GLM procedures of SPSS 17.0 software (version 17.0, SPSS Inc., Chicago, IL). Statistical differences between treatment groups were tested by Duncan’s multiple-range test. Differences were considered significant at \( P \leq 0.05 \) unless otherwise stated. Results were expressed as the mean ± SE. Single df contrasts were made among treatment groups by Duncan’s multiple-range test. Differences were considered significant at \( P \leq 0.05 \) unless otherwise stated. Results were expressed as the mean ± SE. Single df contrasts were made among treatment means to compare the NC versus PC group, and the PC versus T group.

### RESULTS

**Histological Analysis of Jejunal Morphology**

A comparison of jejunal morphology is shown in Table 2. *Salmonella* Typhimurium infection significantly decreased villus height and villus height/crypt depth ratio in the jejunum \( (P < 0.01) \) compared with the NC group. However, birds fed 3-1,3/1,6-glucan had significantly increased villus height and villus height/crypt...
depth ratio in the jejunum (P < 0.01) compared with the Salmonella Typhimurium-infected group, whereas they had no significant effect on crypt depth (P > 0.05).

**Changes in Number of Goblet Cells, sIgA-Expressing Cells, and Total sIgA Level in Jejunum**

Goblet cells were mainly distributed among columnar cells and presented a typical goblet shape (Figure 1A–C). The number of goblet cells in the jejunum (Table 2) was decreased markedly in the Salmonella Typhimurium-infected birds (P < 0.01) compared with that in the NC group. Moreover, birds fed a diet containing β-1,3/1,6-glucan had a significant increase in goblet cell density (P < 0.01) in the jejunum of the Salmonella Typhimurium-infected group compared with the PC group (Table 2). The sIgA-expressing cells were mainly distributed in the area of the mucosal lamina propria of the jejunum. The sIgA-expressing cells were recognizable as lymphocytes by their typical features, such as a nucleus surrounded by a ring of yellow-brown cytoplasm (Figure 2D–F). The density of sIgA-expressing cells at 14 dpi postinfection (dpi; P < 0.05, Table 2) and sIgA content at 7 dpi (P < 0.05; Table 2) and 14 dpi (P < 0.01, Table 2) in the jejunum of the Salmonella Typhimurium-infected group were both increased markedly (P < 0.01, Table 2) compared with those in the NC group. There was a significant increase in the number of sIgA-expressing cells (P < 0.05) at 14 dpi and sIgA level (P < 0.01) at 7 and 14 dpi in the jejunum of the β-1,3/1,6-glucan-treated birds compared with the PC group (Table 2).

**Cecal Colonization and Liver Invasion by Salmonella**

The cloacal swabs from each group before inoculation were all free of Salmonella after enrichment. The uninfected birds remained free of Salmonella throughout the experiment, whereas all liver samples of the PC group were found to be Salmonella-positive at 7 and 14 dpi (Table 3). Cecal Salmonella levels (2.26 cfu/g, 1.30 cfu/g) and the percentage of liver Salmonella-positive samples (6/15, 4/15) in all birds of the T group at 7 and 14 dpi, respectively, were significantly (P < 0.05) decreased compared with those (3.72 cfu/g, 4.08 cfu/g, and 15/15, 15/15) of the Salmonella Typhimurium-challenged group.

**Intestinal TJ mRNA Expression**

With regard to TJ protein gene expression, claudin-1, claudin-4, and occludin gene expression in the jejunum was downregulated significantly (P < 0.01, Table 4) in the Salmonella Typhimurium-infected group compared with the NC group. Birds fed β-1,3/1,6-glucan had significantly higher claudin-1 and occludin gene expression in the jejunum compared with that of the Salmonella Typhimurium-infected group (P < 0.01, Table 4).

**DISCUSSION**

The purpose of this study was to investigate the effect of β-1,3/1,6-glucan on intestinal local mucosal immunity, intestinal epithelial TJ, and bacterial translocation of broilers challenged with Salmonella Typhimurium.

