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Effect of glutamine on the intestinal function and health of broilers challenged with *Salmonella pullorum*

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ABSTRACT

The influence of glutamine (Gln) on the intestinal function and health in broilers challenged with *Salmonella pullorum* was investigated. 240 one -day-old Arbor Acres broilers were divided into four groups in a completely randomized design, each of which included 6 replicates with 10 birds per replicate, for 21 days. The experimental groups were as follows: control group (CON), *S. pullorum* challenged control group (SCC), basal diet plus *S. pullorum* challenged plus 0.5 % Gln (Gln 1) or1.0 % Gln (Gln 2), The results showed that *S. pullorum* had significantly adverse effect on ADG, ADFI and feed to gain ratio (F: G) of broilers compared with the values measured for the CON during days 5 to 7. Moreover, compared with the characteristics of CON, *S. pullorum* showed significantly effects on the relative weight and length of small intestine at 7 d, the activities of sucrose, maltase and lactase in the jejunum mucosa at 4 d, or 7 d, the counts of *Salmonella* and *Lactobacillus* at 4, 7 and 21d and the molar proportion of isobutyric acid at 14 d. The inclusion of Gln significantly elevated the relative weight and length of small intestine, increased intestinal sucrose, maltase and lactase activities, decreased caecal *Salmonella* population and molar proportion of isobutyric acid at 14 d. These results suggested that Gln might exert a favorable effect on intestinal function and health in broilers.

Key words: Broiler, Glutamine, Gut health, Intestine function, *Salmonella pullorum*.

INTRODUCTION

Normal intestinal structure and function are very important, because structural and function abnormalities of the intestinal tract may play a role in preventing illnesses that negatively impact athletic performance. Increased counts of some harmful pathogens, such as *Salmonella pullorum*, may directly affect gut health (*i.e*. intestinal physiology, microbiology), reduce nutrient absorption and bird growth and livability (Sapre *et al*. 1970). It is well established that infection by *Salmonella* is *via* the intestinal mucosa epithelium, might be related to affect the growth of normal intestinal flora and produce some toxins to monitor changes in intestinal function and structure (Wu *et al.* 2018). Thus, it is necessary to prevent intestinal infection by *Salmonellas* to improve the health status and performance of the chickens.

Glutamine (Gln) is the most abundant amion acid and the principal cellular substrate and respiratory fuel of the enterocyte (Horvath *et al*. 1996). Accumulating evidence had shown that its supplementation to diets improve intestinal function and health in humans and other animals (Wu *et al*. 2013). Similarly, some studies have also shown that Gln can improve growth performance, rescue the dysfunction of gut barrier function and modulate the immunity response in endotoxin-challenged animals (Jiang *et al*. 2009). However, it is unclear whether Gln could regulate the intestinal microbiota and the concentration of volatile fatty acids (VFA) in a *S. pullorum* infection model. Therefore it is worth assessing the effects of Gln on the intestinal function and health of broilers via a *S. pullorum* model feeding trial, with the purpose of exploiting Gln as a promising functional feed ingredient.

MATERIALS AND METHODS

Basal experimental diet (corn-soybean-meal, mash; Table 1) were prepared as per the recommendation of National Research Council (NRC 1994). Gln was purchased from Henan Honda Biological Medicine Co., Ltd., China (99% purity, pharmaceutical grade), added and thoroughly mixed into the basal feed.

Arbor Acres broilers (1 day old) were obtained from a hatchery in Luoyang. They were randomly assigned into four groups, each with six replicates, 10 chickens per replicate, for 21 d. The 4 experimental treatments were as follows: 1) unchallenged group (CON); 2) *S. pullorum* -challenged group (SCC), 3) *S. pullorum* -challenged group receiving dietary supplementation of 0.5 % Gln (Gln1) and 1.0 % Gln (Gln2), respectively. All birds were housed in the cages in an environmentally controlled room; the ambient temperature and light were provided according to the normal feeding management procedure of broilers. All procedures

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Table 1: Ingredients and nutrient level in 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 (%)	
AME (MJ/kg)	12.55
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.

and experiments were performed in accordance with the Institutional Animal Care and Use Committee of Henan University of Science and Technology.

