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Original Article

Effects of Glutamine on the Mucosal Structure and Immune Cells in the Intestines of Broiler Chickens Challenged with Salmonella Enteritidis

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■Keywords

Glutamine, intestinal villi, mucosal immunity, Salmonella Enteritidis, broiler.

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ABSTRACT

The aim of this study was to investigate the effects of glutamine (Gln) on the intestinal mucosal structure and immune cells of broilers infected with *Salmonella Enteritidis*. 160 1-d-old commercial Arbor Acres (AA) broilers were randomly selected to receive one of four treatments, each of which had 5 replicates. Each replicate consisted of 8 chicks subjected to a 21-d feeding trial. Group I served as the unchallenged (CON). All birds in groups II (SCC) – IV were challenged with 2.0×10^4 CFU/mL of *S. Enteritidis*. The birds in groups III and IV were treated with 0.5% and 1.0% Gln. The results showed that *S. Enteritidis* infection led to a decrease in the relative length and weight, villus height:crypt depth (VH:CD) of the jejunum and ileum, the number of intraepithelial lymphocyte cells, and number of goblet cells and an increase in the number of mast goblet cells compared with the measurements of these parameters in the CON group ($p<0.05$). In addition, the Gln groups had increased relative length and weight, VH:CD of the jejunum and ileum, numbers of intraepithelial lymphocyte cells, and numbers of goblet cells and decreased crypt depth in the jejunum and ileum and numbers of mast goblet cells compared with the measurements of these parameters in the SCC group ($p<0.05$). It was concluded that Gln added to broiler diets can effectively alleviate the intestinal mucosal damage caused by *S. Enteritidis* infection and improve its normal defense barrier function.

INTRODUCTION

Salmonella is a gram-negative bacteria widely found in nature and is a foodborne pathogen (Hohmann, 2001). *Salmonella* secretes invasion factor E in the small intestine, destroying host gastrointestinal epithelial cells by counteracting the sulfite released by immune cells and then compromising host defense mechanisms (Shanmugasundaram *et al.*, 2015), leading to intestinal damage and decreased growth performance of broilers infected with *Salmonella* (Wang *et al.*, 2012). Moreover, evidence from animal studies showed that *Salmonella* induces an increase in T regulatory cells (Tregs) in the small intestine of the infected broilers. IL-10 secreted by Tregs can suppress the host immune response, enabling *Salmonella* to survive in the small intestine (Paramasivam *et al.*, 2019). Therefore, based on public demand for the rational use of antibiotics, new feed additives must be sought to alleviate the adverse effects of *Salmonella* infection.

Glutamine (Gln) is the most important and most abundant amino acid in the human body. Among its functions, Gln has a role in protecting the intestinal mucosa, participating in the immune response, and in boosting protein synthesis (Bollhalder *et al.*, 2013). Gln is a glucogenic amino acid that can be used as a precursor in the synthesis of nucleic acids, amino sugars and proteins (Souba, 1993). As a nonessential amino

acid, Gln is an essential amino acid when the body is in a special state, such as during high-intensity exercise, during rapid environmental changes, and upon injury (Newsholme, 2001). Adding Gln to the diet prevented the intestinal and liver oxidative damage caused by intestinal ischemia and reperfusion in rats (Hartmann *et al.*, 2017). Rats fed Gln in hypobaric or hypoxic conditions had regulated intestinal flora and reduced intestinal damage and adverse effects (Xu *et al.*, 2014). Currently, the extent of the protective effect of Gln on the important mucosal structure and immune cells in broiler chickens infected with *Salmonella* remains unclear. Therefore, the aim of the present study was to evaluate the effects of glutamine on the mucosal structure and immune cells in the intestine of broiler chickens challenged with *S. Enteritidis*.

MATERIALS AND METHODS

S. Enteritidis

S. Enteritidis serotype was provided by the China Veterinary Culture Collection Center (CVCC 3377, Beijing, China). *S. Enteritidis* was cultured in Brilliant Green Agar at 37°C for 24 h, washed, and then diluted to 2.0 × 10^4 CFU/ mL in sterile normal saline. Colony counts by plating confirmed the viability of the cells.

Glutamine

The glutamine used in the experiment was purchased from Wuxi Jingyao Biotechnology Company Limited (Wuxi, Jiangsu, China) and had a purity of 99%.

Bird management and diets

During the feeding study, the treatment of the birds complied with all of the specific regulations and requirements and was approved by the Institutional Animal Care and Use Committee of Henan University of Science and Technology.

One hundred and sixty 1-d-old commercial Arbor Acres (AA) broilers were obtained from a local commercial hatchery (Luoyang, Henan, China) and randomly allocated into four experimental groups for five replicate studies. The birds were weighed and placed in two separate 3-tier battery cages in an environmentally controlled room; the broilers were initially exposed to ambient temperature, which was gradually decreased from 34 °C to 22 ± 1 °C until the birds reached 21 d of age. Plastic separators were used to prevent horizontal contamination between the cages. The experimental chickens were provided twenty-three h of light from days 1 to 7 and 18 h of light from days 8 to 21.