**Gut Morphology**

The effect of β-1,3/1,6-glucan on chicken gut morphology in an Salmonella Typhimurium-challenged model was observed. There was a significant reduction in villus height and villus height to crypt depth ratio in the jejunum in the Salmonella Typhimurium-infected birds, which indicates that Salmonella Typhimurium challenge can cause intestinal mucosal damage. Similar results have been reported in previous studies (Rahimi et al., 2009; Fasina et al., 2010) in which Salmonella-inoculated birds showed shortening, atrophy, and reduced density of villi. However, we also found that dietary

### Table 2. Effect of dietary β-1,3/1,6-glucan supplementation on jejunal morphology and mucosal immunity in broiler chickens challenged with Salmonella enterica serovar Typhimurium

<table>
<thead>
<tr>
<th>Item</th>
<th>NC</th>
<th>PC</th>
<th>T</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus height, μm</td>
<td>1,239^A</td>
<td>971^C</td>
<td>1,126^B</td>
<td>129.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Crypt depth, μm</td>
<td>198</td>
<td>190</td>
<td>192</td>
<td>24.8</td>
<td>0.899</td>
</tr>
<tr>
<td>V/C^2</td>
<td>6.3^A</td>
<td>5.1^C</td>
<td>5.9^B</td>
<td>0.63</td>
<td>0.031</td>
</tr>
<tr>
<td>Goblet cell density, n/mm^2</td>
<td>791^A</td>
<td>509^C</td>
<td>596^B</td>
<td>135.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sIgA^+ cell numbers/mm^2</td>
<td>39^C</td>
<td>49^B</td>
<td>53^A</td>
<td>9.2</td>
<td>0.045</td>
</tr>
<tr>
<td>sIgA, ng/mL</td>
<td>7 dpi 199^C</td>
<td>221^b</td>
<td>231^a</td>
<td>22.7</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>14 dpi 258^c</td>
<td>287^b</td>
<td>333^a</td>
<td>34.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>

^A Least squares means with different superscripts within the same row are significantly different (P < 0.01).

^B Least squares means with different superscripts within the same row are significantly different (P < 0.05).

^C Each value represents the mean values of 6 birds for each treatment. NC = unchallenged negative control treatment; PC = Salmonella Typhimurium-challenged positive control treatment; T = Salmonella Typhimurium-challenged group with dietary 100 mg/kg of β-1,3/1,6-glucan supplementation; sIgA = secretory IgA; dpi = days postinfection.

^1V/C = villus height to crypt depth ratio.
\(\beta-1,3/1,6\)-glucan administration resulted in increased villus height and villus height to crypt depth ratio in the jejunum after \textit{Salmonella} Typhimurium challenge. These results not only were in agreement with previous studies, demonstrating that birds fed a brewer’s yeast extract containing \(\beta\)-glucans consistently had significantly higher villus height (Morales-Lopez et al., 2009), enhanced mucin-producing goblet cells in the ileum, and to some degree in the jejunum and duodenum (Solis de los Santos et al., 2007), but also showed that dietary \(\beta-1,3/1,6\)-glucan could restore some of the villus loss or damage associated with \textit{Salmonella} challenge. Similar results were reported by Cox et al. (2010a), who showed that dietary \(\beta\)-glucan supplementation at 0.1% reduced lesion severity in the duodenum and jejunum of broiler chickens challenged with \textit{Eimeria}. Intestinal morphology, including duodenal and ileal villus height and crypt depth as well as villus height to crypt depth ratio, is indicative of gut health in broilers. This could mean that increments of villus height that are observed with \(\beta-1,3/1,6\)-glucan treatment are associated with improved gut health. Therefore, the present results indicate that \textit{Salmonella} Typhimurium challenge can damage the integrity of the intestinal mucosal barrier of broiler chickens, whereas the severity of this disruption is decreased after dietary administration of \(\beta-1,3/1,6\)-glucan.

**Goblet Cells, slgA-Expressing Cells, and Bacterial Translocation**

Goblet cells can secrete mucus to the mucosal surface and promote elimination of gut contents; provide the first line of defense against physical and chemical injury caused by ingested food, microbes, and microbial products; and participate in the process of nutrient digestion and absorption (Deplancke and Gaskins, 2001; Iijima et al., 2001). Defective mucous layers result in increased bacterial adhesion to the surface epithelium, increased intestinal permeability, enhanced susceptibility to intestinal pathogenic microbes, and reduced digestion and absorption of nutrients. In the current study, the number of goblet cells was markedly decreased in the jejunum of the \textit{Salmonella} Typhimurium-infected chickens, which indicates that \textit{Salmonella} Typhimurium challenge might have a significant suppressive effect on goblet cell numbers. The result is in agreement with our observation on gut morphology, in which a reduced

