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Dietary Supplementation of Glutamine Improves Metabolic Functions in 1-14 Days Old Broilers Under Cold Stress

Haoneng Guo[1,2], Shuangshuang Xiao[3], Wenfang Lou[1,2], Rifat Ullah Khan[4], Juanjuan Wu[1,2], Bo Huang[2], Sifa Dai[2,3]* and Guanhong Li[1]*

[1]*College of Animal Science and Technology, Jiangxi Agriculture University, Nanchang 330045, PR China*
[2]*Department of Pharmaceutical and Life Sciences, Jiujiang University, Jiujiang 332005, PR China*
[3]*College of Animal Science, Anhui Science and Technology University, Fengyang 233100, PR China*
[4]*College of Veterinary Sciences, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar 25000, Pakistan*

Haoneng Guo and Shuangshuang Xiao contributed equally to this work.

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Glutamine, Chicks, Cold stress, Serum biochemical indices, Serum ions

ABSTRACT

The purpose of this experiment is to study the effect of glutamine (Gln) on the metabolic functions of cold stress chicks (1-14 days old) from the perspective of serum biochemical indicators. A total of 192 healthy 1-day-old broilers with no significant difference in weight, were randomly divided into four groups, each group with four replicates and 12 broilers per replicate. Group I was a control group, the broilers were fed the basal diet, and the broilers in Group II, III, IV were fed a basal diet supplemented with 1.0% and 1.5%, respectively 2.0% Gln. The broilers were kept at 25±2 °C for 14 days. Samples were taken and analyzed at 7 and 14 days of age. On the 7th day, compared with group I, the serum level of LDH in group II, III and IV were significantly decreased. Serum triiodothyronine content in group II, III, and IV was significantly increased. Also, compared with the control, the glucose level was significantly decreased in group II, and the serum uric acid content in II, III, and IV was significantly decreased. On the 14th day, compared with group I, the K^+ in the group III and IV decreased significantly, and the P^{3+} in the group II and IV decreased significantly. The LDH level in group IV was significantly reduced, and the AST level in group II was significantly reduced. The albumin and globulin levels in group IV were decreased significantly. Under this experimental condition, adding Gln to the diet has a certain improvement effects on serum biochemical indices of cold stressed chicks.

INTRODUCTION

In the winter, in most parts of China, the outdoor temperature may be below 0 °C. In general, cold stress can occur when the temperature is below the optimal living standard for chicks. When chicks suffer from cold stress, the body needs to consume a lot of energy to maintain body temperature to resist the external low-temperature environment, only relatively little energy is used for production and maintenance (Zhang *et al*., 2016), resulting in reduced growth performance (Hu *et al*., 2021) and even death. At the same time, cold stress usually causes physiological and biochemical changes in poultry blood (Hangalapura *et al*., 2006; Wang and Xu, 2008), so it is of great significance for broiler breeding to solve or relieve cold stress of chicks. Cold stress reduces the incubation efficiency of laying hens, and the weight of chicks hatched at normal temperature was significantly higher than under cold stress (Kamanli *et al*., 2015). Wang and Xu (2008) showed that the weight gain of pheasants in the cold stress group decreased significantly and feed efficiency increased significantly within one week.

Glutamine (Gln) is added to the chickens diet resulted

* Corresponding author: daisifa-004@163.com, liguanh@163.com
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in improved body weight and feed conversion ratio (Zulkifli *et al*., 2016). Other studies have shown that the addition of Gln in the diet of broilers led to a significant increase in body weight and immune organ weight (Szabó *et al*., 2014). Yi *et al*. (2005) also demonstrated a promoting effect of Gln on the growth performance of broilers at a later stage. Studies have shown that Gln can alleviate the effects of heat stress on the growth performance of broilers and improve their serum physiological and biochemical indices (Dai *et al*., 2012; Hu *et al*., 2016). Previous studies have shown that different content of Gln can significantly improve the performance and immune organ index of 1-14 days old chicks (Xiao *et al*., 2019). However, there are few reports on the effects of Gln on serum biochemical indices of cold stress chicks. The aim of the present study was to evaluate the effect of Gln on the serum biochemical parameters in the first two weeks of the broiler chickens under cold stress.