The chickens were fed basic corn–soybean diets, the components of which are listed in Table 1. The diets were formulated based on the NRC (1994) to meet the nutrient requirements of the broilers.

Table 1 – Ingredients and nutrient level of the experimental diet (g/kg diet as fed basis).

Item	1-21d
Feed Ingredients (g/kg)	
Corn silage	575
Soybean meal	327
Corn gluten meal	30
Soybean oil	28
Limestone	9.5
Dicalcium phosphate	17.5
Salt	3
Choline chloride	3.0
Premix[1]	3.0
L-Lysine	2.5
DL-Methionine	1.5
Total	1,000
Calculated nutrients levels (g/kg)	
AME (MJ/kg)	14.5
CP	232.0
Ca	10.7
Available Phosphorus	4.2
Lys	11.8
Met	4.8
Met+cys	9.1

Note:[1]Each kg of premix contained: Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8 mg; Mn (from manganese sulfate), 110 mg; Zn (Bacitracin Zn), 65 mg; iodine (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg. Vitamin A (transretinyl acetate), 10,000 IU; Vitamin D3 (cholecalciferol), 3,000 IU; Vitamin E (all-rac-α-tocopherolacetate), 30 IU; menadione, 1.3 mg; thiamine 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; calcium pantothenate, 10 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamine), 0.013 mg.

Experimental protocol

The broiler experiments were based on a completely randomized design with four treatment groups, each of which had five replicates. Each replicate consisted of 8 chicks which were subjected to a 21-d feeding trial. The 4 treatments were as follows: Group I was the control group (nonstress treated), and groups II (treated with *S. Enteritidis*), III (treated with *S. Enteritidis* and 0.5% Gln), and IV (treated with *S. Enteritidis* and 1.0% Gln) were the experimental groups. At the age of 3 d, the broilers in experimental groups II, III and IV were administered 2.0 × 10^4 CFU/mL *S. Enteritidis* (0.5 mL) orally, and the chicks of group I were treated with an equal volume of physiological saline.

Sample collection and procedures

At days 4, 7, 14 and 21, eight broilers (after being feed deprived for 12 h) per cage were randomly selected and weighed. All birds were sacrificed by

exsanguination and dissected immediately. Then, the duodenum, jejunum and ileum were collected from each bird and flash frozen in liquid nitrogen and stored at -80°C.

Relative length of the intestine

The relative length of the intestine was calculated using the following formula: Relative length of the intestine (cm / kg) = Intestinal length (cm) / Live weight before slaughter (kg).

Relative weight of the intestine

The relative weight of the intestine was calculated using the following formula: Relative weight of the intestine (g / kg) = Intestinal weight (g) / Live weight before slaughter (kg).

Small intestinal mucosal morphology

The intestines were removed and washed repeatedly with physiological saline. For histological studies, about 2 × 2 cm^2 long samples from the proximal portion of the jejunum and ileum were collected and then placed in 4% formaldehyde prepared in advance. After 24 h, the small intestine tissues were stained with hematoxylin-eosin (HE) to prepare the paraffin sections. The villus height, crypt depth and villus height/crypt depth were calculated.

Observation and counting of the intestinal immune cells

For each broiler, 2 cm middle duodenum, 1 cm jejunum segment near the yolk stalk and 1 cm ileum segment near the ileocecal orifice were sampled. Separate HE- and PAS-stained sections were observed under a Leica-DFC450 C microscope. Five well-stained sections selected from each intestine were observed under a light microscope at 40× and 10× magnification, photographed and counted. The sections with five longest fluff areas were selected from each photograph, and the number of intraepithelial lymphocytes, goblet cells and mast cells per 100 intestinal mucosal column cells was counted by the Image-Pro Plus 5.0 image analysis system.

Statistical analysis

All data were analyzed by one-way ANOVA with post hoc Duncan multiple comparison tests using SPSS statistical software (ver. 21.0 for Windows, SPSS Inc., Chicago, IL). The means and total standard errors are presented. Significance (P value) was evaluated at the 0.05 level.

RESULTS

Relative length and relative weight of the small intestine

In contrast to the control group, *S. Enteritidis* infection decreased the relative lengths of the duodenum (at 7 and 14 d), jejunum (at 4, 7 and 14 d) and ileum (at 7, 14 and 21 d) of the broilers ($p<0.05$) (Table 2). However, the Gln 2 group showed increased relative lengths of the duodenum (at 7 and 14 d), jejunum (at 4, 7 and 14 d) and ileum (at 7, 14 and 21 d) compared with those of the *S. Enteritidis* infection group ($p<0.05$), although there were no differences when they were compared with the relative lengths of the duodenum, jejunum and ileum of the CON group ($p>0.05$), and the Gln 1 group showed increased relative length of the ileum (at 7, 14 and 21 d) compared with that of the *S. Enteritidis* infection group ($p<0.05$), although there was no difference when it was compared with the relative length of the ileum of the CON group ($p>0.05$).