The strain of *S. pullorum* was obtained from the China Veterinary Culture Collection Center (Beijing, China), then cultured anaerobically on Brilliant Green Agar at 37°C for 24 h, washed and then diluted to 2.0×10^4 CFU/ mL in sterile normal saline. Broilers in the SCC, Gln 1 and Gln 2 were orally gavaged (1.0 mL per bird) on d 3 posthatch, while the non-challenged birds were gavaged with the same volume of physiological saline.

One, 4, 11 and 18 days after *S. pullorum* infection, one bird per replicate was randomly selected after fasting for 12 h and sent to the Veterinary Laboratory. Broilers were humanly euthanized, 1 g digesta were aseptically collected from each bird and stored at -70°C for assessing *Salmonella* and *Lactobacillus* colonization of the cecum. The sediment cecal contents were frozen in liquid N until VFA analysis. Subsequently, the duodenum, jejunum and ileum samples were collected, emptied by gentle pressure and each segments length and weight were measured. Additionally, the proximal jejunal mucosa was excised carefully and kept for the disaccharidases activity.

Body weight was measured at 1, 7, 14 and 21 d of age. The amounts of feeds and feed residues were also weighed to determine the average daily feed intake (ADFI) and feed/gain ratio (F/G). Mortalities and health status were visually observed and recorded daily throughout the experiment.

The activities of sucrose, maltase and lactase in the jejunum mucosa supernatant were determined using a corresponding diagnostic kit (Nanjing Jiancheng Bioengineering Institute, China), according to the instructions of the manufacturer. Approximately 1 g of mixed cecal content was diluted and homogenized. The population of *Lactobacilli* was cultured on MRS agar (pH 5.4, Huankai Microbial SCI. and Tech, Co., Ltd. Guangdong, China) at 37°C with CO_2 for 48 h. The population of *Salmonella* was determined on bismuth sulfite agar (Qingdao Hope Bio-Technology Co. Ltd., Qingdao, P. R. China) incubated at 37°C for 24 h. The number of colony forming units (CFU) was expressed as a logarithmic (log10) values per gram of intestinal digesta. The VFA profile was determined by a Varian 3400 gas chromatograph (Varian Associates Inc., Walnut Creek, USA).

Statistical analysis of all data was carried out using SPSS version 22.0. Data for all of the treatment groups were compared using the Turkey's range test. All data were expressed as the mean ± standard error of mean (SEM at levels of significance $P < 0.05$).

RESULTS AND DISCUSSION

Growth performance: Infection with *S. pullorum* caused significantly adverse effects on average daily gain (ADG), ADFI and F: G during days 5 to 7 (Table 2). Birds fed Gln had significantly improved ADG and decreased ADFI and F: G during days 5 to 7 when compared with the SCC. Neither *S. pullorum* infection nor Gln had an effect on ADFI, ADG and F: G during other experimental period.

The relative length and relative weights of small intestine: Broilers challenged by *S. pullorum* had significantly decreased the relative weight and length of duodenum, jejunum and ileum at 7 d compared with the CON group (Table 3). Gln significantly increased the relative weight and length of small intestine, but there were no difference in the relative weight and length of small intestine between the Gln l and Gln 2. *S. pullorum* infection significantly decreased the ileual relative length at 4 d, the relative length of duodenum, jejunum and ileum at 14 d, or 21 d and to reduce the relative weight of ileum at 4 d, or 21 d. Birds fed a diet with Gln had significantly increase in the above - mentioned parameters. However, the relative weight of duodenum and jejunum at 4, 14 d or 21 d did not differ among groups. There were no difference in the relative weight of duodenum, jejunum and ileum at 4 d.

The mucosa disaccharidases activity: The activities of mucosa sucrase, maltase and lactasein SCC were significantly decreased at 4 d, or 7 d compared to CON (Table 4).