MATERIALS AND METHODS

Test materials

L-glutamine (L-Gln) was purchased from Amresco, USA having purity > 99.0 %. Automatic Biochemical Analyzer and centrifuge were purchased from HATTIECS, Federal Republic of Germany.

Animals husbandry and experimental design

A total of 192 healthy, 1-day old Arbor Acres (AA) broilers were randomly divided into four groups (four replicates in each group and 12 chicks in each replicate), with group I as the control group fed with Gln-free basic diet, and groups II, III and IV fed with 1.0, 1.5 and 2.0 Gln, respectively. The temperature and humidity of the chicken coops were controlled by heaters and humidifiers, and the temperature was maintained at 25±2 °C and the humidity was maintained at 60% to 70% for 14 days.

Feeding management and diet formulation

Three days before the start of the experiment, the chicken house was cleaned, fumigated and disinfected. Broilers were fed and watered freely. Immunization was carried out according to broiler husbandry regulations. Daily observation of feed consumption, faeces composition and health condition of the chickens was carried out. The basic diet was corn-soybean meal, which was designed according to the recommendation of National Research Council (1994) (Table I).

Sampling and measurement of indicators

At 7 and 14 days of the experiment, two healthy chicks were randomly selected from each replicate after a 12 h fast, and then slaughtered and sampled. Blood was collected from the carotid artery of the broiler and centrifuged in a 10 ml tube at 3000 r/min for 10 min, then the serum was divided into 0.5 ml tubes and stored at -20°C.

Table I. Ingredients and calculated component (g/kg) of basic diet for chicks (1-14 days old).

	Daily diet
Ingredients	
Corn	600.00
Soybean meal	330.00
Fish meal	20.00
Vit & min permix[1]	50.00
Calculated component	
AME (MJ/kg)	115.50
Crude protein	215.70
Dig. Lys	11.40
Dig. Met	3.50
Calcuim	9.60
Total P	5.70

[1]supplied per kilogram of diet: Vitamin A, 46.29 mg; Vitamin B_1, 30 mg; Vitamin B_2, 160 mg, Vitamin B_6, 50 mg; Vitamin B_{12}, 0.22 mg; Vitamin D, 0.625 mg; Vitamin E, 1.76 mg; Vitamin K, 44 mg; folic acid, 18 mg; niacin, 880 mg; biotin, 2.2 mg; choline, 11 000 mg; zinc, 1 600 mg; iron, 1 600 mg; manganese, 1 600 mg; cuprum, 160 mg; iodine, 7 mg; selenium, 3 mg.

Hitachi 7600 Automatic Biochemical Analyzer was used to measure serum biochemical indicators, namely potassium ion (K^+), sodium ion (Na^+), chloride ion (Cl^-), calcium ion (Ca^{2+}), phosphorus ion (P^{3+}), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), triiodothyronine (T3), thyroxine (T4), albumin (ALB), globulin (GLB), glucose (GLU), triglyceride (TG), and uric acid (UA) with the help of commercial assay kits (Nanjing Jiancheng Biotechnology Research Institute, Nanjing, China).

Statistical analysis of data

Data were analyzed under one-way analysis of variance (ANOVA) using SPSS 18.0. Means were separated by using Tukey test. The results were expressed as means±SEM, and $P<0.05$ was considered as point of significance.

RESULTS

Effect of glutamine on serum antioxidant ability of cold-stressed chicks

The effect of Gln on serum ions of cold stressed broilers on day 7 and 14 are given in Table II. No significance ($P>0.05$) difference was found in serum K^+, Na^+, Cl^-, and P^3 during the first 7 days of the experiment. Serum Ca^{2+} was significantly ($P<0.05$) higher in group IV compared to the control. On day 14, serum K^+ and P^{3+} were significantly ($P<0.05$) lower in group IV compared to the control. The rest of the ions did not change significantly ($P>0.05$) between the control and the treatments.

Table II. Effects of glutamine on serum ions content (mmol/L) of cold-stressed chicks.

