

Dietary hydrolyzed wheat gluten supplementation ameliorated intestinal barrier dysfunctions of broilers challenged with *Escherichia coli* O78

Yujun Wu,^{*} Wenyu Wang,[†] In Ho Kim,[‡] and Ying Yang^{*,1}

^{*}State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, 100193 China; [†]Nantong Tiancheng Modern Agricultural Technology Co. Ltd., Nantong, Jiangsu Province, 226634 China; and [‡]Department of Animal Resource & Science, Dankook University, 330-714 Cheonan, Korea

ABSTRACT This study aimed to investigate a protective effect of hydrolyzed wheat gluten (HWG) on *Escherichia coli* (*E. coli*)-induced intestinal barrier dysfunctions in broilers. Broilers fed a basal diet unsupplemented or supplemented with HWG (0.5% or 1%) were intraperitoneally injected with either *E. coli* O78 suspension (10^8 CFU/mL) or equal volume of vehicle on d 18 of age. Blood and tissue samples were collected 3rd d post infection. The results showed that *E. coli*-infection increased immune-organ indexes of spleen and thymus, enhanced serum diamine oxidase (DAO) level, impaired ileal villus structure and reduced tight junction mRNA levels (*Occludin*, *Claudin-1*, *ZO-1*, $P < 0.05$), while increased mRNA levels of inflammatory cytokines

(*TNF- α* , *IFN- γ* , *IL-1 β* , *IL-6*, *IL-8*) and *TLR4* in the ileum of broilers ($P < 0.05$). The effects of *E. coli* O78 challenge on organ indexes of spleen and thymus, serum DAO level, mRNA levels of tight junctions were alleviated by 1% HWG supplementation, the upregulations of *IL-1 β* and *TLR4* were prevented by 0.5% HWG supplementation ($P < 0.05$). In addition, increased *IFN- γ* of *E. coli*-infected broilers was abrogated by 0.5% or 1% HWG supplementation ($P < 0.05$). In summary, dietary HWG supplementation ameliorated intestinal barrier dysfunctions triggered by *E. coli*-infection in the ileum of broilers. HWG supplementation might be a nutritional strategy to improve the intestinal mucosal barrier function of broilers.

Key words: hydrolyzed wheat gluten, intestinal permeability, inflammatory cytokines, tight junction

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INTRODUCTION

The gastrointestinal tract is critical for nutrients absorption and intestinal homeostasis (Okumura and Takeda, 2017). The monolayer of enterocytes allows digestion and absorption of nutrients, while prevents the invasion of pathogens and toxins from penetrating the intestinal mucosa, otherwise potentially causing cellular injury (Citi, 2018). Dysfunction of the intestinal barrier has been reported to be associated with increased epithelial permeability and bacteria translocation, therefore resulting in the gastrointestinal disorders and even systemic inflammation (Camilleri et al., 2012).

Escherichia coli (*E. coli*) are a group of symbionts colonized in the gastrointestinal tracts of humans and animals (Sarowska et al., 2019). However, replication and colonization of pathogenic *E. coli* are associated with intestinal disorders and decrease digestion and absorption of nutrients in animals (Croxen et al., 2013). Avian pathogenic *E. coli* (APEC), a gram-negative flora, has been reported to adhere to epithelial cells and disrupt the intestinal integrity of birds, including both the meat type broilers, and laying hens (Wang et al., 2016). *E. coli* infection in birds leads to severe diarrhea, decreased feed intake, and reduced growth performance, therefore causing serious economic losses in poultry industries (Mellata, 2013). Several nutritional interventions have been attempted to ameliorate *E. coli*-induced intestinal barrier dysfunctions (Wang et al., 2016; Zhang et al., 2020), eventually improving the intestinal development and health outcomes of broilers.

Hydrolyzed wheat gluten (HWG), obtained by enzymatic hydrolysis of wheat gluten, has been widely used as the functional food to improve the health outcomes of humans and animals due to its high proportions of glutamine (Gln), glutamate (Glu), glycine (Gly) and bioactive peptides (Asrarkulova and Bulushova, 2019).

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Abbreviations: DAO, diamine oxidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Gln, glutamine; Glu, glutamate; Gly, glycine; HWG, hydrolyzed wheat gluten; IFN- γ , interferon- γ ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; IL-8, interleukin-8; NRC, National Research Council; TLR4, Toll like receptor-4; TNF- α , tumor necrosis factor- α ; ZO-1, zonula occludens-1

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¹Corresponding author: cauвет@163.com

Previous study has demonstrated that dietary supplementation with HWG can enhance the immune response and decrease the incidence of diarrhea in weaning piglets (Wang et al., 2011). Due to the high digestibility of HWG, it has been regarded as an ideal protein source for milk replacer for calves (Castro et al., 2016). Additionally, HWG administration alleviates deoxynivalenol-induced intestinal injury by promoting intestinal stem cell proliferation and differentiation in mice (Zhou et al., 2019). Dietary HWG inclusion improves the morphology of small intestinal in broilers (Van Leeuwen et al., 2004). However, it remains unknown whether dietary HWG supplementation can reduce *E. coli* challenge-induced intestinal barrier dysfunctions in broilers. Therefore, this study was conducted to investigate the protective effects of HWG supplementation on intestinal barrier functions and inflammatory response of *E. coli*-challenged broilers.

MATERIALS AND METHODS

E. coli Suspension Preparation

The *E. coli* O78 strain (CVCC1490; China Veterinary Culture Collection Center, Beijing, China) was cultured and used as previously described (Zhang et al., 2020). In brief, *E. coli* was aerobically cultured in Luria Borth liquid medium at 37°C for 16 h. After 10-fold serial dilution, the diluted suspensions were plated on MacConkey agar at 37°C for 24 h for bacteria enumeration. The final bacteria concentration was adjusted to 10⁸ CFU/mL with sterile sodium chloride at pH 7.0.