![Figure 1. Goblet cells with a typical goblet shape appear as a prune color (arrows), and are distributed among columnar cells (periodic acid-Schiff staining, ×40). (A) Unchallenged negative control treatment (NC) group; (B) \textit{Salmonella} Typhimurium-challenged positive control treatment (PC) group; and (C) \textit{Salmonella} Typhimurium-challenged group with dietary 100 mg/kg of \(\beta-1,3/1,6\)-glucan supplementation (T) group.](https://academic.oup.com/ps/article-abstract/92/7/1764/1554157)

![Figure 2. Secretory IgA (slgA)-expressing cells were recognizable as lymphocytes by their typical features such as a nucleus surrounded by a ring of yellow-brown cytoplasm (arrow; immunohistochemical staining, ×40). Scale bars = 100 \(\mu\)m. (D) Unchallenged negative control treatment (NC) group; (E) \textit{Salmonella} Typhimurium-challenged positive control treatment (PC) group; and (F) \textit{Salmonella} Typhimurium-challenged group with dietary 100 mg/kg of \(\beta-1,3/1,6\)-glucan supplementation (T) group.](https://academic.oup.com/ps/article-abstract/92/7/1764/1554157)
villus height to crypt depth ratio was found after *Salmonella* Typhimurium challenge. However, our current study was not in accordance with previous results. Fasina et al. (2010) have reported that *Salmonella* Typhimurium infection increases goblet cell density in the intestine of broiler chickens. The discrepancy may be explained by differences in virulence of challenged *Salmonella* Typhimurium, *Salmonella* Typhimurium challenge dose, sampling period, and analyzed units (villi, crypts, or merged). In contrast, other previous studies (Deplancke and Gaskins, 2001; Iijima et al., 2001; Forder et al., 2007) have shown that changes in mucin profile in response to bacterial colonization play a potential role in protective mechanisms against pathogenic invasion of the intestinal mucosa. Therefore, further investigation is needed into the amount and profile of mucus in the intestine of broiler chickens.

In this study, dietary β-1,3/1,6-glucan addition significantly increased the number of goblet cells in the jejunum of the *Salmonella* Typhimurium-infected chickens compared with *Salmonella* Typhimurium challenge alone. This increase in the number of goblet cells was in agreement with the findings of Solis de los Santos et al. (2007), who found that birds fed a brewer’s yeast extract containing β-glucans had an increase in mucin-producing goblet cells in the ileum, and to some degree in the jejunum and duodenum. Moreover, Cox et al. (2010a) showed that mucin-2 levels produced by goblet cells were upregulated in the jejunum of *Eimeria*-challenged birds fed 0.1% β-glucans compared with the *Eimeria*-challenged controls. Goblet cells and secretion of mucin might play an important role in the protective function by binding to pathogens, preventing their adhesion to the intestinal surface (Ikeda et al., 2002).

Therefore, β-1,3/1,6-glucan alleviates the intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella* Typhimurium, possibly by increasing the number goblet cells in the intestine.

The sIgA serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms, and maintains mucosal homeostasis in the gut (Mantis et al., 2011). In the present study, *Salmonella* Typhimurium infection not only significantly increased sIgA-expressing cells but also enhanced sIgA production. A recent study has shown that *Salmonella* Typhimurium oral challenge can increase sIgA level in the intestine of chickens (Revolledo et al., 2009; Marcq et al., 2011). Moreover, some previous studies (Beal et al., 2004; Withanage et al., 2005) have reported that infection with *Salmonella* leads to increased levels of IgA antibody. The significant changes in sIgA content of chicken intestine shows that *Salmonella* Typhimurium infection can strongly stimulate the mucosal immune system and induce the intestinal mucosal immune response. At the same time, oral β-1,3/1,6-glucan administration not only significantly increases the number of IgA-secreting cells in the lamina propria of the small intestine, but also total sIgA in the intestinal lumen in *Salmonella* Typhimurium-infected birds compared with the PC group. Similar findings have been reported by Guo et al. (2003), who have shown that broiler chickens supplemented with β-1,3/1,6-glucan have an amplified humoral immune response. The increase in the number of goblet cells and sIgA-positive cells is associated with facilitating the expulsion of pathogens and maintaining the integrity of

