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Original Article

Effects of Glutamine on Digestive Function and Redox Regulation in the Intestines of Broiler Chickens Challenged with Salmonella Enteritidis

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ABSTRACT

The aim was to investigate the effect of glutamine (Gln) on broilers challenged with *Salmonella Enteritidis*. 240 1-day-old birds were divided into four groups in a completely randomized design, each of which included 6 replicates with 10 birds per replicate. Group I served as the unchallenged, untreated control (CON). All birds in groups II (SCC) – IV were challenged with 2.0×10^4 CFU/mL of *S. Enteritidis*. Birds in group III and IV were treated with 0.5% (Gln 1) and 1.0% (Gln 2), respectively, of Gln. The results indicated that *S. Enteritidis* infection led to a decrease in the average body weight at d 7, 14, and 21 ($p<0.05$). Chickens fed the Gln showed improved average body weights in comparison with the SCC group ($p<0.05$). At d 4, 7, 14, and 21, the Gln groups increased digestive enzyme (trypsin, lipase and amylase (except the amylase activity of jejunum at d 14 and d 21)) activities in the intestine ($p<0.05$), superoxide dismutase (SOD) (at d 14 jejunum; except at d 4, ileum) and catalase (CAT) (at d 4, and d 21, jejunum; d 4, ileum) activity in the serum (except at d 14) and intestinal mucosa ($p<0.05$), and the mRNA expression of SOD, CAT and nuclear respiratory factor 2 (Nrf2) of the intestinal mucosa compared with the SCC group ($p<0.05$). These results suggest that Gln as a feed additive could be effective for reducing the detrimental effects of *S. Enteritidis* infection of broilers.

INTRODUCTION

Salmonella Enteritidis is capable of colonizing the gut effectively (Eliana *et al.*, 2012), and it can produce systemic or septicaemic disease in young chickens. *Salmonella* attaches to the intestinal cells and epithelium, causing alteration of the redox status of the jejunal mucosa and superoxide dismutase (SOD) and glutathione reductase activity in the infected hosts (Popkova *et al.*, 1984; Alaeldein *et al.*, 2017). Moreover, evidence from animal study has shown that *S. Enteritidis* infection can also adhere to glycoproteins of the intestinal epithelium and induce the activation of immune cells, lead to injury of the colonic mucous epithelium, thereby causing diffuse extraintestinal infections and an inflammatory response with diarrhea (Wu *et al.*, 2018). The pathogenesis of this condition is complex and is strongly implicated in compromised growth and mortality of the birds (Taunay *et al.*, 1996). Therefore, control programs for intestinal colonization caused by *S. Enteritidis* are essential to protect the health of the birds and ensure the safety of consumers. Glutamine (Gln) is considered as a conditionally essential amino acid (Newsholme, 2001) and is a critical fuel source and a potent mitogen for intestinal enterocytes (Jacobs *et al.*, 1988). Previous studies have shown that Gln treatment can improve the average body weight, feed conversion ratio, abdominal morphological structures, intestinal cell metabolism, and the activity of digestive enzyme as a

result of reducing the pH in the alimentary tract (Lin & Zhou, 2006; Wu *et al.*, 2019). Recent work also demonstrated that pretreatment with Gln attenuated cytokine release and improved the activity of the gut antioxidant defense system, which involves protecting the epithelial barrier function, in an animal model of endotoxemia (Kazantzidou *et al.*, 2010; Geng, 2011; Li *et al.*, 2014). However, little research has been conducted on the regulatory mechanism of the internal redox state on Gln in *Salmonella*-challenged birds. Hence, the purpose of this research was to find the possible antioxidant properties of Gln on serum and intestinal tissues to reduce the colonization of *S. Enteritidis* in the intestine and the impact of Gln on the digestive function after the challenge in broiler chicks.

MATERIALS AND METHODS

S. Enteritidis

S. Enteritidis serotype was provided by 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 viable cells.

Broiler, management and experimental diets

Two hundred and forty 1-day-old commercial Arbor Acres broilers (mixed male and female), were obtained from a local hatchery in Luoyang, China. The birds were weighed and placed in two separate 3-tier battery cages in an environmentally controlled room; the broilers were exposed to ambient temperatures that gently decreased from 34°C to 22 ± 1°C until 21 d of age. Plastic separators were used to prevent horizontal contamination between the cages. The experimental chickens were provided twenty-three hours of light at days 1 to 7, and 18 h light at days 8 to 21. The birds were fed mash diet formulated according to the NRC (1994) recommendations to meet or exceed the nutrient and energy requirements of broilers (Table 1) and allowed access to feed and water *ad libitum*. Gln (pharmaceutical grad: 99% purity, Henan Honda Biological Medicine Co., Ltd., China) was supplemented and thoroughly mixed into the basal feed. Fresh feed was prepared and stored in sealed bottles at room temperature. The experiment was done under the supervision of the Institutional Animal Care and Use Committee of Henan University of Science and Technology.