Animals and Experimental Design

The experimental protocol was approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China). A total of 360 1-day-old male Arbor Acre broilers were randomly allocated into 3 dietary treatments (0%, 0.5% or 1% HWG supplemented diets) with 8 replicates of 15 birds per replicate. Broilers in 0% HWG group were fed the basal diet (Table 1), which met the nutritional requirements of broilers (National Research Council, 1994), 0.5% or 1% HWG group were respectively fed 0.5% or 1% HWG supplemented diets. The HWG used in the study was provided by Zhengzhou Xinwei Nutritional Technology Co., Ltd. (Zhengzhou, China). The composition of hydrolyzed wheat gluten has been provided in the Table S1. The inclusion amount was based on the manufacturer suggestions, and nutrient contents were maintained across all diets. On d 18 of the age, 4 broilers per replicate from each treatment (3 dietary treatments) were randomly selected and isolated, then divided into 2 groups (*E. coli* infection or not) according to 3 × 2 factorial design. Broilers in infected and uninfected groups were intraperitoneally injected with 1 mL of either *E. coli* suspension (10⁸ CFU/mL) or equal volume of vehicle. All birds had free access to individual feed and water throughout the whole study period.

Table 1. Composition and nutritional levels of basal diet (air-dry basis).

Ingredients	Contents (%)	Nutritional levels ¹	Contents (%)
Corn	59.63	ME (calculated, MJ/kg)	12.35
Soybean meal	30.05	Crude protein (%)	21.05
Soybean oil	1.46	Lysine (%)	1.18
Corn gluten meal	4.69	Methionine (%)	0.55
Calcium hydrophosphate	1.90	Threonine (%)	0.81
Limestone	0.91	Tryptophan (%)	0.20
<i>DL</i> -Methionine	0.16	Calcium (%)	1.02
<i>L</i> -Lysine (78%)	0.20	Nonphytate Phosphorus (%)	0.46
NaCl	0.35		
Choline chloride (50%)	0.30		
Multivitamin ²	0.02		
Multimineral ³	0.30		
Santoquin	0.03		
Total	100.00		

¹Nutritional levels were measured, except for ME.

²The multivitamin provided the following per kg of diets: VA 12500 IU, VD₃ 2500 IU, VE 18.75 mg, VK₃ 2.65 mg, VB₁ 2 mg, VB₂ 6 mg, VB₆ 6 mg, VB₁₂ 0.025 mg, biotin 0.0325 mg, folic acid 1.25 mg, pantothenic acid 1.25 mg, and nicotinic acid 50 mg.

³The multimineral provided the following per kg of diets: Cu 8 mg, Zn 75 mg, Fe 80 mg, Mn 100 mg, Se 0.15 mg, and I 0.35 mg.

The broilers were reared in the battery cages with a plastic wire-floor (0.06 m²/bird), placed in an environmentally controlled room. The room temperature was maintained at 33°C to 35°C during the first 3 d and then gradually decreased to 22°C by d 21. The lighting program was performed with 23 h lighting and 1 h darkness for the first 7 d, followed by 20 h light and 4 h darkness from d 8 until the end of the experiment.

Sample Collection

On the third day post challenge, one bird per replicate was randomly selected and weighed for sampling. Blood samples were aseptically collected from the wing vein. After centrifugation at 3,000 rpm for 10 min at 4°C, serum samples were obtained and stored at -80°C until further analysis. After euthanized by sodium pentobarbital (30 mg/kg BW), immune-related organs (spleen, thymus, and bursal) were separated and weighed to calculate the organ indexes (organ weight/body weight, %). Then, ileal segments were fixed in 4% paraformaldehyde solution for morphological analysis, and ileal mucosa was collected and stored at -80°C after snap-frozen for further genes quantification.

Serum Diamine Oxidase (DAO) Analysis

All serum samples were thawed and completely mixed before analysis. The serum DAO was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (HY-60106, Beijing Sino-UK Institute of Biological Technology, Beijing, China).

Intestinal Morphology Analysis

Intestinal villus height and crypt depth were measured according to previous report (Qiu et al., 2021).

Fixed intestinal tissues were dehydrated through a graded ethanol series (70–100%), then embedded in paraffin wax. Serial sections (5 μm of thickness) were cut by rotary microtome (LEICA RM2135, Leica Microsystems, CA), and stained with hematoxylin and eosin. Morphological structures of intestine were observed and evaluated under the bright field (100 \times) on a microscope (Carl Zeiss Microscopy LLC, NY). Five vertically cross-cutting sections were selected for each sample. The vertical distances from the villous tip to villous–crypt junction was regarded as villus height, and from the villous–crypt junction to the lower limit of the crypt was seen as crypt depth. Ten well-orientated villi and their associated crypt per section were measured using an image analyzer (Lucia Software, Lucia, Za Drahov, Czechoslovakia). The mean value of 50 measurements per sample was submitted for statistical analysis.

RNA Extraction and Quantitative Real-Time PCR

Total RNA of ileal mucosa was extracted by Trizol reagent (Invitrogen, CA) following the RNA extraction instruction, while cDNA was obtained by using PrimeScript RT Kit (Takara, Shiga, Japan). Quantitative real-time PCR was performed according to the SYBR Premix Ex Taq II instructions (Takara, Shiga, Japan) and the reaction was conducted on an ABI-Prism 7500 Real-Time PCR System (Applied Biosystems, CA). Primers for RT-qPCR were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Table S2). Amplifications were performed in triplicate for each sample. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was regarded as the housekeeping gene and the relative expressions of target genes to that of the reference gene (GAPDH) was calculated according to the $2^{-\Delta\Delta C_t}$ method.