Enteritidis infection decreased the relative weight of the duodenum (at 7 d), jejunum (at 7 d) and ileum (at 4, 7, 14 and 21 d) of broilers ($p<0.05$). The Gln 2 group had increased relative weights of the duodenum (at 7 d), jejunum (at 7 d) and ileum (at 4, 7, 14 and 21 d) compared with these weights of the *S. Enteritidis* infection group ($p<0.05$), although there were no differences when they were compared with the relative weights of the duodenum (at 7 d), jejunum (at 7 d) and ileum (at 4, 7, 14 and 21 d) of the CON group ($p>0.05$), and the Gln 1 group showed an increased relative weight of the ileum (at 4 d) compared with that of the *S. Enteritidis* infection group ($p<0.05$), although there was no difference when it was compared with the relative length of the ileum (at 4 d) of the CON group ($p>0.05$). The secondary relationship between age and relative lengths of broiler small intestine is not obvious based on data regression analysis ($R^2 < 0.700$). Based on the regression analysis of the data, a quadratic fitted curve between age and relative lengths of broiler small intestine is obtained y (duodenum, 0.5% Gln) = $-0.0095x^2+0.6318x+6.2015$ ($R^2= 0.8773$, the best effect is added at 14 d); y (jejunum, 0.5% Gln) = $-0.0359x^2+1.6066x+14.975$ ($R^2= 0.9322$, the best effect is added at 7 d); y (ileum, 0.5% Gln) = $-0.0413x^2+1.648x+5.0902$ ($R^2= 0.7906$, the best effect is added at 7 d); y (duodenum, 1.0% Gln) = $-0.0362x^2+1.1796x+7.5453$ ($R^2= 0.651$); y (jejunum, 1.0% Gln) = $-0.0691x^2+2.333x+16.15$ ($R^2= 0.7805$, the best effect is added at 7 d); y (ileum,1.0% Gln) = $-0.079x^2+2.7382x+1.2632$ ($R^2= 0.9127$, the best effect is added at 7 d).

Table 2 – Effect of dietary Gln on the relative length and relative weights of small intestine in broilers infected with S. Enteritidis.

Items		Diet Treatments[1]			
		CON	SCC	Gln1	Gln2
Relative length (cm/kg)					
Duodenum	4 d	191.33±9.74	176.25±12.06	184.32±9.55	194.67±11.39
	7 d	204.10±2.77^{b}	180.79±8.90^{a}	196.09±3.92^{ab}	215.45±4.84^{b}
	14 d	210.62±1.32^{b}	189.67±0.64^{a}	200.94±2.35^{ab}	220.38±4.78^{b}
	21 d	223.79±4.25^{ab}	197.15±0.45^{a}	213.88±1.65^{a}	230.96±3.24^{b}
Jejunum	4 d	469.85±22.26^{b}	440.12±1.15^{a}	470.60±20.08^{ab}	490.68±12.06^{b}
	7 d	470.26±15.23^{b}	449.41±11.15^{a}	462.69±1.12^{a}	483.45±2.04^{b}
	14 d	480.34±25.57^{b}	454.27±20.95^{a}	472.09±9.02^{ab}	512.15±2.38^{b}
	21 d	502.47±51.85	470.63±25.49	490.21±8.51	517.80±35.46
Ileum	4 d	221.70±23.22	207.07±32.22	214.48±15.01	238.35±16.43
	7 d	236.08±10.89^{b}	186.07±20.01^{a}	233.75±16.38^{b}	245.18±15.63^{b}
	14 d	236.45±0.30^{b}	221.03±0.33^{a}	235.20±1.05^{b}	261.28±0.07^{b}
	21 d	250.45±0.44^{b}	225.34±0.32^{a}	268.94±1.02^{b}	268.25±0.08^{b}
Relative weights (g/kg)					
Duodenum	4 d	9.10±0.51	8.70±0.99	8.87±1.35	10.65±1.21
	7 d	11.27±1.83^{b}	9.11±1.30^{a}	9.65±0.62^{ab}	15.82±2.02^{b}
	14 d	13.56±0.56	11.29±1.49	13.49±1.22	15.88±0.03
	21 d	15.25±0.52	13.14±0.09	15.19±1.15	16.65±0.07
Jejunum	4 d	21.16±2.95	20.18±2.24	20.44±2.12	22.49±1.20
	7 d	27.71±3.63^{b}	21.59±1.14^{a}	25.13±0.63^{ab}	32.37±1.79^{b}
	14 d	31.43±1.10	28.85±3.83	30.02±1.07	33.31±0.02
	21 d	33.10±0.01	30.25±2.21	32.98±1.03	35.26±0.01
Ileum	4 d	9.50±0.90^{b}	7.06±1.03^{a}	9.38±1.39^{b}	9.51±0.87^{b}
	7 d	17.89±0.98^{b}	12.20±0.80^{a}	17.45±2.18^{ab}	19.06±0.58^{b}
	14 d	19.87±0.14^{b}	16.07±3.25^{a}	18.36±0.60^{ab}	22.61±0.01^{b}
	21 d	22.86±0.16^{b}	18.98±2.10^{a}	21.99±0.12^{ab}	24.36±0.02^{b}

[1]CON = noninfect control group, SCC = *S. Enteritidis* infect control group received the basal diet, Gln1= *S. Enteritidis* infect control group received the basal diet plus 0.5 % Gln; Gln2= *S. Enteritidis* infect control group received the basal diet plus 1.0 % Gln.