Table 1 – Ingredients and nutrient level of the experimental diet (%).

Feed Ingredients (%)	1-21d
Corn silage	54.6
Corn gluten meal	35.5
Soybean oil	3.5
Limestone	1.2
Dicalcium phosphate	1.5
Salt	3.0
50% Choline chloride	0.14
Premix[1]	0.24
L-Lysine	0.12
DL-Methionine	0.2
Total	100
Calculated nutrients levels (%)	
Apparent metabolisable energy (AME) (MJ/kg)	12.55
Crude protein (CP)	21.00
Ca	0.90
Available Phosphorus	0.45
Lys	1.15
Met+cys	0.80

Note:[1]Each kg of premix contained: Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8 mg; Mn (from manganese sulfate), 100 mg; Zn (Bacitracin Zn), 65 mg; iodine (from calcium iodate), 0.35 mg; Se (from sodium selenite), 0.15 mg. Vitamin A (transretinyl acetate), 12,500 IU; Vitamin D_3 (cholecalciferol), 2,500 IU; Vitamin E,18.5 mg; Vitamin K_3,2.65 mg; thiamine 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B_{12} (cobalamine), 0.013 mg.

Experimental protocol

Broilers were used in a completely randomized design with four treatment groups, each of which included six replicates with ten birds (half male and half female) per replicate.

Group I served as the unchallenged, untreated control (the negative control group, CON). At three days of age, all the other birds in group II (SCC = *S. Enteritidis* infect control group), III (Gln1= *S. Enteritidis* infect control group received the basal diet plus 0.5 % Gln), and IV (Gln2= *S. Enteritidis* infect control group received the basal diet plus 1.0 % Gln) received by oral gavage 2.0 × 10^4 CFU each (0.5 mL) of *S. Enteritidis* suspension. Each chick in the unchallenged group received an equivalent amount of sterile normal saline. The trial lasted 21d.

Growth performance

At days 1, 3, 4, 7, 14, and 21, the birds were weighed individually after an overnight fast, and then the data was used to calculate the average body weights on days 3, 4, 7, 14, and 21.

Sample collection and procedures

Before sampling, broiler chickens starved for 12 h. On d 1, 7, 14 and 21, six chicks per cage were

randomly selected, and immediately euthanized with cervical dislocation. 5.0 mL blood samples were taken from the caudal veins, and samples were centrifuged for 15 min at 1,800 × *g* for the collection of the serum.

During necropsy the jejunum and ileum were dissected free of the mesentery. Approximately 2 and 10 cm long segment of the jejunum and the ileum were resected at mid-jejunum and mid-ileum, gut contents from the jejunum and the ileum were collected into the plastic collection tubes and stored at -80 °C to determine digestive enzyme activity. Then, each residual intestine segment was opened longitudinally, cleaned with a physiological saline solution, and the mucosa samples were gently scraped with a glass microscope slide, and separated into two subsamples. One mucosa subsample was stored quickly at -80 °C to assess RNA quality. The other mucosa scraping was subsampled, then placed on ice until they were centrifuged for 10 min at 1,200 to 1,500× *g*, the suspension was gathered, and then stored immediately at -80°C for future antioxidant enzyme assays.

Biochemical assay determination of serum and intestine mucosa parameters

According to the instructions of the manufacturer, the digestive enzymes included amylase (catalog No. C016-1), trypsin (catalog No. A054), and lipase (catalog No. A080-2) detected by the detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Total protein concentration was assayed according to the method of Bradford (1976). The detection kits from the same source were used to detect the concentration of Catalase (CAT, catalog No. A007-1), malondialdehyde (MDA, catalog No. A003-1), SOD (catalog No. A001-1) and protein (catalog No. A045-3).