### Table 3. Effect of dietary β-1,3/1,6-glucan supplementation in broiler diets on liver *Salmonella* invasion and cecal *Salmonella* colonization levels of chickens challenged with *Salmonella enterica* serovar Typhimurium

<table>
<thead>
<tr>
<th>Item</th>
<th>NC</th>
<th>PC</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver invasion²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 dpi</td>
<td>0/15</td>
<td>15/15</td>
<td>6/15</td>
</tr>
<tr>
<td>14 dpi</td>
<td>0/15</td>
<td>15/15</td>
<td>4/15</td>
</tr>
<tr>
<td>Cecal <em>Salmonella</em> colonization³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 dpi</td>
<td>0c</td>
<td>3.72a</td>
<td>2.26b</td>
</tr>
<tr>
<td>14 dpi</td>
<td>0c</td>
<td>4.08a</td>
<td>1.30b</td>
</tr>
</tbody>
</table>

[a–cDifferent superscript letters associated with mean values in the same column indicate significant differences between treatments (P < 0.05).

1All chickens from the PC and T group were challenged with *Salmonella* Typhimurium at 7 d of age, and 15 chickens were killed at 7 and 14 dpi. NC = unchallenged negative control treatment; PC = *Salmonella* Typhimurium-challenged positive control treatment; T = *Salmonella* Typhimurium-challenged group with dietary 100 mg/kg of β-1,3/1,6-glucan supplementation.

2Individual chickens were considered the experimental unit. Values represent the number of birds sampled at each sampling day.

3Individual chickens were considered the experimental unit. Values represent the least squares means at each sampling day, expressed in log10 cfu per gram of cecal content.

### Table 4. Relative expression of cytokines and tight junction protein mRNA expression in the jejunum of broiler chickens at 21 d of age treated with dietary β-1,3/1,6-glucan and challenged with *Salmonella enterica* serovar Typhimurium

<table>
<thead>
<tr>
<th>Item</th>
<th>NC</th>
<th>PC</th>
<th>T</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin-1</td>
<td>1.10D</td>
<td>0.44C</td>
<td>2.09A</td>
<td>0.135</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Claudin-4</td>
<td>1.05A</td>
<td>0.47B</td>
<td>0.51B</td>
<td>0.089</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Occludin</td>
<td>1.01A</td>
<td>0.53C</td>
<td>1.61A</td>
<td>0.143</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

[A–CLeast squares means with different superscripts within the same row are significantly different (P < 0.01).

1Each value represents the mean values of 6 birds for each treatment. NC = unchallenged negative control treatment; PC = *Salmonella* Typhimurium-challenged positive control treatment; T = *Salmonella* Typhimurium-challenged group with dietary 100 mg/kg of β-1,3/1,6-glucan supplementation.
the mucous protective layers, especially in enteric disease (Brandtzaeg, 2010). Therefore, oral β-1,3/1,6-glucan protects the intestinal barrier function of chickens infected with Salmonella Typhimurium by enhancing mucosal sIgA production.

Some studies have shown that the presence of bacteria in organs is a function of the integrity of the intestinal barrier, bacterial overgrowth, and status of the host immune system (Berg, 1995). In the current study, there was a significant increase in the percentage of Salmonella Typhimurium-positive liver samples and Salmonella Typhimurium colonization levels in the cecum, which suggests that Salmonella Typhimurium can adhere to the gut, spread to the liver, spleen, and blood, and lead to impaired intestinal mucosa. Similarly, previous studies have indicated that Salmonella Typhimurium can colonize chicken intestine and invade the liver and spleen (Revolledo et al., 2009), increase intestinal permeability, and impair the intestinal mucosal barrier (Turnbull and Snoeyenbos, 1974; Fasina et al., 2010). Moreover, several in vitro studies (Kops et al., 1996; Jepson et al., 2000; Kohler et al., 2007) have shown that infection of intestinal epithelial cell lines by Salmonella Typhimurium increases intestinal permeability and facilitates bacterial translocation. In the current study, dietary β-1,3/1,6-glucan addition significantly inhibited cecal colonization and liver invasion by Salmonella. Similarly, several previous studies have shown that β-glucan alone or combination with other probiotics provides strong protection against organ invasion and intestine colonization by Salmonella in chickens (Lowry et al., 2005; Chen et al., 2008; Revolledo et al., 2009). Nevertheless, it is of interest to note that dietary β-1,3/1,6-glucan supplementation failed to eradicate completely Salmonella Typhimurium gut infection during the 2 wk of measurement postinfection, which agrees with the study of Marcq et al. (2011). Therefore, our results showed that dietary β-1,3/1,6-glucan offers a high level of protection to chickens against Salmonella challenge probably via increasing the number of goblet cells and sIgA-expressing cells, and the amount of total IgA in the intestinal fluid.