Statistical Analysis

The data were evaluated using SPSS 19.0 software by two-way ANOVA, and the differences were considered significant at $P < 0.05$. When a significant dietary effect or an interaction between diet and challenge was observed, the data were further analyzed by using one-way ANOVA with Duncan's post hoc test.

RESULTS

Effects of Dietary Supplementation With HWG on Immune-Related Organs Indexes of Broilers

To evaluate the impacts of the dietary HWG supplementation or *E. coli* O78 challenge on the immune-related organs (spleen, thymus and bursal) of broilers, the organs were collected and the organ indexes were calculated (Figure 1). The results showed that dietary supplementation with 0.5% or 1% HWG did not alter the immune-related organs indexes of unchallenged broilers ($P > 0.05$), the spleen index and thymus index of broilers were significantly increased after *E. coli* O78 challenge ($P < 0.05$). For dietary HWG effects, 1% HWG supplementation significantly decreased thymus index of *E. coli*-challenged broilers ($P < 0.05$), while dietary supplementation with 0.5% HWG had no effects on immune-related organ indexes of *E. coli*-challenged broilers ($P > 0.05$).

Effects of Dietary Supplementation With HWG on Serum DAO Level of Broilers

As the biomarker of the intracellular permeability, serum DAO level of broilers was tested (Figure 2). For the unchallenged broilers, dietary 0.5% or 1% HWG supplementation did not change the serum DAO activity ($P > 0.05$). *E. coli* O78 challenge significantly increased serum DAO activity of broilers ($P < 0.05$). Dietary 1% HWG supplementation significantly reduced serum DAO activity of *E. coli*-challenge broilers ($P < 0.05$), while 0.5% HWG group showed no significant difference ($P > 0.05$).

Effects of Dietary Supplementation With HWG on Ileal Morphological Structure of Broilers

To identify intestinal morphological integrity after dietary HWG supplementation or *E. coli* challenge, ileum segments of broilers were fixed and evaluated. As shown in Figure 3, dietary supplementation with 0.5% or 1% HWG exhibited few significant modifications on

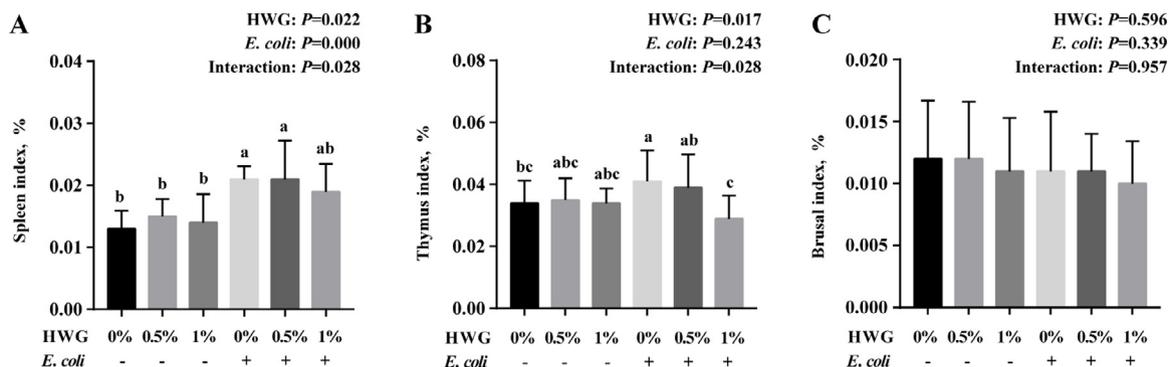


Figure 1. Effects of dietary HWG supplementation on immune-related organs indexes of broilers on d 21 of age. Spleen index (A), thymus index (B) and bursal index (C). Values are means \pm SEM, $n = 8$. Means without a common letter differ, $P < 0.05$.

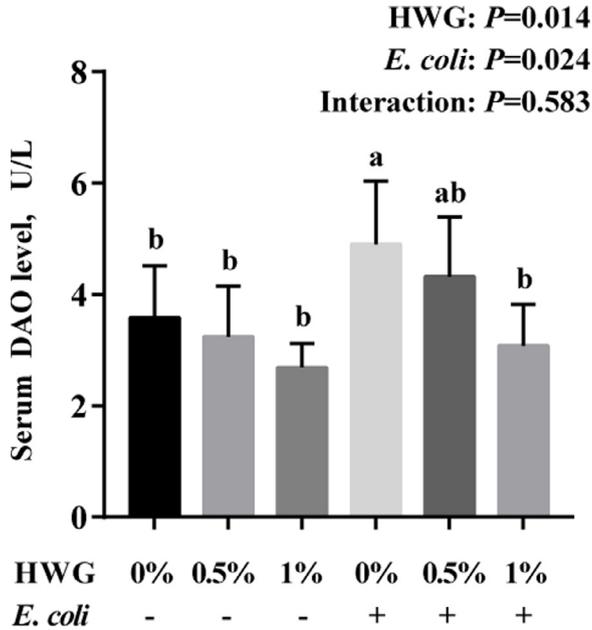


Figure 2. Effects of dietary HWG supplementation on serum DAO level of broilers on d 21 of age. Values are means \pm SEM, $n = 8$. Means without a common letter differ, $P < 0.05$. Abbreviation: DAO, diamine oxidase.

the ileal morphological structure of unchallenged broilers. *E. coli* O78 challenge induced mucosal structure damage, which was alleviated by dietary 0.5% or 1% HWG supplementation for broilers, including the villi height and villi height/crypt depth ratio.