RNA Isolation and real-time quantitative PCR

Total RNA of the intestinal mucosa samples (2 μg) was isolated by TRIzol reagent (Invitrogen Trading (Shanghai) Co., Ltd., China) and ground using a homogenizer, according to the instructions of the manufacturer. The RNA pellets were dried at 55°C for 10 min and resuspended in buffer with ten mM tris hydrochloride and one mM EDTA at a pH of 8.0. The RNA integrity was electrophoresed by 1% agarose-formaldehyde gel. The amplified RNA quantity was detected using a spectrophotometer (Gene Quant 1300/100, General Electric Company, USA). Then, 1 μg RNA was immediately reverse transcribed into cDNA by using reverse transcription kits. Subsequently, labeled cRNAs were disrupted, incubated, diluted, and assembled RNA microarray. The PCR was performed in duplicate on each sample. The specific primers for nuclear respiratory factor 2 (Nrf2), SOD, and CAT are summarized in Table 2. According to the instructions of the manufacturer, qRT-PCR reactions were run with the SYBR® Green RT-PCR Kit. The following PCR amplification parameters were used, namely, 2 min at 50°C; 10 min at 95 °C; and cycle numbers were 40 at 95°C for 10 s, 20 s at 60°C, 65°C for 1 min. The relative fold-change from different parts of small intestine was calculated by the $2^{-\Delta\Delta ct}$ method, which accounts for gene-specific efficiencies and was normalized to the mean expression of the abovementioned index (Livak & Schmittgen, 2001; Liu *et al.*, 2010).

Table 2 – Specific Primer sequences for target and β-actin genes.

GeneName	Gene NO.	Primer Sequence	Fragment Size
SOD	NM_205064.1	5′-TTGTCTGATGGAGATCATGGCTTC-3′ 5′-TGCTTGCCTTCAGGATTAAAGTGA-3′	98 bp
CAT	NM_001031215.1	5′-GTTGGCGGTAGGTCTGGTCT-3′ 5′-GTGGTCAAGGCATCTGGCTTCTG-3′	182 bp
Nrf2	NM_205117.1	5′-ATCACCTCTTCTGCACCGAA-3′ 5′-GCTTTCTCCCGCTCTTTCTG-3′	258 bp
β-actin	NM_2055518.1	5′-AGCGAACGCCCCCAAAGTTCT-3′ 5′-AGCTGGGCTGTTGCCTTCACA-3′	139 bp

Statistical methods

All analyses were performed using statistical software SPSS version 21.0 (SPSS Inc., Chicago, IL, USA, 2012), and differences between means were examined using the Duncan multiple range test of SPSS. All results were expressed as the mean with the standard deviation. Statistically significance was declared for $p<0.05$.

RESULTS

Average body weight and mortality

There were no significant differences among the CON, SCC, and Gln groups on days 3 and 4 ($p>0.05$) (Table 3). However, the present study indicated the lowest average body weight gain in *S. Enteritidis* infected birds (group SCC) compared with control

Table 3 – Effect of dietary Gln on the average body weight (g) of broilers infected with *S. Enteritidis.*

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
3 d	83.59±1.10	83.60±1.27	84.76±1.08	84.95±1.21
4 d	102.28±2.94	100.24±3.08	101.06±1.97	102.09±3.14
7 d	190.63±6.00[b]	155.24±5.21[a]	181.38±4.62[b]	188.72±5.53[b]
14 d	488.55±10.05[b]	435.07±9.38[a]	479.09±10.11[b]	486.75±8.64[b]
21 d	955.50±18.53[b]	849.67±12.24[a]	920.23±14.39[b]	947.68±16.20[b]
Mortality rates (%)	0	3.33	1.67	0

[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.

and Gln groups on days 7, 14, and 21. *Salmonella*-challenged birds with Gln supplementation (groups Gln 1 and Gln 2) showed better average body weights ($p<0.05$) compared with SCC group, but there was no difference on the average body weight between Gln groups and CON group ($p>0.05$).

Challenge doses (2.0×10^4 CFU/ml) of *S. Enteritidis* caused mortality rates ranging from 0% in the CON group to 3.33% in the SCC group (Table 3). Compared with the *S. Enteritidis* challenge group, Gln treatment decreased the mortality rates of appliances. The CON group did not experience any deaths during the experiment duration. Moreover, birds were clinically dull, depressed, tired, and anorexia after *S. Enteritidis* challenge in the SCC group. Birds control group remained in good health status during the experiment period, and they did not show any clinical signs of the disease, including diarrhea.