	Control	Group II	Group III	Group IV
After 7 d				
K^+	6.41±0.28	6.42±0.25	6.28±0.18	6.37±0.30
Na^+	139.45±2.97	136.48±1.12	138.40±2.01	134.80±2.69
Cl^-	108.88±2.41	107.85±1.04	108.43±2.06	104.50±1.95
Ca^{2+}	$1.99±0.08^b$	$2.06±0.08^{ab}$	$2.17±0.03^{ab}$	$2.20±0.06^a$
P^{3+}	2.34±0.13	2.35±0.19	2.39±0.07	2.29±0.05
After 14 d				
K^+	$5.72±0.34^a$	$5.23±0.05^{ab}$	$4.99±0.17^{bc}$	$4.52±0.31^c$
Na^+	144.58±2.02	141.00±4.57	138.50±1.66	135.48±3.13
Cl^-	111.58±1.93	109.25±3.74	107.63±2.26	104.83±3.44
Ca^{2+}	2.22±0.04	2.12±0.04	2.20±0.04	2.09±0.05
P^{3+}	$2.82±0.12^a$	$2.36±0.18^b$	$2.49±0.09^{ab}$	$2.32±0.07^b$

$^{a-b}$ means with different superscripts within each row differ significantly ($P<0.05$). Control fed a basal diet. Group II fed a basal diet supplemented with 1.0% Gln. Group III fed a basal diet supplemented with 1.5% Gln. Group IV fed a basal diet supplemented with 2.0% Gln. Each value represents mean of four replicate pens of two chicks each (n=8).

Effect of glutamine on serum enzymes activities

The effect of Gln on serum ALT, AST, LDH, and CK on day 7 and 14 are given in Table III. Serum LDH content decreased significantly ($P<0.05$) in the treatment groups compared to the control. No significant changes were recorded in other parameters on day 7.

On day 14, serum AST decreased significantly ($P<0.05$) in group II compared to the control. Similarly, LDH was also reduced significantly ($P<0.05$) in group IV compared to control.

Effect of glutamine on serum hormones contents

Compared with control, serum triiodothyronine level content in group II, III, and IV was significantly increased ($P<0.05$) on the 7th day of the experiment (Table IV).

Effect of glutamine on serum protein content

Table V shows us that on the 14th day, albumin and globulin decreased significantly ($P<0.05$) in group IV compared to the control.

Table III. Effects of glutamine on serum enzyme activity (U/L) of cold-stressed chicks.

	Control	Group II	Group III	Group IV
After 7 d				
ALT	1.50± 0.29	1.50± 0.29	2.00± 0.41	1.75± 0.25
AST	251.00± 10.21	220.25± 11.54	235.50± 23.93	229.50± 7.14
LDH	1728.25± 127.72^a	1318.50± 144.50^b	1214.00± 42.44^b	1355.25± 58.36^b
CK	2690.50± 284.96	2322.00± 219.87	2068.25± 330.05	2179.25± 49.74
After 14 d				
ALT	3.25± 0.25	2.75± 0.48	2.25± 0.25	2.25± 0.48
AST	277.50± 13.49^a	244.00± 12.23^b	269.67± 4.91^{ab}	249.00± 8.34^{ab}
LDH	1828.25± 163.41^a	1534.25± 154.13^{ab}	1806.50± 146.71^{ab}	1352.00± 149.67^b
CK	2769.75± 208.18	2564.00± 280.18	2816.00± 400.61	2704.33± 429.87

$^{a-b}$ for statistical details and for details of groups see Table II. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase.

Table IV. Effects of glutamine on serum hormone levels (nmol/L) of cold-stressed chicks.

	Control	Group II	Group III	Group IV
After 7 d				
T3	1.40±0.20	2.03±0.36	1.75±0.20	1.72±0.19
T4	16.76±2.90	17.67±2.26	18.26±1.08	17.36±2.15
After 14 d				
T3	1.43±0.35	1.80±0.21	1.67±0.24	1.41±0.25
T4	18.45±1.93	18.09±0.80	19.11±2.26	19.74±3.23

For statistical details and for details of groups see Table II. T3, triiodothyronine; T4, thyroxine.