Effects of Dietary Supplementation With HWG on mRNA Levels of Tight Junctions in the Ileum of Broilers

As the important components of intestinal barrier functions, the relative mRNA levels of tight junctions in the ileum of broilers were quantified (Figure 4). Dietary 0.5% or 1% HWG supplementation significantly upregulated the relative mRNA level of *ZO-1* ($P < 0.05$), while not caused any changes in relative mRNA levels of *Occludin* and *Claudin-1* in the ileum of unchallenged broilers ($P > 0.05$). In addition, the relative mRNA levels of *Occludin*, *Claudin-1*, and *ZO-1* were reduced in the ileum of broilers after *E. coli* O78 challenge ($P < 0.05$). Although dietary supplementation with 0.5% HWG showed no differences in relative mRNA levels of tight junctions in *E. coli*-infected broilers ($P > 0.05$), dietary supplementation with 1% HWG significantly upregulated

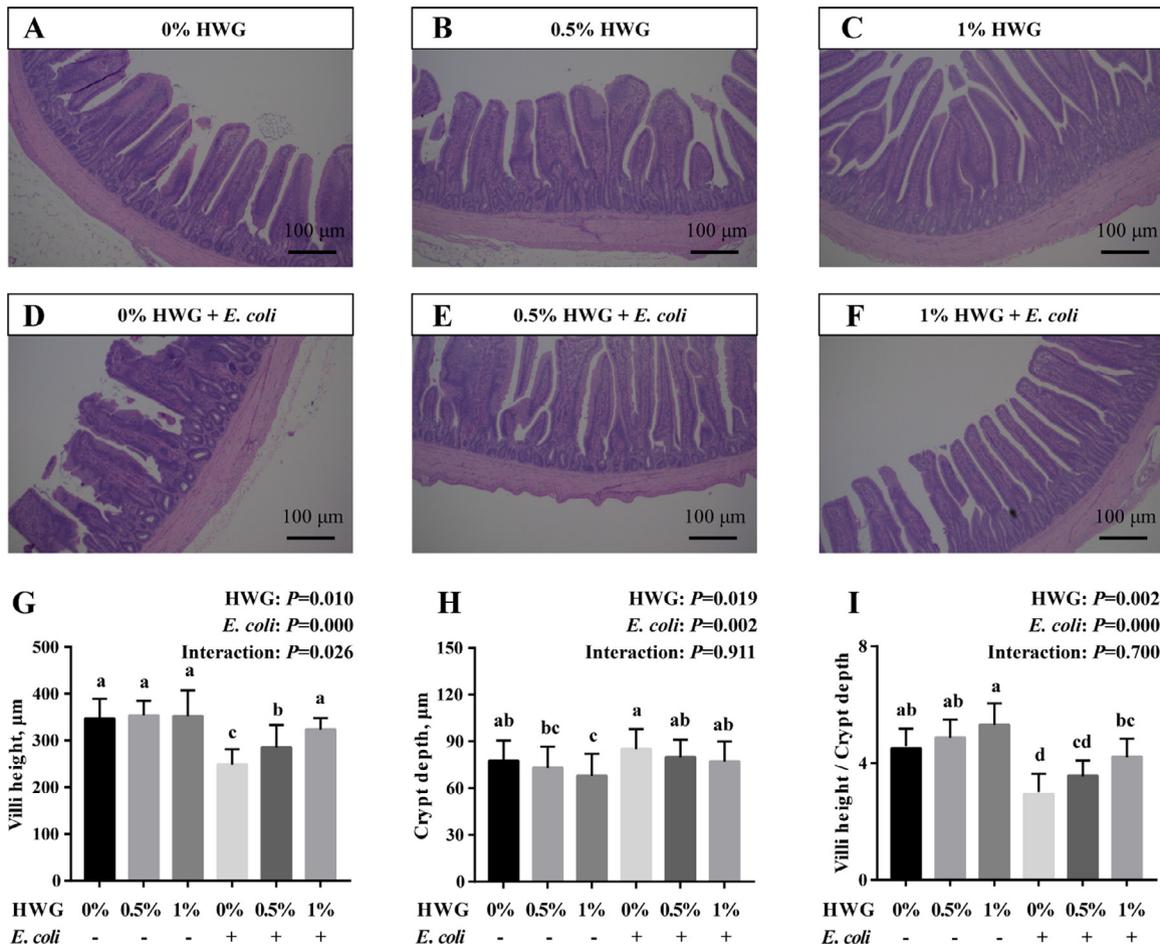


Figure 3. Effects of dietary HWG supplementation on ileal morphology of broilers on d 21 of age.

Ileum of 0% HWG group (A), 0.5% HWG group (B), 1% HWG group (C), 0% HWG + *E. coli* group (D), 0.5% HWG + *E. coli* group (E) and 1% HWG + *E. coli* group (F). Villi height (G), crypt depth (H) and villi height/crypt depth ratio (I) of ileal intestine. HWG, hydrolyzed wheat gluten; *E. coli*, *E. coli* O78. Values are means \pm SEM, $n = 6$. Means without a common letter differ, $P < 0.05$.

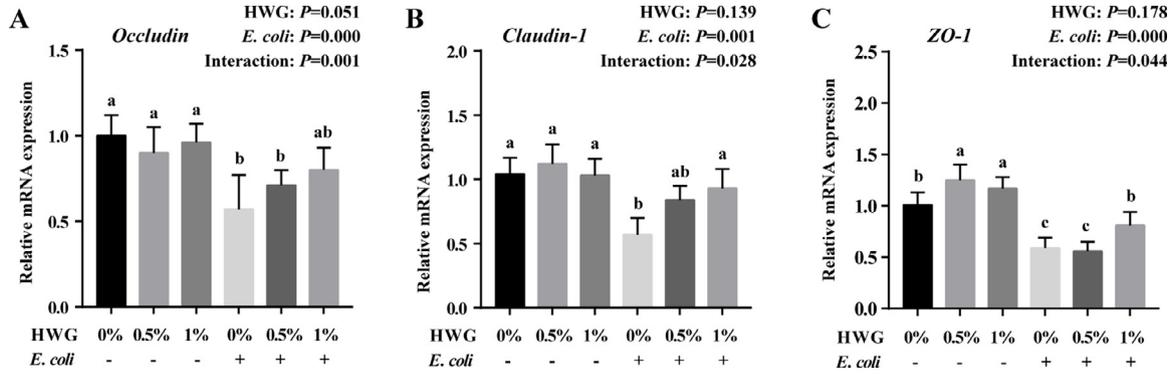


Figure 4. Effects of dietary HWG supplementation on mRNA levels of ileal tight junctions of broilers on d 21 of age.