2, a, b Values within the same row that do not share a common superscript are significantly different at $p<0.05$; n = 8

Morphology of the intestinal mucosa

Our findings regarding the morphology of the intestinal mucosa are given in Table 3. At days 4, 7, 14, and 21, in contrast to the control group, the *S. Enteritidis* infection broiler group showed significantly decreased villus height and VH:CD in the jejunum (except at d 21) and ileum ($p<0.05$). Compared with those of the *S. Enteritidis* challenged groups, the villus height and VH:CD in the jejunum (except at d 21) and ileum in the Gln treatment groups were higher than they were in the *S. Enteritidis* infection group ($p<0.05$), but there were no differences when they were compared with the villus height and VH:CD in the jejunum (except at d 21) and ileum in the CON treatment group ($p>0.05$).

At days 4, 7, 14, and 21, the crypt depths in the jejunum (except at d 21) and ileum were significantly increased in the *S. Enteritidis* infection group compared with those of the CON group ($p<0.05$). However, the Gln 1 and Gln 2 groups showed decreased crypt depths in the jejunum (except at d 21) and ileum ($p<0.05$) compared with the crypt depths in the jejunum and ileum of the *S. Enteritidis* infection group, although there were no significant differences when they were compared with the crypt depths in the jejunum (except d 21) and ileum of the CON group ($p>0.05$). The secondary relationship between age and VH:CD of broiler small intestine is not obvious based on data regression analysis ($R^2 < 0.700$).

Number of intraepithelial lymphocytes

The number of intraperitoneal lymphocyte cells in the duodenum, jejunum and ileum was significantly decreased in the *S. Enteritidis* infection group compared with that of the CON group at 4, 7, 14 and 21 d of age ($p<0.05$) (Table 4). Compared with the *S. Enteritidis* challenged groups, the number of intraperitoneal lymphocyte cells in the duodenum, jejunum and ileum in the Gln treatment was higher than that in the *S. Enteritidis* infection group ($p<0.05$), and there was no difference when it was compared with the number of intraperitoneal lymphocyte cells in

Table 3 – Effect of dietary Gln on the morphology (μm) of the intestinal mucosa in broilers infected with S. Enteritidis.

Items		Diet Treatments[1]			
		CON	SCC	Gln1	Gln2
4 d					
Jejunum	Villus height	651.65±30.10[b]	556.31±16.71[a]	643.01±20.31[b]	648.13±24.07[b]
	Crypt depth	90.12±8.37[a]	112.06±9.18[b]	96.68±7.64[a]	95.01±8.35[a]
	Villus height:crypt depth	7.23±1.02[b]	4.96±0.95[a]	6.65±1.10[b]	6.82±0.87[b]
Ileum	Villus height	418.37±20.38[b]	386.29±16.47[a]	405.34±16.31[b]	410.13±19.16[b]
	Crypt depth	108.04±8.01[a]	123.68±10.14[b]	112.19±8.31[a]	110.92±7.52[a]
	Villus height:crypt depth	3.87±0.27[b]	3.12±0.16[a]	3.61±0.19[b]	3.70±0.11[b]
7 d					
Jejunum	Villus height	756.12±30.15[b]	612.03±21.20[a]	728.92±17.91[b]	744.91±22.83[b]
	Crypt depth	96.54±10.03[a]	117.04±8.81[b]	98.37±8.67[a]	95.94±9.20[a]
	Villus height:crypt depth	7.83±0.86[b]	5.23±0.69[a]	7.41±0.81[b]	7.76±0.93[b]
Ileum	Villus height	452.64±18.67[b]	401.75±10.75[a]	442.18±12.06[b]	448.61±11.14[b]
	Crypt depth	118.34±7.38[a]	138.48±9.01[b]	119.26±9.27[a]	117.13±8.64[a]
	Villus height:crypt depth	3.83±0.86[b]	2.91±0.75[a]	3.71±0.63[b]	3.83±0.81[b]
14 d					
Jejunum	Villus height	1004.59±25.34[b]	869.23±34.18[a]	990.37±19.43[b]	999.17±30.75[b]
	Crypt depth	122.81±7.45[a]	130.17±6.21[b]	125.07±5.98[a]	123.75±4.25[a]
	Villus height:crypt depth	8.18±0.59[b]	6.68±0.76[a]	7.92±0.63[b]	8.07±0.49[b]
Ileum	Villus height	572.14±25.01[b]	477.05±18.43[a]	549.18±15.27[b]	557.83±10.46[b]
	Crypt depth	130.28±12.01[a]	152.92±7.63[b]	136.34±10.04[a]	133.71±8.25[a]
	Villus height:crypt depth	4.39±0.58[b]	3.12±0.67[a]	4.03±0.81[b]	4.17±0.75[b]
21 d					
Jejunum	Villus height	1135.31±65.07	1100.37±42.51	1112.65±54.64	1127.38±70.13
	Crypt depth	134.36±16.87	138.36±18.67	136.09±10.02	134.32±9.64
	Villus height:crypt depth	8.45±1.02	7.95±2.25	8.18±2.99	8.39±1.11
Ileum	Villus height	744.12±16.37[b]	607.25±13.84[a]	721.08±19.62[b]	738.94±16.34[b]
	Crypt depth	136.12±9.31[a]	154.27±7.69[b]	140.02±8.63[a]	138.18±10.02[a]
	Villus height:crypt depth	5.47±0.25[b]	3.94±0.39[a]	5.15±0.46[b]	5.35±0.27[b]