Table V. Effects of glutamine on serum protein content (g/L) of cold-stressed chicks.

	Control	Group II	Group III	Group IV
After 7 d				
ALB	10.20±0.46	10.20±0.67	11.27±0.53	10.35±0.64
GLB	14.20±0.17	14.27±0.79	15.55±0.40	14.45±0.77
A/G	0.72±0.04	0.72±0.04	0.72±0.03	0.72±0.03
After 14 d				
ALB	$12.37±0.21^a$	$10.55±0.49^{ab}$	$12.00±0.33^a$	$9.90±1.02^b$
GLB	$14.57±0.44^a$	$12.82±0.39^{ab}$	$14.35±0.84^a$	$12.15±0.97^b$
A/G	0.85±0.01	0.82±0.02	0.84±0.04	0.81±0.04

$^{a-b}$ for statistical details and for details of groups see Table II. ALB, albumin; GLB, globulin; A/G, calculate as albumin to globulin.

Table VI. Effects of glutamine on serum GLU, TG, and UA levels (mmol/L) of cold-stressed chicks.

	Control	Group II	Group III	Group IV
After 7 d				
GLU	11.49±0.63[a]	9.48±0.72[b]	10.99±0.54[ab]	9.68±0.45[ab]
TG	0.40±0.06	0.33±0.08	0.33±0.04	0.34±0.01
UA	577.00± 57.37[a]	284.50± 7.24[c]	430.25± 24.52[b]	425.00± 64.24[b]
After 14 d				
GLU	9.72±0.31	10.51±0.80	10.72±0.56	9.58±0.18
TG	0.43±0.04	0.31±0.05	0.32±0.03	0.42±0.12
UA	585.33± 36.67[a]	303.25± 44.05[b]	360.00± 53.06[b]	542.33± 30.99[a]

[a-b] for statistical details and for details of groups see Table II. GLU, glucose; TG, triglyceride; UA, uric acid.

Effect of glutamine on serum glucose (GLU), triglyceride (TG) and uric acid (UA) contents

On the 7th day, compared with control, the GLU level was significantly decreased in group II ($P<0.05$). The serum UA content in group II was significantly ($P<0.05$) lower compared to the control. The concentration of UA decreased significantly ($P<0.05$) in group II and III compared to the control on day 14th of the study (Table VI).

DISCUSSION

Serum biochemical index of animals can reflect the metabolism and health status to a certain extent. Huang *et al.* (2017) study showed that serum Ca^{2+} and P^{3+} levels were significantly reduced after 10 days of age and were raised at 5°C lower than the control for 3 days. Hangalapura *et al.* (2006) also found that cold stress significantly increased serum T_3 and T_4 levels of broilers. Serum AST and ALT activities increased significantly in chickens exposed to chronic cold stress (Zhang *et al.*, 2014). Zhen *et al.* (2011) showed that the levels of serum CK and LDH of 4-months-old female quails were significantly increased after being exposed to cold stress for 4 h on daily basis. Different degrees of cold stress had different effects on the serum biochemical index of chickens.

With the increasing demand for animal protein, broiler farms are also increasing in many countries of the world. In the early stage, broilers are vulnerable to cold stress. Cold stress causes serious problems to chicks, ranging from reduced growth performance to high mortality. Poultry researchers have reported improvement in reducing cold stress through strengthening management improving environmental facilities and supplementation of feed additives (Su *et al.*, 2018). Feed additives such as minerals (Yang *et al.*, 2019; Medeiros-Ventura *et al.*, 2020), probiotics (Huff *et al.*, 2015; Su *et al.*, 2018), and vitamins (Ozkan *et al.*, 2007; Ferreira *et al.*, 2015) to the diet can relieve cold stress in broilers. According to the results of previous studies, adding Gln to the diet of broilers can promote the growth performance and provides resistance to the cold stress (Dai *et al.*, 2012; Hu *et al.*, 2016).