Relative mRNA expressions of *Occludin* (A), *Claudin-1* (B) and *ZO-1*(C). *ZO-1*, zonula occluden-1. Values are means \pm SEM, n = 8. Means without a common letter differ, $P < 0.05$.

the relative mRNA levels of *Claudin-1* and *ZO-1* in the *E. coli*-challenged broilers ($P < 0.05$).

Effects of Dietary Supplementation With HWG on Relative mRNA Levels of Inflammatory Cytokines in Ileum of Broilers

To investigate the immune response to the dietary HWG supplementation or *E. coli* challenge, relative mRNA levels of inflammatory cytokines in the ileum were measured (Figure 5). As shown, dietary supplementation with 0.5% or 1% HWG did not alter the

relative mRNA levels of inflammatory cytokines (*TNF- α* , *IFN- γ* , *IL-1 β* , *IL-6*, *IL-8*, and *TLR4*) of the unchallenged broilers ($P > 0.05$), while *E. coli* O78 challenge significantly increased the relative mRNA levels of these inflammatory cytokines ($P < 0.05$). In addition, dietary supplementation with 0.5% HWG significantly reduced the relative mRNA levels of *IFN- γ* , *IL-1 β* , and *TLR4* in *E. coli*-challenged broilers ($P < 0.05$), while no significant effects were found of the *TNF- α* , *IL-6*, and *IL-8* ($P > 0.05$). Dietary supplementation with 1% HWG significantly decreased the relative mRNA level of *IFN- γ* in *E. coli*-challenged broilers ($P < 0.05$), while no significant effects were found on the relative mRNA levels of *TNF- α* , *IL-1 β* , *IL-6*, *IL-8*, and *TLR4* ($P > 0.05$).

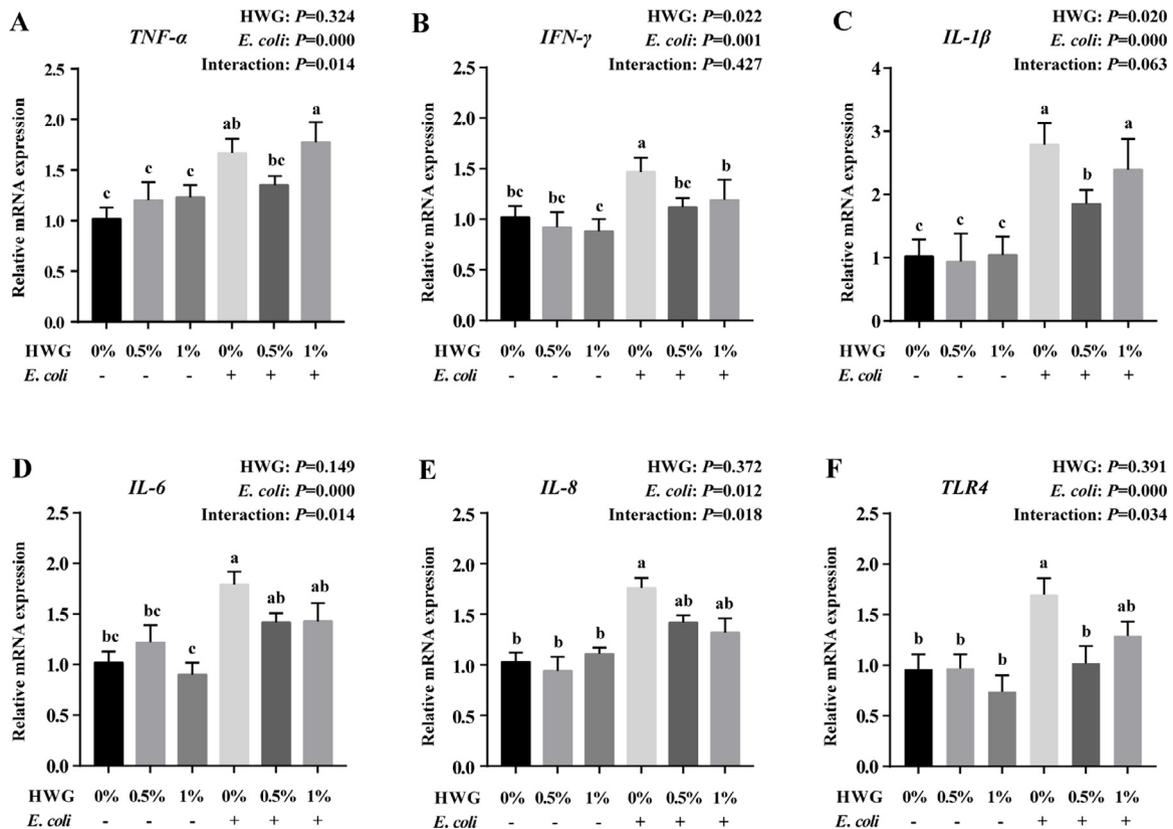


Figure 5. Effects of dietary HWG supplementation on ileal mRNA levels of inflammatory cytokines of broilers on d 21 of age.