[1]CON = noninfect control group, SCC = *S. Enteritidis* infect control group received the basal diet, Gln1= *S. Enteritidis* infect control group received the basal diet plus 0.5 % Gln; Gln2= *S. Enteritidis* infect control group received the basal diet plus 1.0 % Gln.

2, a, b Values within the same row that do not share a common superscript are significantly different at $p<0.05$; n = 8.

the duodenum, jejunum and ileum in the CON group ($p>0.05$). Based on the regression analysis of the data, a quadratic fitted curve is obtained y (duodenum, 0.5% Gln) = $-0.0334x^2+ 1.3319x+6.9874$ ($R^2= 0.9156$, the best effect is added at 7 d); y (jejunum, 0.5% Gln) = $-0.0205x^2+0.9898x+7.5121$ ($R^2= 0.892$, the best effect is added at 7 d); y (ileum, 0.5% Gln) = $-0.0169x^2+0.8621x+6.2603$ ($R^2= 0.9202$, the best effect is added at 7 d); y (duodenum, 1.0% Gln) = $-0.0199x^2+0.9827x+9.2279$ ($R^2= 0.9032$, the best effect is added at 14 d); y (jejunum, 1.0% Gln) = $-0.0186x^2+0.9601x+7.8006$ ($R^2= 0.9155$, the best effect is added at 7 d); y (ileum,1.0% Gln) = $-0.0199x^2+0.9208x+6.4477$ ($R^2= 0.9289$, the best effect is added at 7 d).

Number of intestinal goblet cells

At days 4, 7, 14 and 21, the number of intestinal goblet cells in the duodenum (except at d 21), jejunum (except at d 21) and ileum (except at d 4) was significantly decreased in the *S. Enteritidis* infection group compared with that of the CON group ($p<0.05$) (Table 5). However, *Salmonella*-challenged broilers treated with Gln supplements (groups Gln 1 and Gln 2) had an increased number of intestine goblet cells in the duodenum (except at d 21), jejunum (except at d 21) and ileum (except at d 4) compared with that of the *S. Enteritidis* infection group ($p<0.05$), although there was no difference when it was compared with the number of intestinal goblet cells in the duodenum, jejunum and ileum of the CON group ($p>0.05$). Based on the regression analysis of the data, a quadratic fitted curve is obtained y (duodenum, 0.5% Gln) = $-0.0085x^2+0.6466x+6.8477$ ($R^2= 0.9336$, the best effect is added at 7 d); y (jejunum, 0.5% Gln) = $-0.0015x^2+0.4553x+9.3286$ ($R^2= 0.8641$, the best effect is added at 7 d); y (ileum, 0.5% Gln) = $0.0094x^2+0.0757x+12.837$ ($R^2= 0.9028$, the best effect is added at 7 d); y (duodenum, 1.0% Gln) = $0.0128x^2+0.7704x+6.6386$ ($R^2= 0.9117$, the

Table 4 – Effect of dietary Gln on the number of the intraepithelial lymphocyte cells in broilers infected with *S. Enteritidi*s (Entries/100 absorptive cells).

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
4 d				
Duodenum	12.04±0.28[b]	8.38±0.51[a]	11.61±1.04[b]	11.93±0.95[b]
Jejunum	11.20±1.24[b]	7.92±0.28[a]	10.88±0.81[b]	11.08±1.57[b]
Ileum	9.73±0.27[b]	6.67±0.62[a]	9.24±0.40[b]	9.65±0.52[b]
7 d				
Duodenum	15.84±0.31[b]	10.23±0.52[a]	14.97±0.86[b]	15.06±0.71[b]
Jejunum	14.67±0.27[b]	9.37±0.61[a]	13.89±0.81[b]	14.07±0.53[b]
Ileum	12.37±0.28[b]	8.69±0.55[a]	11.81±0.34[b]	12.20±0.61[b]
14 d				
Duodenum	19.20±0.13[b]	11.42±0.63[a]	18.91±0.79[b]	19.12±0.88[b]
Jejunum	17.26±0.29[b]	10.63±0.45[a]	17.07±0.67[b]	17.33±0.27[b]
Ileum	15.32±0.31[b]	9.67±0.30[a]	14.81±0.27[b]	15.27±0.35[b]
21 d				
Duodenum	21.56±1.83[b]	13.67±2.15[a]	20.28±1.78[b]	21.06±1.89[b]
Jejunum	20.17±1.41[b]	11.67±2.18[a]	19.32±2.23[b]	19.86±1.63[b]
Ileum	17.25±1.38[b]	10.08±2.10[a]	16.97±1.85[b]	17.06±1.94[b]

[1]CON = noninfect control group, SCC = *S. Enteritidis* infect control group received the basal diet, Gln1= *S. Enteritidis* infect control group received the basal diet plus 0.5 % Gln; Gln2= *S. Enteritidis* infect control group received the basal diet plus 1.0 % Gln.