Table 4 – Effect of dietary Gln on the digestive enzyme activitys of broilers infected with *S. Enteritidis.*

Items		Diet Treatments[1]			
		CON	SCC	Gln1	Gln2
4 d					
Jejunum	Trypsin (U/g)	1165.53±111.27[b]	907.52±92.24[a]	1095.65±102.84[b]	1170.14±101.29[b]
	Lipase (U/mg)	148.27±10.45[b]	116.37±9.46[a]	139.34±6.37[b]	143.62±5.97[b]
	Amylase (U/mg)	57.12±1.62[b]	50.76±2.04[a]	56.85±2.83[b]	57.86±1.23[b]
Ileum	Trypsin (U/g)	1652.16±111.13[b]	1108.75±109.34[a]	1588.60±100.72[b]	1619.76±110.07[b]
	Lipase (U/mg)	100.10±10.01[b]	54.32±5.17[a]	94.18±6.31[b]	103.41±9.17[b]
	Amylase (U/mg)	54.76±4.38[b]	25.24±3.21[a]	51.87±1.40[b]	53.45±5.24[b]
7 d					
Jejunum	Trypsin (U/g)	1079.65±102.32[b]	804.75±82.04[a]	980.19±83.82[b]	1076.90±91.09[b]
	Lipase (U/mg)	136.54±22.23[b]	106.56±20.13[a]	129.65±16.71[b]	138.80±24.28[b]
	Amylase (U/mg)	48.96±3.69[b]	43.19±2.67[a]	47.61±5.07[b]	49.18±3.37[b]
Ileum	Trypsin (U/g)	1720.46±102.31[b]	1327.43±99.67[a]	1608.69±100.04[b]	1719.94±110.20[b]
	Lipase (U/mg)	111.09±12.86[b]	60.46±10.21[a]	99.86±9.84[b]	108.04±10.04[b]
	Amylase (U/mg)	46.89±6.76[b]	21.37±6.20[a]	42.19±2.43[b]	47.31±3.01[b]
14 d					
Jejunum	Trypsin (U/g)	898.45±45.31[b]	614.24±51.76[a]	880.13±30.27[b]	894.46±23.27[b]
	Lipase (U/mg)	96.48±16.34[b]	63.69±11.20[a]	90.69±13.45	95.41±9.19
	Amylase (U/mg)	39.10±2.65	36.96±2.99	38.56±6.41	40.27±4.65
Ileum	Trypsin (U/g)	1808.93±52.89[b]	1235.67±110.24[a]	1761.30±123.45[b]	1800.62±98.67[b]
	Lipase (U/mg)	130.89±13.59[b]	100.02±5.67[a]	120.17±4.63[b]	129.84±3.48[b]
	Amylase (U/mg)	28.56±5.43[b]	14.53±3.07[b]	26.74±4.69[b]	30.01±3.76[b]
21 d					
Jejunum	Trypsin (U/g)	826.01±32.14[b]	536.84±21.18[a]	817.81±11.76[b]	828.34±22.32[b]
	Lipase (U/mg)	69.97±7.15[b]	44.38±5.95[a]	61.67±6.46[b]	69.43±3.39[b]
	Amylase (U/mg)	10.73±5.98	8.59±8.67	9.76±9.75	10.43±7.31
Ileum	Trypsin (U/g)	2000.53±120.43[b]	1567.38±139.47[a]	1896.41±140.08[b]	1997.86±101.72[b]
	Lipase (U/mg)	155.86±16.30[b]	107.85±18.97[a]	142.30±10.34[b]	154.97±11.27[b]
	Amylase (U/mg)	15.76±4.21[b]	7.68±3.02[a]	13.47±5.00[b]	14.89±2.68[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.

Activities of digestive enzymes

At d 4, 7, 14, and 21, *S. Enteritidis* infection significantly decreased the trypsin, lipase, and amylase (jejunum excluded at d 14 and d 21) activity in the jejunum and the ileum as compared with the control (p<0.05) (Table 4). The Gln1 and Gln 2 groups showed significantly increased trypsin, lipase, and amylase activity of the jejunum and ileum compared with the *S. Enteritidis* infection group (p<0.05). However, there were no differences on the trypsin, lipase, and amylase activities between the CON group and the SCC group (p>0.05).

Antioxidant indices

At d 4, 7, and 21, contrasting with the control group, *S. Enteritidis* infection decreased the serum CAT and SOD concentration of broilers (p<0.05) (Table 5). However, Gln 1 and Gln 2 groups showed increased serum CAT and SOD concentration as compared with

Table 5 – Effect of dietary Gln on the serum and mucosa antioxidation function of broilers infected with *S. Enteritidis.*