Compared with the white phoenix chicks fed at normal temperature, when the normal feeding temperature is 5°C, the Ca^{2+} and P^{3+} in the serum decreased significantly after feeding for three days. After 15 days of experiment, the Ca^{2+} and P^{3+} in the serum did not change. When the normal feeding temperature is 10°C, three days after feeding, the serum Ca^{2+} content decreases significantly, and the P^{3+} does not change significantly. After 15 days of feeding, the serum Ca^{2+} content does not change significantly, and the P^{3+} content increases significantly (Huang *et al.*, 2017). From the present study, it can be seen that cold stress can alter the broiler serum ions. The results of this study show that adding Gln to the diet of chicks tends to increase the serum Ca^{2+} content and significantly reduce serum K^+, P^{3+} in 14-day-old chicks. The results of this study are similar to those of Zhang *et al.* (2018). Gln can maintain the serum ion balance of stress broilers and alleviate the harm caused by cold stress to broilers.

According to the results of previous studies, from the perspective of serum enzyme activity, cold stress has adverse effects on broilers, which may cause liver damage to broilers. The results of this experiment show that adding 1.0% ~ 2.0% Gln to the diet of chicks can reduce the serum LDH, CK, and ALT activity of chicks. This experiment is similar to the results of the Hu *et al.* (2016). Gln can reduce the serum LDH and CK activity, and relieve the damage caused by stress to the body.

T_3 and T_4 hormones play an important role in the growth and development of the body. They can promote the metabolism of proteins, sugars, and fats, thus promoting the growth and development of the body. Xiao *et al.* (2019) showed that dietary supplementation with different concentrations of Gln significantly increased ADFI and ADG, reduced FCR, and improved immune organ index in chicks of different ages. A certain increase in T_3 and T_4 levels in serum can relieve the damage caused by cold stress to the body. The results of this experiment show that supplementation of 1.0% ~ 2.0% Gln can improve T_3 and T_4 levels in the serum of chicks. The results of this study are similar to those of Dai *et al.* (2012) and Hu *et al.* (2016). Gln can improve T_3 and T_4 levels in the serum of stress broilers and mitigate the adverse effects of stress on broilers.

Supplementation with 1.5% Gln was added to the diet of chicks, and the serum albumin and globulin content of 7 days old chicks increased by 10.49 and 9.51%, respectively. Gln can improve the serum albumin and globulin levels of stressed animals, which may be due to the addition of exogenous Gln, which enhances the synthesis efficiency of proteins in animals (Zhang *et al*., 2015). The results of this study also show that 2.0% Gln is added to the diet of chicks, and the serum albumin and globulin content of 14-days-old chicks are significantly reduced. This may be that the high doses of exogenous Gln are added to provide nutrients, and then the deposition of albumin and globulin in the serum are accelerated into body proteins, and the potential mechanism needs to be further studied.

The increase in serum glucose content is due to the increase in glycogen decomposition in broilers at low temperatures (Zhang *et al*., 2014), which also indicates that animal bodies need to consume more sugar to convert into heat energy in cold environments to resist cold environments (Van *et al*., 2010). Summarizing the results of previous studies, it can be concluded that cold stress can increase the serum glucose, triglyceride, and uric acid content of broilers. The results of this study show that add Gln to the diet, and the serum glucose content of 7-day-old chicks decreased to varying degrees, and the serum glucose content decreased significantly. Adding exogenous Gln to cold stress chicks can provide energy for the body as an energy substance. The serum glucose content of chicks in the Gln group decreased. Add 1.0% ~ 2.0% Gln to the chick's diet, and the serum triglyceride and uric acid content of chicks decreased to varying degrees.

In the chick stage (1 to 14-days old), too low temperature does have negative impact on serum biochemical indicators. Under this experimental condition, supplementation with Gln improves the serum biochemical indexes of cold stress chicks. Among them, adding 1.0 % Gln is the best dosage.

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Ethical approval

The project was approved by the Laboratory Animal Ethics Committee of Jiangxi Agriculture University (Nanchang, Jiangxi, China).

Statement of conflict of interest

The authors have declared no conflict of interest.

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