2, a, b Values within the same row that do not share a common superscript are significantly different at $p<0.05$; n = 8.

Table 5 – Effect of dietary Gln on the number of the intestine's goblet cells in broilers infected with *S. Enteritidi*s (Entries/100 absorptive cells)

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
4 d				
Duodenum	9.28±0.23[b]	6.53±0.17[a]	8.95±0.14[b]	9.19±0.22[b]
Jejunum	11.13±0.18[b]	8.30±0.30[a]	10.65±0.21[b]	10.08±0.27[b]
Ileum	13.59±0.20	9.65±0.24	13.07±0.18	13.46±0.20
7 d				
Duodenum	12.10±0.15[b]	8.10±0.20[a]	11.56±0.27[b]	11.97±0.11[b]
Jejunum	14.01±0.32[b]	10.27±0.40[a]	13.27±0.30[b]	13.98±0.28[b]
Ileum	15.36±0.24[b]	12.11±0.14[a]	14.21±0.19[b]	15.18±0.25[b]
14 d				
Duodenum	14.73±0.12[b]	11.48±0.30[a]	13.87±0.14[b]	14.58±0.16[b]
Jejunum	15.39±0.62[b]	12.67±0.60[a]	14.92±0.17[b]	15.27±0.22[b]
Ileum	16.21±0.42[b]	13.01±0.23[a]	15.51±0.37[b]	16.12±0.18[b]
21 d				
Duodenum	17.89±1.05	15.34±2.96	16.96±1.37	17.28±1.54
Jejunum	19.76±0.91	17.92±1.87	18.39±1.64	19.27±1.89
Ileum	20.49±1.06[b]	14.31±0.84[a]	18.64±0.92[b]	19.87±0.87[b]

[1]CON = noninfect control group, SCC = *S. Enteritidis* infect control group received the basal diet, Gln1= *S. Enteritidis* infect control group received the basal diet plus 0.5 % Gln; Gln2= *S. Enteritidis* infect control group received the basal diet plus 1.0 % Gln.

2, a, b Values within the same row that do not share a common superscript are significantly different at $p<0.05$; n = 8.

best effect is added at 7 d); y (jejunum, 1.0% Gln) = $-0.0062x^2+0.6343x+ 8.4476$ ($R^2= 0.8577$, the best effect is added at 7 d); y (ileum,1.0% Gln) = $0.011x^2+0.0695x+13.426$ ($R^2 = 0.9011$, the best effect is added at 7 d).

Number of mast cells

At days 4, 7, 14 and 21, the number of intestinal goblet cells in the duodenum (except at d 21), jejunum and ileum significantly increased in the *S. Enteritidis* infection group compared with that of the CON group ($p<0.05$) (Table 6). The Gln 1 and Gln 2 groups each showed a significantly decreased number of intestinal goblet cells in the duodenum (except at d 21), jejunum and ileum compared with that of the *S. Enteritidis* infection group ($p<0.05$). However, there were no differences in the number of intestinal goblet cells in the duodenum, jejunum and ileum of the Gln 1

Table 6 – Effect of dietary Gln on the number of the mast cells in broilers infected with S. Enteritidis (Entries/100 absorptive cells).

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
4 d				
Duodenum	42.18±1.19[a]	51.43±1.82[b]	43.37±2.08[a]	42.85±1.64[a]
Jejunum	30.92±1.50[a]	39.07±1.21[b]	32.01±2.17[a]	31.24±1.54[a]
Ileum	29.42±1.01[a]	37.35±1.14[b]	30.64±2.07[a]	30.20±1.30[a]
7 d				
Duodenum	49.10±2.60[a]	60.01±1.08[b]	50.31±2.01[a]	49.68±2.71[a]
Jejunum	33.61±1.30[a]	44.83±1.11[b]	34.08±2.01[a]	33.91±2.22[a]
Ileum	32.15±2.17[a]	41.28±1.62[b]	33.63±3.11[a]	32.79±1.09[a]
14 d				
Duodenum	57.36±3.64[a]	68.31±5.30[b]	58.52±4.37[a]	57.21±3.42[a]
Jejunum	38.07±1.69[a]	47.17±1.92[b]	39.48±1.91[a]	38.91±1.34[a]
Ileum	37.46±1.39[a]	42.05±2.01[b]	38.69±1.83[a]	38.19±2.04[a]
21 d				
Duodenum	68.25±5.17	77.34±2.30	69.07±2.19	68.33±3.01
Jejunum	42.18±1.37[a]	51.05±1.38[b]	43.16±2.54[a]	42.49±1.17[a]
Ileum	41.07±1.47[a]	50.56±2.38[b]	42.06±2.01[a]	41.38±2.37[a]

[1]CON = noninfect control group, SCC = *S. Enteritidis* infect control group received the basal diet, Gln1= *S. Enteritidis* infect control group received the basal diet plus 0.5 % Gln; Gln2= *S. Enteritidis* infect control group received the basal diet plus 1.0 % Gln.