**TJ Proteins**

Tight junctions are composed of at least 3 types of transmembrane proteins: occludin, claudins, and junctional adhesion molecules (Tsukita et al., 2001; Schneeberger and Lynch, 2004). Occludin and the claudin family are the most important components in the regulation of epithelial barrier function in the intestine (Fanning et al., 1998). Disruption (i.e., relocation or redistribution) of the TJ will lead to injured intestine barrier structure and increased intestinal permeability (Tsukita et al., 2001; Schneeberger and Lynch, 2004). To clarify the molecular mechanism of β-1,3/1,6-glucan preventing Salmonella Typhimurium-induced loss of epithelial barrier function, we detected changes in expression of claudin-1, claudin-4, and occludin at the mRNA level. We indicated that Salmonella Typhimurium challenge decreased claudin-1, claudin-4, and occludin mRNA expression in the jejunum. This finding is supported by previous in vitro studies (Boyle et al., 2006; Kohler et al., 2007) and in broiler (Zhang et al., 2012) and mouse (Clark et al., 1998; Sears, 2000) studies. The reduction of claudin-1, claudin-4, and occludin mRNA expression might imply that less claudin-1 and occludin was present at TJ during Salmonella Typhimurium infection in our study. Downregulation of TJ proteins results in enhancement of paracellular permeability and disruption of the intestinal barrier, thereby allowing the diffusion of macromolecules such as antigens, bacterial toxins (endotoxin), and pathogens from the intestinal lumen into the circulation (Kucharczik et al., 2001; Schneebberger and Lynch, 2004). The high percentage of Salmonella Typhimurium liver translocation in our study was associated with this disruption of TJ during Salmonella Typhimurium challenge. Our results also indicated that β-1,3/1,6-glucan upregulated claudin-1 and occludin mRNA expression, but had no effect on claudin-4 mRNA expression in the jejunum of the Salmonella Typhimurium-challenged birds. These results suggest that regulation of claudin-1 and occludin expression by β-1,3/1,6-glucan may be involved in ameliorating increased intestinal permeability induced by Salmonella Typhimurium challenge.

Cytokines play an important role in the modulation of the intestinal epithelial TJ barrier (Al-Sadi et al., 2009; Groschwitz and Hogan, 2009; Turner, 2009). Previous studies have shown that proinflammatory cytokines such as IL-13, interferon-γ, IL-6, chemokine IL-8, and anti-inflammatory cytokines (IL-10 and transforming growth factor-β) expression are regulated in the intestinal tissues during Salmonella challenge (Withanage et al., 2004, 2005; Fasina et al., 2008). Dietary β-1,3/1,6-glucan also can modulate intestinal cytokine profiles, limit intestinal inflammation, and reduce tissue lesion severity in broiler chickens during coccidiosis (Cox et al., 2010a,b). Further investigation is needed into changes in intestinal pro- and anti-inflammatory cytokines in the gut of broiler chickens treated with dietary β-1,3/1,6-glucan supplementation and challenged with Salmonella Typhimurium. This study will be helpful to explain clearly the molecular mechanism of β-1,3/1,6-glucan regulation of the intestinal TJ.

In conclusion, dietary β-1,3/1,6-glucan supplementation can reduce intestinal mucosal barrier impairment of broiler chickens challenged with Salmonella Typhimurium, and the partial mechanism might be related to the increased expression of claudin-1 and occludin, and increased goblet cell numbers and sIgA level in the jejunum of broiler chickens. These results provide new information on the role of β-1,3/1,6-glucan as an oral mucosal adjuvant of the immune system to improve gut health, especially in the prevention or amelioration of Salmonella infection.
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β-1,3/1,6-GLUCAN AND INTESTINAL MUCOSAL BARRIER IMPAIRMENT