Table 2: Effect of dietary Gln supplementation on the growth performance of broilers infected with *S. pullorum.*

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
1 to 4 d				
ADFI[2] (g/bird/d)	20.17±0.12	20.03±0.23	20.54±1.01	20.12±0.81
ADG[2] (g/bird/d)	15.40±0.14	14.84±0.14	15.33±0.62	15.48±0.51
F:G[2]	1.31±0.03	1.35±0.09	1.34±0.10	1.30±0.07
5 to 7 d				
ADFI[2] (g/bird/d)	27.48±0.16[a]	28.29±0.13[b]	27.64±0.14[a]	27.24±0.08[a]
ADG[2] (g/bird/d)	19.77±0.14[b]	18.86±0.10[a]	19.33±0.05[b]	19.74±0.13[b]
F:G[2]	1.39±0.01[a]	1.50±0.11[b]	1.43±0.05[a]	1.38±0.09[a]
8 to 14 d				
ADFI[2] (g/bird/d)	31.71±1.29	32.73±0.89	32.33±0.52	30.20±0.91
ADG[2] (g/bird/d)	21.57±0.64	21.39±0.80	21.22±0.77	21.57±0.74
F:G[2]	1.47±0.09	1.53±0.06	1.50±0.08	1.45±0.08
15 to 21 d				
ADFI[2] (g/bird/d)	40.65±0.72	38.50±0.48	44.54±0.81	43.52±0.27
ADG[2] (g/bird/d)	24.64±0.58	21.88±0.76	26.20±0.47	25.90±0.61
F:G[2]	1.65±0.08	1.76±0.06	1.70±0.09	1.68±0.03

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

[2]ADG=average daily gain; ADFI = average daily feed intake; F/G = average daily feed intake/ average daily gain.

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

Moreover, Gln significantly increased the activities of mucosa sucrase, maltase and lactase compared with the SCC, however, there were no differences in the activities of sucrase, maltase and lactase between the Gln l and Gln 2 at 4 d, or 7 d. Compared with the CON, the activities of mucosa sucrase and maltase significantly decreased in the SCC group at 14 d, or 21 d. Gln significantly increased the activities of sucrase and maltase, but there were no difference in the activities of sucrase and maltase between the Gln l and Gln 2 at 14 d, or 21 d. Moreover, the mucosa lactase activity at 14 d or 21 d did not differ among groups.

Microbial population: *S. pullorum* infection significantly reduced the population of *Lactobacillus* and promoted the growth of *Salmonella* colonies compared with the control during the experimental period (Table 5). However, Gln significantly increased the counts of *Lactobacillus* (except day 21) and reduced the *Salmonella* population compared with the SCC, but there were no difference in the counts of *Lactobacillus* and *Salmonella* between the Gln l and Gln 2.

VFAs: Neither *S. pullorum* infection nor Gln supplementation had an effect on the molar proportion of acetic acid, butyric acid, propionic acid and isobutyric acid (except day 14) during the experimental period (Table 6). Compared with the CON, the molar proportion of isobutyric acid at 14 d was significantly increased in the SCC. The incorporation of Gln significantly increased the molar proportion of isobutyric acid at 14 d and there were no differences in proportions of isobutyric acid between the Gln l and Gln 2.

Salmonella infections leads to a negative effect on production performance, intestinal flora colonization and gut health in broilers, which was confirmed in this study. In the present study, *Salmonella* significantly infection decreased the production performance of the chicks at days 5 to 7 and had no effect on the production performance at other experimental period, but there were some apparent clinical symptoms, such as reluctance to move, head dropping, wing prolapse, lethargy, lack of appetite, diarrhea and decreased water intake appeared at days 8 to 14 or 15 to 21. These results were in agreement with previous studies showing that broilers were extremely sensitive to *S. pullorum* infections in the first 7 d of their life (Sapre *et al*. 1970), who noted that *S. pullorum* infection can cause intestinal flora disorder and reduction in production performance in young birds. The experimental results on chicken growth performance in this study were slightly different from the findings of others (Bohez *et al.* 2008). These differences between findings may be due to chick age at challenge, strain variations, challenge dosages, animal management and environmental conditions (Singh *et al*. 2018). However, Gln supplementation significantly improved ADG, ADFI and F: G and reduced the number of *S. pullorum* in the challenged chickens at days 5 to 7 and the above mentioned clinical symptoms vanished at days 8 to 14 or 15 to 21, indicating that Gln might exert a protective role in controlling *Salmonella* infection, which was similar to a previous study (Xue *et al*. 2018). These studies indicated that Gln may improve the growth performance of broilers under stress conditions, which could be attributed to the beneficial effect of Gln on the digestion of nutrients and development of the small intestine (Uni *et al*. 1999).