2, a, b Values within the same row that do not share a common superscript are significantly different at $p<0.05$; n = 8.

group, Gln 2 group, and CON group ($p>0.05$). Based on the regression analysis of the data, a quadratic fitted curve is obtained y (duodenum, 0.5% Gln) = $-0.0097x^2+ 1.6926x+37.552$ ($R^2= 0.9801$, the best effect is added at 7 d); y (jejunum, 0.5% Gln) = $0.0014x^2+0.7209x+29.052$ ($R^2= 0.943$, the best effect is added at 14 d); y (ileum, 0.5% Gln) = $-0.0197x^2+1.16x +26.374$ ($R^2= 0.8541$, the best effect is added at 14 d); y (duodenum, 1.0% Gln) = $-0.0015x^2+1.4668x+37.914$ ($R^2= 0.9791$, the best effect is added at 7 d); y (jejunum, 1.0% Gln) = $-0.0152x^2+1.0414x+27.336$ ($R^2= 0.9771$, the best effect is added at 14 d); y (ileum,1.0% Gln) = $-0.0193x^2+1.1461x+ 25.847$ ($R^2 = 0.9011$, the best effect is added at 14 d).

DISCUSSION

The duodenum, jejunum, and ileum are important digestive and absorbing organs that play a vital role. Previous studies have shown that broiler stress can significantly reduce the relative weight and relative length of each intestinal tract, affecting intestinal development (AI-Fataftah *et al.*, 2014; Garriga *et al.*, 2006; Wu *et al.*, 2019). In our experiment, results were obtained to support these previous findings, and the relative length and relative weight of the small intestine were reduced in the broilers infected with *S. Enteritidis*. This outcome may be related to the fact that *Salmonella* can cause intestinal inflammation and increase proinflammatory cytokines, which in turn can increase intestinal damage and nutrient loss (Wang *et al.*, 2019; Dinarello, 2000). However, the results from the dietary Gln supplementation experiments showed that the abovementioned clinical symptoms disappeared; the relative length and relative weight of each intestinal segment of the small intestine increased compared with those of the SCC group after 1.0% Gln was added to the diet given to the broilers infected with *Salmonella*, and when 0.5% Gln was added, the effect of the infection was weak. This finding may indicate that the addition of Gln to the broiler diets improved the intestinal physiology and promoted the recovery of the damaged intestinal tract (Bortoluzzi *et al.*, 2017). This outcome may be due to glutamine serving as a major source of energy for proliferating intestinal cells (Akiba *et al.*, 2009).

The small intestine is an important place where the body absorbs nutrients. The intestinal mucosa plays an important role in the digestion and absorption of nutrients. The increase in villus height is beneficial for the absorption of nutrients, but the increase in crypt depth is not conducive. It is generally believed that the absorption of nutrients increases as the ratio of VH:CD increases (Awad *et al.,* 2009). In our present study, *S. Enteritidis* infection led to a significant decrease in villus height and a significant increase in crypt depth such that the VH:CD was reduced in the broilers infected with *Salmonella*. Similarly, previous reports showed that the VH:CD in the small intestine significantly reduced upon *Salmonella* infection (Andrade *et al.*, 2013; Shao

et al., 2014; Jazi *et al.*, 2018; Wu *et al.*, 2020). This outcome may be the result of *Salmonella* damaging the intestinal mucosa, affecting the absorption of nutrients, weakening the body's resistance, and then aggravating the intestinal mucosa. Our present experimental results indicated that, after feeding broilers with Gln supplemented diets, the villus height increased, the crypt depth decreased, and the VH:CD increased compared to these parameters in the SCC group. Similar findings were reported for broilers fed Gln (Bartell *et al.*, 2007; Wu *et al.*, 2018; Barekatain *et al.*, 2019), mice fed Gln (Huang *et al.*, 2005; Sukhotnik *et al.*, 2007), and piglets fed Gln (Wu *et al.*, 1996; Wang *et al.*, 2008). According to the findings described above, it can be concluded that adding Gln to feed can help protect the intestinal mucosa. This result may be related to the addition of Gln to the diet increasing the expression of genes that prevent oxidative activity (Wu *et al.*, 2019).