Items		Diet Treatments[1]			
		CON	SCC	Gln1	Gln2
4 d					
Serum	CAT (U/mL)	8.76±0.34[b]	3.80±1.02[a]	8.66±0.31[b]	8.80±0.50[b]
	SOD (U/mL)	100.27±5.86[b]	82.58±4.29[a]	106.46±6.21[b]	109.78±3.37[b]
	MDA (nmol/mL)	2.31±0.29	2.85±0.50	2.29±0.32	2.08±0.43
Jejunum	CAT (U/mg prot)	14.38±1.23[b]	4.27±0.86[a]	13.77±1.05[b]	14.17±0.82[b]
	SOD (U/mg prot)	225.85±20.15	160.38±12.76	199.98±10.00	210.73±13.84
	MDA (nmol/ mg prot)	0.12±0.01[a]	0.26±0.07b	0.08±0.02[a]	0.11±0.01[a]
Ileum	CAT (U/mg prot)	10.63±1.79[b]	4.98±1.14[a]	9.94±1.59[b]	10.77±1.83[a]
	SOD (U/mg prot)	145.17±13.02	95.71±20.13	148.61±21.60	152.27±21.20
	MDA (nmol/ mg prot)	0.47±0.05a	0.72±0.01[b]	0.50±0.07[a]	0.45±0.02[a]
7 d					
Serum	CAT (U/mL)	10.52±1.28[b]	4.05±1.15[a]	9.89±1.00[b]	10.37±0.56[b]
	SOD (U/mL)	108.89±4.32[b]	30.27±3.01[a]	106.64±3.81[b]	109.94±5.58[b]
	MDA (nmol/mL)	3.18±0.43[a]	4.86±0.32[b]	3.22±0.26[a]	3.11±0.19
Jejunum	CAT (U/mg prot)	4.02±1.62	2.09±0.86	3.72±1.01	3.98±1.90
	SOD (U/mg prot)	290.75±20.19	203.08±11.32	246.31±20.93	289.72±24.37
	MDA (nmol/ mg prot)	0.20±0.01[a]	0.38±0.05[b]	0.25±0.02[a]	0.19±0.03[a]
Ileum	CAT (U/mg prot)	2.35±0.17	1.99±0.20	2.24±0.42	2.57±0.75
	SOD (U/mg prot)	104.57±1.58[b]	60.86±1.72[a]	98.88±2.79[b]	102.30±7.69
	MDA (nmol/ mg prot)	0.07±0.01	0.12±0.02	0.06±0.04	0.05±0.01
14 d					
Serum	CAT (U/mL)	18.69±2.56	15.27±1.86	18.39±1.02	18.78±1.37
	SOD (U/mL)	145.62±8.61	130.98±4.56	141.56±6.20	151.07±6.14
	MDA (nmol/mL)	2.96±0.29[a]	4.57±0.20[b]	3.11±0.17[a]	2.94±0.32[a]
Jejunum	CAT (U/mg prot)	5.60±1.02	5.05±0.51	5.78±1.42	5.83±1.11
	SOD (U/mg prot)	86.53±2.12[b]	53.67±2.19[a]	82.57±3.01[b]	86.49±4.21[b]
	MDA (nmol/ mg prot)	0.35±0.10[a]	0.58±0.13[b]	0.42±0.01[a]	0.37±0.06[a]
Ileum	CAT (U/mg prot)	9.67±1.13	5.98±0.52	8.69±1.23	9.54±0.75
	SOD (U/mg prot)	117.68±5.07[b]	66.73±8.63[a]	105.91±2.75[b]	116.75±6.38[b]
	MDA (nmol/ mg prot)	0.38±0.03[a]	0.59±0.10b	0.42±0.02[a]	0.39±0.03[a]
21 d					
Serum	CAT (U/mL)	9.15±1.36[b]	4.56±1.21[a]	8.30±0.62[b]	9.51±0.73[b]
	SOD (U/mL)	180.68±6.50[b]	100.01±5.59[a]	175.69±7.41[b]	183.46±7.37[b]
	MDA (nmol/mL)	1.83±0.12[a]	3.56±0.20[b]	2.14±0.19[a]	1.90±0.11[a]
Jejunum	CAT (U/mg prot)	14.58±1.12[b]	7.46±1.95[a]	13.39±2.54[b]	14.49±1.70[b]
	SOD (U/mg prot)	175.32±15.39	133.94±10.27	159.60±2.49	169.97±7.83
	MDA (nmol/ mg prot)	0.39±0.01[a]	0.98±0.15[b]	0.49±009[a]	0.43±0.08[a]
Ileum	CAT (U/mg prot)	8.89±1.00	5.62±0.71	7.76±1.72	8.65±2.02
	SOD (U/mg prot)	119.86±15.20[b]	69.92±9.12[a]	99.96±11.60[a]	113.51±9.18[a]
	MDA (nmol/ mg prot)	0.30±0.02[a]	0.62±0.12[b]	0.36±0.10[a]	0.30±0.08[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.

the *S. Enteritidis* infection group ($p<0.05$), although there were no differences when compared with the CAT and SOD of the CON group ($p>0.05$). *S. Enteritidis* infection increased the serum MDA in the SCC group at d 7, 14, and 21 compared with the CON group. However, the Gln 1 and Gln 2 groups showed decreased serum MDA as compared with the *S. Enteritidis* infection group ($p<0.05$), but the MDA content of Gln groups showed no differences when compared with those of the CON group ($p>0.05$). Serum CAT (14 d), SOD (d 14), and MDA (d 4) were not affected after infection, and no significant differences were seen among the treatment groups ($p>0.05$).