Moreover it was observed that the relative length and weight of the small intestine were affected after the *Salmonella* infection, they are consistent with the studies of Wu *et al*. (2018), which might be attributed to *Salmonella* infection alteration of digestive, absorptive functions and hyperplasia of the mucosa. Because the small intestine is the target organs for the stress, bacteria, toxins and endotoxins disrupt normal intestine cell function by inhibiting cell division. In the present study, Gln supplementation prevented many of the adverse effects on the development of the small intestine caused by *S. pullorum*. It may be explained that Gln provides sufficient energy for intestinal cell proliferation, differentiation and protein synthesis (Giancamillo *et al*. 2003). On the other hand, these results could be attributed to increasing the digestibility of feeds, the passage rate of material from the gastrointestinal tract. Additionally, Gln was able to relieve the hyperplasia, irritate the digestive tract and increase different parts of the muscle layer of the submucosa (Rosa *et al*. 2015). These results might explain the improvement in the performance and intestine development of broilers supplemented with Gln.

The activities of the small intestinal mucosal disaccharidases such as maltase, lactase and sucrase have been related to the degree of mucosal injury and intraluminal

Table 3: Effects of dietary Gln supplementation on the relative length (cm/kg) and relative weights (g/kg) of small intestine of broilers infected with *S. pullorum*.

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
4 d				
Relative length				
Duodenum	191.33±9.74	176.25±12.06	184.32±9.55	194.67±11.39
Jejunum	502.47±51.85	470.63±25.49	490.21±8.51	517.80±35.46
Ileum	221.70±23.22	207.07±32.22	214.48±15.01	238.35±16.43
Relative weights				
Duodenum	9.10±0.51	8.70±0.99	8.87±1.35	10.65±1.21
Jejunum	21.16±2.95	20.18±2.24	20.44±2.12	22.49±1.20
Ileum	9.50±0.90^{b}	7.06±1.03^{a}	9.38±1.39^{b}	9.51±0.87^{b}
7 d				
Relative length				
Duodenum	204.10±2.77^{b}	180.79±8.90^{a}	196.09±3.92^{b}	210.45±4.84^{b}
Jejunum	480.34±25.57^{b}	454.27±20.95^{a}	472.09±9.02^{b}	512.15±2.38^{b}
Ileum	236.08±10.89^{b}	186.07±20.01^{a}	233.75±16.38^{b}	245.18±15.63^{b}
Relative weights				
Duodenum	11.27±1.83^{b}	8.11±1.30^{a}	10.65±1.62^{b}	11.82±2.02^{b}
Jejunum	27.71±3.63^{b}	21.59±1.14^{a}	25.13±0.63^{ab}	32.37±1.79^{a}
Ileum	17.89±0.98^{b}	12.20±0.80^{a}	17.45±2.18^{ab}	19.06±0.58^{a}
14 d				
Relative length				
Duodenum	210.62±2.32^{b}	189.67±3.64^{a}	200.94±2.35^{b}	218.38±4.78^{b}
Jejunum	469.85±22.26^{b}	449.41±11.15^{a}	470.60±20.08^{ab}	490.68±12.06^{a}
Ileum	236.45±0.30^{b}	221.03±0.33^{a}	235.20±1.05^{a}	261.28±0.07^{a}
Relative weights				
Duodenum	13.56±0.56	11.29±1.49	13.49±1.22	15.88±0.03
Jejunum	31.43±1.10	28.85±3.83	30.02±1.07	33.31±0.02
Ileum	19.87±0.14^{b}	16.07±3.25^{a}	18.36±0.60^{b}	20.61±0.01^{b}
21 d				
Relative length				
Duodenum	223.79±4.25^{b}	187.15±0.45^{a}	213.88±1.65^{b}	230.96±3.24^{b}
Jejunum	470.26±15.23^{b}	440.12±1.15^{a}	472.69±1.12^{b}	483.45±2.04^{b}
Ileum	250.45±0.44^{b}	225.34±0.32^{a}	268.94±1.02^{b}	268.25±0.08^{b}
Relative weights				
Duodenum	15.25±0.52	13.14±0.09	15.19±1.15	16.65±0.07
Jejunum	33.10±0.01	30.25±2.21	32.98±1.03	35.26±0.01
Ileum	22.86±0.16^{b}	18.98±2.10^{a}	22.99±0.12^{b}	24.36±0.02^{b}