Relative mRNA expressions of *TNF- α* (A), *IFN- γ* (B), *IL-1 β* (C), *IL-6* (D), *IL-8* (E) and *TLR4* (F). Values are means \pm SEM, n = 8. Means without a common letter differ, $P < 0.05$. Abbreviations: *IFN- γ* , interferon- γ ; *IL-1 β* , interleukin-1 β ; *IL-6*, interleukin-6; *IL-8*, interleukin-8; *TLR4*, Toll like receptor-4; *TNF- α* , tumor necrosis factor- α .

DISCUSSION

The intestinal structural and functional development of broilers in the early stage are vulnerable to pathogenic bacteria invasion, which can induce the intestinal mucosal damage and barrier dysfunction (Shao et al., 2013). Pathogenic *E. coli* infection is one of the most prominent problems with high morbidity and mortality in broiler industries, severely suppressing the growth of broilers and lowering the economic benefits (Kim et al., 2020). Previous studies indicate that early nutritional interventions can effectively promote immunological maturation, enhance intestinal barrier functions, and inhibit pathogens colonization, which consequently improve the broilers growth performance (Zhang et al., 2017; Lee et al., 2018). Hydrolyzed wheat gluten (HWG) is a protein mixture with high digestibility, comprising plentiful peptides and various functional amino acids, such as Gln, Glu and Gly (Wang et al., 2006). Although HWG has been widely used in piglets, calves, aquaculture and pets, few studies have been conducted in poultry industries. In this study, 2 doses of HWG were supplemented into the diets to explore the protective effects on intestinal barrier functions and immune response of broilers based on the *E. coli*-challenge model.

In general, pathogenic *E. coli*-infection is associated with reduced feed intake and intestinal mucosal damage (Mellata, 2013). In the present study, we found that *E. coli* O78 infection resulted in increased immune-related organs indexes (spleen index and thymus index) and impaired gut mucosal morphology of broilers, indicating that animal model of *E. coli* infection has been successfully established (Fadl et al., 2020). Previous studies have demonstrated that functional amino acids, like Gln and tryptophan (**Trp**), exerted positive role in anti-inflammation and cell proliferation, which might account for the amelioration of immune organic compensatory hypertrophy and the intestinal mucosal impairment of *E. coli* O78-induced broilers (Maiorka et al., 2016; Wang et al., 2020).

The intestinal permeability largely depends on the intestinal barrier integrity (Kelly et al., 2015). Intracellular DAO, serving as a biomarker of intestinal permeability, can be released into the circulation in large quantities, resulting in high level DAO in the blood when the intestinal barrier is damaged (Cai et al., 2019). In our study, elevation of serum DAO level after *E. coli*-challenge suggested that the intestinal mucosal barrier was damaged (Ding et al., 2019). The reduced serum DAO level in *E. coli*-challenged broilers after dietary 1% HWG supplementation can be inferred that Gln and bioactive small peptides in HWG were utilized by intestinal epithelial cells to maintain intestinal mucosal integrity and barrier functions (Zhou et al., 2019).

Tight junctions, including claudins, occludin, junctional adhesion molecule (**JAM**) and tricellulin, have been identified as main transmembrane proteins, interacting with cytosolic scaffold proteins, such as zonula occludens (**ZO**), corporately responsible for intestinal

barrier functions and permeability (Suzuki, 2013). In this study, the mRNA upregulation of *ZO-1* in the ileum of unchallenged broilers indicated that HWG could promote intestinal physical barrier under the general physiological conditions (Zihni et al., 2016). Previous studies have reported that pathogenic *E. coli* infection reduced the tight junctions and increased the intestinal permeability (Bhat et al., 2020; Bucker et al., 2020). In the current study, downregulated mRNA levels of *Claudin-1* and *ZO-1* by *E. coli*-challenge were prevented by dietary 1% HWG supplementation, suggesting that HWG could elevate the gene expressions of tight junctions to protect intestinal barrier integrity from the *E. coli* O78 invasion (Apper et al., 2016). In addition, the protective effects of Gln and Gly on epithelial tight junctions have been observed in our previous studies, in which dietary HWG may exert the main roles in the barrier defense (Wang et al., 2015; Li et al., 2016).

Accumulating evidence highlights that intestinal epithelial cells not only serve as a barrier, but also integrate the external and internal signals to coordinate the immune responses (McGuckin et al., 2009; Rescigno, 2011). The intestinal barriers impairment induced by pathogens or toxins are commonly accompanied with the inflammation (Camilleri et al., 2012). Generally, pathogenic *E. coli* recognizes the membrane TLR4 with its unique lipopolysaccharide (**LPS**) and activates downstream signaling pathway to produce large number of inflammatory cytokines, therefore triggering the inflammation (Keestra et al., 2013). In the present study, mRNA levels of ileal *TLR4* and inflammatory cytokines (*TNF- α* , *IFN- γ* , *IL-1 β* , *IL-6*, and *IL-8*) were elevated after *E. coli* O78 infection, indicating that *E. coli* infection triggered the inflammatory response of broilers (Wang et al., 2016). Interestingly, the upregulated mRNA levels of ileal *IFN- γ* , *IL-1 β* , and *TLR4* were abolished in *E. coli*-infected broilers by dietary HWG supplementation, suggesting that HWG might inhibit TLR4 signaling pathway to suppress the production of inflammatory cytokines, consequently alleviated the *E. coli*-induced inflammation (Liu et al., 2017). Previous studies have demonstrated that functional amino acids, such as Gln and Gly, could relieve the LPS-induced intestinal injury (Xu et al., 2018; Zhang et al., 2019). In the present study, high proportion of Gln, Gly and proline in the HWG may be responsible for the intestinal inflammatory alleviation of *E. coli*-infected broilers. Moreover, bioactive peptides (i.e., Gly-Glu), abundant in HWG, have been proved to mitigate the LPS-induced inflammation via depressing the production of proinflammatory cytokines (Jiang et al., 2009).