There are a large number of intraepithelial lymphocytes with strong immune function in the intestinal mucosa, and these lymphocytes play an important role in resisting infection through immunity and maintaining the integrity of the intestine (Shi *et al.*, 2015). Previous studies have shown that the number of intraepithelial lymphocytes gradually decreases in stressed broilers (Shi *et al.*, 2015). Our study obtained similar results: under physiological conditions, the number of intestinal intraepithelial lymphocytes in the broilers significantly reduced upon *Salmonella* infection. This finding may be related to the ability of *Salmonella* to invade cells and induce the intracellular expression of inflammatory cytokines to cause inflammation, thus destroying the intestinal mucosa (Galan, 1998). Our study indicates that the number of intestinal intraepithelial lymphocytes significantly increased in the broilers fed a diet with 0.5% or 1.0% Gln. Similarly, Swaid's study (Swaid *et al.*, 2013) showed that the addition of Gln to the diet prevented mucosal damage and improved intestinal recovery after intestinal injury in rats, and in Kew S's study (Kew *et al.*, 1999), the addition of Gln to mouse diets enhanced T lymphocyte proliferation. These results were likely attributable to the ability of Gln to reduce the level of the inflammatory factor TNF-α and to decrease the apoptosis of intestinal epithelial cells (Wu *et al.*, 2019). It may also be related to the ability of Gln to provide energy for intestinal epithelial lymphocytes and promote the repair of the damaged intestinal tracts (Schoor *et al.*, 2010).

Intestinal goblet cells play an important role in intestinal immunity; they can secrete mucin and some bioactive molecules, forming an outer layer of cells that prevents the invasion of bacteria and protects the body from damage (Kim *et al.*, 2010). In this study, broilers infected with *S. Enteritidis* had a reduced number of intestine goblet cells compared with those in the uninfected broilers, a finding that was similar to that of a previous study (Zhen *et al.*, 2018). These findings may be related to the colonization of the intestinal tract by *Salmonella* and the downregulation of jejunal mucin, which in turn causes intestinal inflammation and impaired barrier function. This finding indicates that *S. Enteritidis* infection endangers intestinal health in broilers. However, broilers receiving Gln-supplemented diets showed increased goblet cell numbers in the small intestine, indicating that supplementing Gln had a positive role in controlling *S. Enteritidis* infection. Similar to our results, those of another study in which Gln was added to the diet of weaned piglets resulted in a significantly increased number of goblet cells in the duodenum and ileum epithelium (Xing *et al.*, 2017), which may have been related to the ability of Gln to enhance the proliferation of the intestinal stem cells and the promotion of their differentiation into goblet cells (Chen *et al.*, 2019). In contrast, it has been reported that adding Gln to the diets of weaned mice had no effect on the number of intestinal goblet cells (Chen *et al.*, 2018), but another study showed that feeding Gln in tumor-bearing rats reduced the number of goblet cells in the duodenum and jejunum (Martins *et al.*, 2016). This finding is different from that of our experiments and may be related to different species and infection patterns.

Broiler small intestine mast cells are important immune-active cells that can attract granulocytes and lymphocytes to the site of stimulation through paracrine cytokines, regulate intestinal homeostasis, and participate in the immune process after infection in the body (Bischoff *et al.*, 2009). Öhman and Vivinus-Nébot studies (Öhman *et al.*, 2010; Vivinus *et al.*, 2012) have shown that mast cells are associated with irritable bowel syndrome, and intestinal mucosal mastocytosis is one of its characteristics. In our study, the number of small intestine mast cells in the *Salmonella*-infected broilers increased significantly, indicating that the broiler intestinal tracts were damaged. Similar findings have been reported in nematode-infected mice. This outcome may be related to promoted mast cell activation, an additional effect of their nonspecific release under stress, infection and inflammatory conditions (Befus *et al.*, 1979; Bienenstock *et al.*, 1979; Gue *et al.*, 1997). Moreover, our present study

found that Gln supplementation in *S. Enteritidis*-infected broilers significantly reduced the number of small intestinal mast cells. A previous study showed similar results: Gln was sufficient to reduce the number of gastric mast cells in gastric-damaged mice (Mitra *et al.*, 1977). This finding may be related to the ability of glutamine to reduce the release of lipid mediators and the expression of proinflammatory cytokines, thereby inhibiting mast cell activation (Lechowski *et al.,* 2013).

In addition, our study found that the effect of Gln addition was best at 7 days of age based on data regression analysis. This may be related to the intestinal microorganisms of broilers. Previous studies reported that the diversity of intestinal microbiota of chickens increases with the growth of age, and the ileum microflora can be divided into three stages: 3-5d, 5-12d and 12-17d, while the stability of the first stage is the worst (Gong *et al.*, 2013; Hume *et al.*, 2003; Wielen *et al.*,2002), and the intestinal microorganisms of broilers at 15-22 d gradually tend to mature (Ranjitkar *et al.*, 2016). Moreover, our present study found that there were no significant differences in some indicators of 21-day-old broiler chickens between groups. Kogut's (2009) research shows that determining an animal's ability to respond to a particular pathogenic microorganism depends on the age of the animal. This may be due to the natural and adaptive immunity of the animal's intestine, the structural integrity of the mucosal tissues, and the physiological functions becoming more and more perfect with age.

CONCLUSION

In our study, 0.5% and 1.0% Gln addition to broiler diets effectively alleviated intestinal mucosal damage caused by *Salmonella* infection and improved its normal defense barrier function, with 1.0% Gln having the better effect. In addition, the effect of Gln addition was best at 7 days of age based on data regression analysis.

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