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

loss of mucosal cellular material and could reflect the functional integrity of the intestinal mucosa. As shown in Table 4, *S. pullorum* infection model of the broilers results in a marked stimulation of intestinal mucosa disaccharidase levels. These results were consistent with the finding that mucosal disaccharidases activity was present in human after *Eimeria necatric*, which could be due to the change in the small intestine epithelium. Moreover, this is partly related to ontogenic decline in brush-border disaccharidases activity. In the present study, we examined the effect of Gln on the disaccharidase levels in broiler challenged with *S.pullorum*. These results showed that intestinal disaccharidase activities are higher in the Gln group (except for jejunal lactase activity at 14 d or 21 d), indicating that Gln could provide a protective effect to the intestinal mucosa. It is possible that Gln may regulate its transfer across the basolateral-membrane vesicles into the circulation and deposition in the small intestine. Furthermore, Gln could be beneficial to the intestinal mucosa through its metallo-regulatory functions, stimulation of mucosal cellular growth and differentiation and trophic effect.

A complex intestinal microbial communities and its activity are believed to maintain the physiological, immunological, protective functions of the intestinal tract and improve gut health, as well as provide some benefits to their host. In previous studies, *Salmonella* caused a high level

Table 4: Effect of dietary Gln supplementation on the jejunum mucosa disaccharidases (U/g) of broilers infected with *S. pullorum.*

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
4 d				
Sucrase	76.09±4.20[b]	54.36±6.49[a]	70.89±7.20[b]	74.36±5.49[b]
Maltase	258.27±30.71[b]	176.09±11.20[a]	244.36±21.49[b]	260.86±21.00[b]
Lactase	9.65±0.76[b]	3.47±0.23[a]	8.81±0.82[b]	9.48±0.71[b]
7 d				
Sucrase	132.81±11.37[a]	99.25±12.09 [a]	99.25±12.09 [a]	88.37±10.73[a]
Maltase	285.27±19.43[a]	200.17±9.07[b]	280.23±16.17[a]	293.46±10.70[a]
Lactase	10.93±1.18[b]	6.38±1.04[a]	10.79±0.99[b]	10.88±1.21[b]
14 d				
Sucrase	155.28±30.00[b]	76.09±11.20[a]	149.42±13.67[b]	188.18±10.73[b]
Maltase	318.92±26.42[b]	217.35±9.78[a]	300.21±16.35 [b]	315.20±16.91[b]
Lactase	11.01±1.12	9.65±0.76	10.93±1.18	11.04±1.57
21 d				
Sucrase	185.21±32.56[b]	92.35±21.03[a]	169.46±33.67[b]	198.67±20.89[b]
Maltase	447.85±28.64[b]	330.86±24.74 [a]	428.92±30.11[b]	455.20±15.20[b]
Lactase	14.65±3.76	12.53±1.12	13.94±1.18	14.87±1.53

[1]CON = noninfect control group, SCC = *S. pullorum* infect control group received the basal diet, Gln1= *S. pullorum* infect control group received the basal diet plus 0.5 % Gln; Gln2= *S. pullorum* 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 supplementation on the cecal microbiota (log CFU) of broilers infected with *S. pullorum.*

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
4 d				
*L*actobacillus	6.53±0.11[b]	5.23±0.19[a]	6.29±0.14[b]	6.47±0.10[b]
Salmonella	6.80±0.13[a]	8.86±0.17[c]	7.58±0.21[b]	7.21±0.27[ab]
7 d				
*L*actobacillus	7.11±0.12[c]	5.95±0.20[a]	6.60±0.08[b]	6.92±0.15[bc]
Salmonella	7.18±0.21[a]	8.35±0.19[b]	7.46±0.17[a]	7.23±0.25[a]
14 d				
*L*actobacillus	7.38±0.13[b]	6.42±0.17[a]	7.19±0.23[b]	7.43±0.14[b]
Salmonella	5.61±0.32[a]	7.43±0.29[c]	6.57±0.10[b]	5.75±0.27[a]
21 d				
*L*actobacillus	6.87±0.19	6.77±0.10	6.80±0.20	7.07±0.15
Salmonella	6.70±0.21[a]	7.89±0.10[b]	7.00±0.25[a]	6.79±0.17[a]