S. Enteritidis infection significantly increased the mucosa MDA contents of the jejunum and ileum at d 4, 7 (apart from the ileum), 14, and 21 when compared with the MDA contents of the CON group ($p<0.05$). Compared with the *S. Enteritidis*-challenged groups, the MDA contents of the jejunum and ileum mucosa in the Gln-treated groups were lower than those of the *S. Enteritidis* infection broilers ($p<0.05$). There was no difference on the activity of SOD in the intestinal mucosa at d 4 and jejunal mucosa at d 7 or 21 d among the three groups ($p>0.05$). *S. Enteritidis* infection decreased the SOD activity of the intestinal mucosa at d 14 and the ileal mucosa at d 7, 14, and 21 when compared with the SOD activity of the CON group ($p<0.05$). However, Gln 1 and Gin 2 groups showed increased SOD activity ($p<0.05$) when compared with the SOD activity of the *S. Enteritidis* infection group. There was no difference on the activity of CAT in the intestinal mucosa at d 7 and 14, and ileal mucosa at d 21 among the three groups ($p>0.05$). The CAT activity of the intestinal mucosa at d 4 and the jejunal mucosa at d 14 and 21 in the SCC group ($p<0.05$) was less than that of the CON group ($p<0.05$). However, the Gln 1 and Gln 2 groups showed increased SOD activity ($p<0.05$) compared with the SCC group.

mRNA expression levels in the intestinal mucosa

Compared with the CON treatment, the *S. Enteritidis* infection group showed a significant decrease in the mRNA expression levels of CAT, SOD, and Nrf2 at 4, 7, and 14 days of age ($p<0.05$) (Table 6). Compared with the *S. Enteritidis* challenged groups, the mRNA expression levels of CAT, SOD, and Nrf2 in the Gln treatment were higher than those of the *S. Enteritidis* infection group ($p<0.05$), but there were no differences when compared with the mRNA expression levels of CAT, SOD, and Nrf2 in the CON treatment ($p>0.05$).

There were rather large differences in the CAT, SOD, and Nrf2 mRNA expression levels of the ileal mucosa at 21 days of age. Appliances in the SCC group exhibited decreased CAT, and Nrf2 mRNA expression levels in the jejunal and ileal mucosa as compared with the CON group ($p<0.05$). The Gln treatment group showed upregulated mRNA expression levels of the above-mentioned genes in comparison with the *S. Enteritidis* infection group ($p<0.05$), but there were no differences when compared with the jejunal and ileal mucosa of the CON group ($p>0.05$). At 21 days of age, there were no differences on the mRNA expression levels of SOD in the jejunal and ileal mucosa among the groups ($p>0.05$).

DISCUSSION

Previous studies have reported that young chickens infected with *Salmonella* present cachexia, shivering, clustering, head dropping, wing prolapse, loss of appetite, difficulty breathing, loss of body weight, diarrhea, and intestinal lesions from inflammation and damage to villi, leading to high mortality rates (Suzuki, 1994; Ma *et al.*, 2014; Zhou *et al.*, 2014). In our experiment, oral infection with *Salmonella* was not severe enough to lead to high mortality in the chickens (3.33%), but it did effectively activate the antioxidant system to protect against infection. Moreover, the results of our experiment study revealed that the infection with *S. Enteritidis* led to a difference in the average body weight between the SCC and CON group at d 7, 14, and 21. The results suggest that the infection was successful and are consistent with some published studies (Zhou *et al.*, 2014; Wu *et al.*, 2018) that found that the oral vaccination of *S. Enteritidis* in broilers negatively affected the average body weight. The experimental results on chicken growth performance and mortality in this study were slightly different from the findings of others (Bohez *et al.*, 2008; Haider *et al.*, 2012). These differences between the experimental results are probably due to chick age at the infection, strain variations, challenge dosages, animal management, and environmental conditions (Liu *et al.*, 2018; Wu *et al.*, 2018). However, dietary Gln supplementation showed that the abovementioned clinical symptoms disappeared; the mortality decreased, and the average body weight of the birds under infection conditions improved to levels similar to the healthy broilers.