Taken together, dietary HWG supplementation depressed inflammatory response of *E. coli*-infected broilers by reducing the mRNA levels of *TLR4* and inflammatory cytokines (*TNF- α* , *IFN- γ* , *IL-1 β* , *IL-6*, and *IL-8*), alleviated intestinal mucosal damage and barrier dysfunctions of *E. coli*-challenged broilers through upregulating the mRNA levels of tight junctions (*Claudin-1* and *ZO-1*). Our findings will provide

the guidelines for the HWG application in poultry industries. However, more insightful mechanisms of the protective effects of HWG on the *E. coli* infection need further investigation.

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Ethical Approval: This research involves only animals (broilers), without any studies involving human participants. The animal use protocols in this study were reviewed and approved by the China Agricultural University Animal Care and Use Committee (Beijing, China).

DISCLOSURES

The authors declared no conflicts of interests.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.psj.2021.101615](https://doi.org/10.1016/j.psj.2021.101615).

REFERENCES

- Apper, E., D. Weissman, F. Respondek, A. Guyonvarch, F. Baron, P. Boisot, A. Rodiles, and D. L. Merrifield. 2016. Hydrolysed wheat gluten as part of a diet based on animal and plant proteins supports good growth performance of Asian seabass (*Lates calcarifer*), without impairing intestinal morphology or microbiota. *Aquaculture* 453:40–48.
- Asrarkulova, A. S., and N. V. Bulushova. 2019. Wheat gluten and its hydrolysates. Possible fields of practical use. *Appl. Biochem. Microbiol.* 54:825–833.
- Bhat, M. I., S. Kapila, and R. Kapila. 2020. *Lactobacillus fermentum* (MTCC-5898) supplementation renders prophylactic action against *Escherichia coli* impaired intestinal barrier function through tight junction modulation. *LWT-Food Sci. Technol.* 123:109118.
- Bucker, R., S. S. Zakrzewski, S. Wiegand, R. Pieper, A. Fromm, M. Fromm, D. Gunzel, and J. D. Schulzke. 2020. Zinc prevents intestinal epithelial barrier dysfunction induced by alpha-hemolysin-producing *Escherichia coli* 536 infection in porcine colon. *Vet. Microbiol.* 243:108632.
- Cai, J., H. Chen, M. Weng, S. Jiang, and J. Gao. 2019. Diagnostic and clinical significance of serum levels of D-lactate and diamine oxidase in patients with Crohn's disease. *Gastroenterol. Res. Pract.* 2019:8536952.
- Camilleri, M., K. Madsen, R. Spiller, B. G. Van Meerveld, and G. N. Verne. 2012. Intestinal barrier function in health and gastrointestinal disease. *Neurogastroenterol. Motil.* 24:503–512.
- Castro, J. J., G. H. Hwang, A. Saito, D. A. Vermeire, and J. K. Drackley. 2016. Assessment of the effect of methionine supplementation and inclusion of hydrolyzed wheat protein in milk protein-based milk replacer on the performance of intensively fed Holstein calves. *J. Dairy Sci.* 99:6324–6333.
- Citi, S. 2018. Intestinal barriers protect against disease. *Science* 359:1097–1098.
- Croxen, M. A., R. J. Law, R. Scholz, K. M. Keeney, M. Wlodarska, and B. B. Finlay. 2013. Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin. Microbiol. Rev.* 26:822–880.
- Ding, S., Y. Wang, W. Yan, A. Li, H. Jiang, and J. Fang. 2019. Effects of *Lactobacillus plantarum* 15-1 and fructooligosaccharides on the response of broilers to pathogenic *Escherichia coli* O78 challenge. *PLoS One* 14:e0212079.
- Fadl, S. E., G. A. El-Gammal, O. A. Sakr, A. Salah, A. A. Atia, A. M. Prince, and A. M. Hegazy. 2020. Impact of dietary Mannan-oligosaccharide and beta-Glucan supplementation on growth, histopathology, *E. coli* colonization and hepatic transcripts of TNF-alpha and NF-κB of broiler challenged with *E. coli* O78. *BMC Vet. Res.* 16:204.
- Jiang, Z. Y., L. H. Sun, Y. C. Lin, X. Y. Ma, C. T. Zheng, G. L. Zhou, F. Chen, and S. T. Zou. 2009. Effects of dietary glycyl-glutamine on growth performance, small intestinal integrity, and immune responses of weaning piglets challenged with lipopolysaccharide. *J. Anim. Sci.* 87:4050–4056.
- Keestra, A. M., M. R. de Zoete, L. I. Bouwman, M. M. Vaezrad, and J. P. van Putten. 2013. Unique features of chicken Toll-like receptors. *Dev. Comp. Immunol.* 41:316–323.
- Kelly, J. R., P. J. Kennedy, J. F. Cryan, T. G. Dinan, G. Clarke, and N. P. Hyland. 2015. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front. Cell Neurosci* 9:392.
- Kim, Y. B., M. Y. Yoon, J. S. Ha, K. W. Seo, E. B. Noh, S. H. Son, and Y. J. Lee. 2020. Molecular characterization of avian pathogenic *Escherichia coli* from broiler chickens with colibacillosis. *Poult. Sci.* 99:1088–1095.
- Lee, Y. S., S. H. Lee, U. D. Gadde, S. T. Oh, S. J. Lee, and H. S. Lillehoj. 2018. *Allium hookeri* supplementation improves intestinal immune response against necrotic enteritis in young broiler chickens. *Poult. Sci.* 97:1899–1908.
- Li, W., K. Sun, Y. Ji, Z. Wu, W. Wang, Z. Dai, and G. Wu. 2016. Glycine regulates expression and distribution of Claudin-7 and ZO-3 proteins in intestinal porcine epithelial cells. *J. Nutr.* 146:964–969.
- Liu, G., W. Ren, J. Fang, C. A. A. Hu, G. Guan, N. A. Al-Dhabi, J. Yin, V. Duraipandiyar, S. Chen, Y. Peng, and Y. Yin. 2017. L-Glutamine and L-arginine protect against enterotoxigenic *Escherichia coli* infection via intestinal innate immunity in mice. *Amino Acids* 49:1945–1954.
- Maiorka, A., A. V. F. Silva, E. Santin, F. Dahlke, L. D. G. Bruno, I. C. Boleli, M. Macari, and H. Trautenmuller. 2016. Effect of broiler breeder age and glutamine supplementation on the development of the intestinal mucosa of 7-day-old chicks. *Braz. J. Poult. Sci.* 18:17–22.
- McGuckin, M. A., R. Eri, L. A. Simms, T. H. Florin, and G. R. Smith. 2009. Intestinal barrier dysfunction in inflammatory bowel diseases. *Inflamm. Bowel. Dis.* 15:100–113.
- Mellata, M. 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathog. Dis.* 10:916–932.
- National Research Council. 1994. Nutrient requirements of poultry. National Academy Press.
- Okumura, R., and K. Takeda. 2017. Roles of intestinal epithelial cells in the maintenance of gut homeostasis. *Exp. Mol. Med.* 49:e338.
- Qiu, K., Y. Ma, U. E. Obianwuna, J. Wang, H. Zhang, G. Qi, and S. Wu. 2021. Application of selenium conjugated to animal protein in laying hens' diet for the production of selenium-enriched eggs. *Foods* 10:1224.
- Rescigno, M. 2011. The intestinal epithelial barrier in the control of homeostasis and immunity. *Trends Immunol* 32:256–264.
- Sarowska, J., B. Futoma-Koloch, A. Jama-Kmiecik, M. Frej-Madrzak, M. Ksiaczzyk, G. Bugla-Ploskonska, and I. Choroszy-Krol. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. *Gut Pathog* 11:10.
- Shao, Y., Y. Guo, and Z. Wang. 2013. Beta-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica serovar* Typhimurium. *Poult. Sci.* 92:1764–1773.
- Suzuki, T. 2013. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol. Life Sci.* 70:631–659.
- van Leeuwen, P., J. M. Mouwen, J. D. van der Klis, and M. W. Verstegen. 2004. Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. *Br. Poult. Sci.* 45:41–48.