[1]CON = noninfect control group, SCC = *S. pullorum* infect control group received the basal diet, Gln1= *S. pullorum* infect control group received the basal diet plus 0.5 % Gln; Gln2= *S. pullorum* 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 6: Effect of dietary Gln supplementation on the volatile fatty acids (mmol/L) in caecum of broilers infected with *S. pullorum*

Items	Diet Treatments[1]			
	CON	SCC	Gln1	Gln2
4 d				
Acetic acid	2.41±0.12	2.20±0.17	2.55±0.19	2.43±0.22
Propionic acid	0.65±0.10	0.58±0.12	0.70±0.13	0.64±0.19
Butyric acid	1.52±0.22	1.39±0.24	1.45±0.11	1.58±0.27
Isobutyric acid	0.059±0.010	0.067±0.024	0.061±0.014	0.052±0.020
7 d				
Acetic acid	2.25±0.27	2.10±0.11	2.23±0.20	2.30±0.42
Propionic acid	0.52±0.14	0.45±0.17	0.50±0.06	0.54±0.21
Butyric acid	1.26±0.32	0.98±0.20	1.20±0.17	1.28±0.22
Isobutyric acid	0.045±0.011	0.053±0.020	0.049±0.013	0.042±0.025
14 d				
Acetic acid	1.95±0.30	1.62±0.17	1.79±0.22	1.93±0.19
Propionic acid	0.41±0.11	0.31±0.06	0.39±0.20	0.48±0.21
Butyric acid	1.03±0.20	0.88±0.12	0.95±0.19	1.06±0.21
Isobutyric acid	0.028±0.004[a]	0.043±0.007[b]	0.032±0.005[a]	0.030±0.008[a]
21 d				
Acetic acid	1.79±0.24	1.57±0.13	1.68±0.15	1.80±0.18
Propionic acid	0.38±0.10	0.30±0.13	0.38±0.18	0.43±0.11
Butyric acid	0.95±0.14	0.82±0.17	0.93±0.23	0.98±0.20
Isobutyric acid	0.020±0.001	0.028±0.005	0.025±0.003	0.022±0.004

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

of infection in the caecum and decreased the number of *Lactobacillus* colonies (Wang *et al.* 2012). In this study, the *Salmonella* count in SCC was higher than that of uninfected groups, whereas *Salmonella* inhibited the growth of *Lactobacillus*. These changes may contribute to the processes of mucosal injury. These results suggest that *S. pullorum* may influence the gut microbial community composition, or damage to the intestinal mucosal barrier. However, Gln supplementation increased the *Lactobacillus* number and decreased the *Salmonella* number, implying that Gln promoted the restoration of the normal gut microbial community of the *S. pullorum*-infected chicks, prevent the colonization of pathogens and alleviate intestinal mucosal barrier injury, which is in line with the improved intestinal function development (Xu *et al.* 2014). These results indicate that Gln can counteract *Salmonella* infection, promoting the growth of *Lactobacilli* to levels comparable to those in healthy chickens. The mechanism of these beneficial effects of Gln on the microbial community needs further study.

VFAs is produced in the colon and plays a key role in the development of broilers cecalmicroflora during growth, which is associated with the protection against mucosal oxidative stress, strengthening of the colonic defense barrier, anti-inflammatory properties and gut health. In our study, cecal concentrations of acetic acid, butyric acid and propionic acid in the SCC chicks were numerically, but not statistically: lower than the CON. However, compared with the CON, the molar proportion of isobutyric acid at 14 d was increased in the SCC. These results showed that the small intestine of broilers increased susceptibility to *Salmonellae's* cecal colonization, which has been attributed to insufficient concentration of cecal VFAs to prevent colonization. Gln increased slightly the concentrations of acetic acid, butyric acid and propionic acid, indicate that cecal concentrations of VFA can be affected by dietary constituents, but these alterations do not appear to be severe enough to affect *Salmonella* colonization. Unfortunately, sufficient information is not available on the effects of Gln on intestinal microflora and VFAs, thus further studies will be needed.

CONCLUSION

Present study concluded that Gln regulates *Salmonella* infected microglia activation, improve the growth performance of broiler, increased the relative weight and length of small intestine, also modulates the mucosal disaccharidases activity and cecal VFAs concentration.

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