Our present experiment results indicated that Gln increased the average body weight, which was

Table 6 – Effect of dietary Gln on the expressioin of genes broilers infected with *S. Enteritidis.*

Items		Diet Treatments[1]			
		CON	SCC	Gln1	Gln2
4 d					
Jejunum	CAT	0.84±0.06[b]	0.61±0.07[a]	0.80±0.03[b]	0.86±0.03[b]
	SOD	0.92±0.12[b]	0.52±0.10[a]	0.87±0.07[b]	0.92±0.04[b]
	Nrf2	1.15±0.06[b]	0.68±0.09[a]	1.04±0.07[b]	1.15±0.08[b]
Ileum	CAT	0.80±0.08[b]	0.49±0.06[a]	0.78±0.10[b]	0.82±0.04[b]
	SOD	0.80±0.01[b]	0.44±0.05[a]	0.78±0.01[b]	0.81±0.02[b]
	Nrf2	1.09±0.04[b]	0.62±0.05[a]	0.97±0.09[b]	1.08±0.10[b]
7 d					
Jejunum	CAT	0.88±0.03[b]	0.64±0.07[a]	0.84±0.01[b]	0.90±0.02[b]
	SOD	0.96±0.12[b]	0.53±0.10[a]	0.88±0.07[b]	0.94±0.04[b]
	Nrf2	1.07±0.10[b]	0.60±0.06[a]	0.98±0.07[b]	1.05±0.03[b]
Ileum	CAT	0.86±0.10[b]	0.56±0.08[a]	0.82±0.05[b]	0.88±0.11[b]
	SOD	0.84±0.04[b]	0.49±0.04[a]	0.80±0.01[b]	0.85±0.03[b]
	Nrf2	1.00±0.06[b]	0.59±0.05[a]	0.91±0.03[b]	0.97±0.04[b]
14 d					
Jejunum	CAT	1.08±0.12[b]	0.72±0.11[a]	0.96±0.08[b]	1.07±0.10[b]
	SOD	1.19±0.10[b]	0.58±0.10[a]	1.05±0.11[b]	1.22±1.00[b]
	Nrf2	0.95±0.02[b]	0.50±0.04[a]	0.89±0.06[b]	0.96±0.08[b]
Ileum	CAT	0.90±0.07[b]	0.65±0.02[a]	0.83±0.04[b]	0.92±0.03[b]
	SOD	0.88±0.04[b]	0.50±0.09[a]	0.82±0.03[b]	0.89±0.01[b]
	Nrf2	0.92±0.07[b]	0.55±0.04[a]	0.88±0.01[b]	0.92±0.03[b]
21 d					
Jejunum	CAT	1.22±0.15[b]	0.96±0.10[a]	1.22±0.09[a]	1.27±0.11[a]
	SOD	1.12±0.11	0.90±0.09	1.11±0.12	1.14±0.10
	Nrf2	0.89±0.09[b]	0.52±0.05[a]	0.80±0.07[b]	0.88±0.06[b]
Ileum	CAT	0.96±0.04[b]	0.70±0.07[a]	0.87±0.06[b]	0.94±0.02[b]
	SOD	0.93±0.07	0.91±0.09	0.93±0.05	0.95±0.08
	Nrf2	0.81±0.03[b]	0.49±0.06[a]	0.75±0.06[b]	0.80±0.04[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.

similar to a previous study by Xue *et al.* (2018). These studies indicated that supplementing the diet with Gln may improve the weight gain of broilers under stress conditions, which may partly depend on the advantageous effect of Gln on the growth and development of the digestive organs (Uni *et al.*, 1999). Some studies reported that Gln could increase the intestinal fold height and number, maintaine the integrity and function of the gut, and reduce the adhesion and invasion of pathogenic bacterial (Dai *et al.*, 2013; Xu *et al.*, 2014). In contrast, it has been reported that diet containing Gln did not improve the average body weight gain of broilers under stress conditions (Shakeri *et al.*, 2016). These different results may be directly related to the proper addition of Gln, the rearing period of the appliances (Shakeri *et al.*, 2016), the levels and sources of stress, etc. Our results also showed that diet Gln enhanced the average body weight associated with antioxidant (CAT and SOD) and digestive enzyme activity.