- Wang, B., S. Sun, M. Liu, H. Chen, N. Liu, Z. Wu, G. Wu, and Z. Dai. 2020. Dietary L-tryptophan regulates colonic serotonin homeostasis in mice with dextran sodium sulfate-induced colitis. *J. Nutr.* 150:1966–1976.
- Wang, H., C. Zhang, G. Wu, Y. Sun, B. Wang, B. He, Z. Dai, and Z. Wu. 2015. Glutamine enhances tight junction protein expression and modulates corticotropin-releasing factor signaling in the jejunum of weanling piglets. *J. Nutr.* 145:25–31.
- Wang, J., M. Zhao, X. Yang, and Y. Jiang. 2006. Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. *J. Cereal Sci.* 44:93–100.
- Wang, W., Z. Li, Q. Han, Y. Guo, B. Zhang, and R. D’Inca. 2016. Dietary live yeast and mannan-oligosaccharide supplementation attenuate intestinal inflammation and barrier dysfunction induced by *Escherichia coli* in broilers. *Br. J. Nutr.* 116:1878–1888.
- Wang, X., Y. Feng, G. Shu, Q. Jiang, J. Yang, and Z. Zhang. 2011. Effect of dietary supplementation with hydrolyzed wheat gluten on growth performance, cell immunity and serum biochemical indices of weaned piglets (*Sus scrofa*). *Agr. Sci. China* 10:938–945.
- Xu, X., X. Wang, H. Wu, H. Zhu, C. Liu, Y. Hou, B. Dai, X. Liu, and Y. Liu. 2018. Glycine relieves intestinal injury by maintaining mTOR signaling and suppressing AMPK, TLR4, and NOD signaling in weaned piglets after lipopolysaccharide challenge. *Int. J. Mol. Sci.* 19:1980.
- Zhang, B., Z. Lv, H. Li, S. Guo, D. Liu, and Y. Guo. 2017. Dietary L-arginine inhibits intestinal *Clostridium perfringens* colonisation and attenuates intestinal mucosal injury in broiler chickens. *Br. J. Nutr.* 118:321–332.
- Zhang, M., J. Kou, Y. Wu, M. Wang, X. Zhou, Y. Yang, and Z. Wu. 2020. Dietary genistein supplementation improves intestinal mucosal barrier function in *Escherichia coli* O78-challenged broilers. *J. Nutr. Biochem.* 77:108267.
- Zhang, X., X. Tan, Y. Liu, W. You, G. Liu, X. Liu, Q. Jin, C. Wei, F. Wan, and H. Zhao. 2019. Alanyl-glutamine ameliorates lipopolysaccharide-induced inflammation and barrier function injury in bovine jejunum epithelial cells. *Biochem. Cell Biol.* 97:670–680.
- Zhou, J. Y., S. W. Zhang, H. L. Lin, C. Q. Gao, H. C. Yan, and X. Q. Wang. 2019. Hydrolyzed wheat gluten alleviates deoxynivalenol-induced intestinal injury by promoting intestinal stem cell proliferation and differentiation via upregulation of Wnt/beta-catenin signaling in mice. *Food Chem. Toxicol.* 131:110579.
- Zihni, C., C. Mills, K. Matter, and M. S. Balda. 2016. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat. Rev. Mol. Cell Biol.* 17:564–580.