Digestive enzyme activity is considered to be a reliable indicator of digestive capacity, intestinal development, and the nutritional status of the individual. A variety of factors can affect digestive enzyme activity, including different dietary supplements, the type, and degree of stress, intestinal microflora, degree of intestine development, and feeding conditions (Xue *et al.*, 2018). Previous reports revealed that digestive enzyme activity decreased after *Salmonella* infection (Zhang *et al.*, 2016; Tang *et al.*, 2018), probably due to its capacity to reduce the populations of beneficial microorganisms and impaired intestinal mucosa and function, which dramatically influences the early intestinal microbial balance and purpose in chickens. Our present study found that Gln supplementation in the *S. Enteritidis* infected broiler groups promoted the activities of digestive enzyme (trypsin, lipase, and amylase). Similarly, a similar increase in digestive enzyme activity was noted in other animals fed Gln-supplemented feed (Xu *et al.*, 2014). These effects

were likely due to Gln administration, which interacted with food matrix components and was involved in nutrient metabolism and absorption. Besides, Gln also induced protection of the intestinal mucosal integrity by preventing the bacteria translocation, regulating the balance of intestinal microorganisms and consequently promoting the digestion and absorption of feed nutriment. Finally, the benefit of Gln may be related to the promoted development of the pancreas in broilers to some extent (Pinkus & Berkositz, 1980). The results of this experiment also showed that the change in the digestive enzyme activity was different with age, which is probably connected with the maturation of the digestive system, causing different pH values and gut permeability at different ages (Mahagna *et al.*, 1995; Shapiro & Nir, 1995). However, digestive enzyme activity was higher in the chicken jejunum than in the ileum. These effects might be ultimately connected with the changes in indigestion due to changes in the histological structure of the digestive tract, acidic pH values, and microbiota composition (Sohail *et al.*, 2012; Wu *et al.*, 2018). The effect of glutamine on the digestive enzyme activity in different parts of the small intestine needs further study.

Stress can disrupt cellular homeostasis, thereby inducing the excessive production or accumulation of reactive oxygen species (ROS) or lipid peroxides, causing oxidative injury in animals (Liu *et al.*, 2015). Some studies have shown that oxidative stress of the intestine and substantially reconstructed mucosal barriers occur in *S. Enteritidis* -challenged birds (Wu *et al.*, 2018). Our present study showed that *S. Enteritidis* infection led to reduced plasma and intestinal mucosa SOD and CAT activity. Elevated plasma and intestinal mucosa MDA concentrations in the *S. Enteritidis* infected treatment, caused intestinal mucosal barriers and caused the body to experience an unbalanced oxidant/antioxidant status in *Salmonella*-challenged broilers, compared with the CON treatment group. These results are in agreement to previous findings that suggested increased concentrations of MDA detected in bacteria-infected chickens (Wu *et al.*, 2018).

However, the administration of Gln to infected animals can eliminate ROS, such as superoxide dismutase, hydrogen peroxide, and hydroxyl radicals, to reduce oxidant lesions, which may be associated with the reactive oxygen-derived free radical ability (Chen *et al.*, 2009; Hu *et al.*, 2014). Dietary supplementation with Gln decreased MDA concentrations and increased the SOD and CAT activities in *S. Enteritidis*-infected broilers in the present study. The current results are consistent with the published reports (Brasse-Lagnel *et al.*, 2009; Liu *et al.,* 2015), which revealed that the Gln attenuated oxidative damage to the intestines and body. Also, the detection of molecular stress biomarkers indicated that the administration of Gln mediated the expression of genes associated with antioxidant activity (CAT, SOD, and Nrf2). These results suggest that dietary Gln scavenges free radicals and increases antioxidation, likely through the mediation of reactions that reduce oxidized protein levels and normalize index enzyme activities, which may consequently attenuate renal oxidative damage in *Enteritidis*-infected broilers, in the intestinal mucosa and sera of ovens (Fathi *et al.*, 2014).

Moreover, Gln is a beneficial precursor for glutathione production in the plasma and intestinal mucosa (Cheng *et al.*, 2011). Therefore, the upregulation of the intestinal antioxidant genes expression levels can also be explained by the use of Gln on the antioxidant enzymes activation of the intestinal mucosa. However, further studies will be needed to clarify the detailed antioxidant mechanism of Gln using genetic manipulation technologies.

CONCLUSION

Overall, our results indicated that the administration of Gln generally improved the physiological status, growth performance, and the activity of digestive enzyme in broiler chicks. It promoted the antioxidative stability and stress resistance capacities of infected broilers by modulating both actions and the mRNA expression of antioxidant enzymes and decreased MDA, which protect the intestinal mucosa from oxidative damage. It is evident that positively enhanced digestive enzymes and antioxidant activities partly contributed to the beneficial effect of dietary Gln on the average body weight of broilers.

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