Pakistan J. Zool., pp 1-8, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20221013091007

Ameliorative Effect of Dietary Glutamine on Antioxidant Capacity of Broiler Chicks During Starter Phase Under Cold Stress

Wenfang Lou[1,2], Wenhao Wu[1], Haoneng Guo[1,2], Shuangshuang Xiao[3], Rifat Ullah Khan[4], Xianhua Zhang[1], Guanhong Li[2] and Sifa Dai[1]*

[1]*Department of Pharmaceutical and Life Sciences, Jiujiang University, Jiujiang, China*
[2]*College of Animal Science and Technology, Jiangxi Agriculture University, Jiangxi Province Key Laboratory of Animal Nutrition, Engineering Research Center of Nutritional Feed Development, Nanchang, China*
[3]*College of Animal Science, Anhui Science and Technology University, Fengyang, China*
[4]*College of Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan*

Wenfang Lou and Wenhao Wu contributed equally to this work.

Article Information
Received 13 October 2022
Revised 22 December 2022
Accepted 19 January 2023
Available online 25 May 2023
(early access)

Authors' Contribution
WL and SX conducted the experiment. HG, RUK edited the paper. WW, XZ sampling and analysis. SD, GL supervision, fund acquisition, editing and writing.

Key words
Glutamine, Cold stress, Chicks, Antioxidant capacity

Online First Article

ABSTRACT

This study investigated the effect of glutamine (Gln) on antioxidant capacity of cold-stressed chicks. A total of 192 1-day-old Arbor Acres (AA) broilers were divided into 4 groups: group I was fed basic diet, groups II, III, and IV were fed with addition of 1.0%, 1.5%, and 2.0% Gln. The house temperature was maintained at 25 ± 2 ℃. Compared with group I, on day 7, the activities of catalase (CAT) and glutathione peroxidase (GSH-PX) in serum and jejunum, GSH-Px in duodenum of group II, the CAT and GSH-Px activities in ileum of group III, the GSH-PX and total antioxidant capacity (T-AOC) activities in jejunum in group IV were increased ($P < 0.05$). The malonic dialdehyde (MDA) content in serum was decreased ($P < 0.05$). On day 14, the jejunum CAT and superoxide dismutase (SOD) activities in group II were increased ($P < 0.05$), and the serum MDA content was reduced ($P < 0.05$). CAT activity of jejunum and ileum, and GSH-PX activity of serum and jejunum were increased in group III ($P < 0.05$). The jejunum GSH-PX activity was increased in group IV ($P < 0.05$). The experiment confirmed that adding Gln to the diet can improve the antioxidant capacity of cold-stressed chicks.

INTRODUCTION

Chicks are not fully feathered and their thermoregulatory mechanisms are not fully developed, so chicks need higher ambient temperatures during the early life. The optimum ambient temperature for chicks aged from 1 to 14 days has been estimated to be 29 ~ 35℃. Temperature below 30 ℃ causes thin carcass, shortening and bleeding of intestinal villi and decrease the immunity (Zhao *et al*., 2013). However, chicks are easily exposed to the stress of low temperature when transferred from incubation chamber to brood chamber. Research shows that, cold stress can destroy the balance of oxidants and antioxidant in broilers, increases free radical levels in the body and results in oxidative stress (Wang *et al*., 2019). Cold stress reduces body immunity, increases morbidity and mortality in broilers (Yang *et al*., 2016; Tsiouris *et al*., 2015). Cold stress can also significantly increase pH value and *Clostridium perfringens* count of cecal contents of broilers (Tsiouris *et al*., 2015). In addition, cold stress can significantly reduce the performance of broilers and cause changes in intestinal immune function (Zhou *et al*., 2021; Zhao *et al*., 2013). As a result, the production performance and product quality are deteriorated in broilers (Zhang *et al*., 2014; Hao *et al*., 2015; Nguyen *et al*., 2015). Intestinal extrinsic nutrients are very important to cold-stressed chicks (Nguyen *et al*., 2015). Therefore, it is of great significance to study the protective effect of nutritional additives on cold-stressed chicks.

Glutamine (Gln), a conditional essential amino acid,

* Corresponding author: daisifa-004@163.com
0030-9923/2023/0001-0001 $ 9.00/0

Copyright 2023 by the authors. Licensee Zoological Society of Pakistan.
This article is an open access ∂ article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

is the most abundant amino acid in broiler skeletal muscles (Hu *et al*., 2020). In addition to its role as a building block of proteins and its importance in the conversion of amino acids to ammonia, glutamine has many non-nutritional functions (Li *et al*., 2019) and is thought to be necessary during stress or clinical situations (Stehle and Kuhn, 2015; Coqueiro *et al*., 2019; Oh *et al*., 2020). Glutamine is the main substrate used by intestinal cells, and the demand for Gln is significantly increased when stress, trauma, infection or disease occurs in body tissues (Wischmeyer, 2007). Previously, it was reported that dietary supplementation of 1.0 to 1.5% Gln could significantly increase the villus height of small intestine of broilers, and 0.5-1.0% glutamine can significantly improve the growth performance of broilers (Hassan and Amir, 2013; Wu *et al*., 2021). However, dietary supplementation of 10 g/kg Gln can promote intestinal development and improve growth performance of broilers with necrotic enteritis (Xue *et al*., 2018). Supplementation of 0.5 to 1.0% Gln in cold stressed broilers can promote the development of small intestine, significantly increase the daily gain and improve feed conversion ratio of broilers at 7 to 42 days of age (Abdulkarimi *et al*., 2019). Studies have shown that glutamine can improve the performance of broilers under heat stress, improve the oxidative stress induced by heat stress, and promote the performance of chicks under cold stress (Dai *et al*., 2018; Hu *et al*., 2020; Liu *et al*., 2021; Xiao *et al*., 2019). It also suggests that Gln plays an important role in broiler production. Our previous study confirmed that Gln can improve the performance of cold-stressed broilers (1-14 d), but there are no reports on the changes of antioxidant performance in different parts of the body (Xiao *et al*., 2019). Therefore, the purpose of this study was to investigate the effects of Gln on serum, liver and small intestine antioxidant capacity of cold-stressed chicks.

MATERIALS AND METHODS

Test material

L-Glutamine was purchased from Amresco, USA and contains >99.0 %.

Experimental design and treatments

A total of 192 healthy, 1-day-old Arbor Acres (AA) broilers were randomly divided into 4 groups, having 4 replicates in each group and 12 chicks in each replicate. Group I acted as the control group (basal diet without Gln), Groups II, III and IV were fed basal diet with 1.0, 1.5 and 2.0% Gln, respectively. The temperature and humidity of the chicken house are controlled by heater and humidifier, the temperature is maintained at 25±2℃, the humidity is maintained at 60-70%, lasted 14 d.

The chicken house was cleaned and fumigated three days before the experiment, and the chicken equipment were cleaned and disinfected. Broilers had free access to feed and drinking water. Disinfection and immunization were carried out according to the standard protocol of broiler management. Feed consumption, feces and health status of broilers were observed daily. The basal experimental diet was corn-soybean meal which was prepared according to NRC (1994) recommendations. The specific ingredients and chemical analysis are shown in Table I.

Table I. The ingredients and chemical composition of basic diet for chicks (1-14 days old).

	Daily diet
Ingredients (%)	
Corn	60.00
Soybean meal	33.00
Fish meal	2.00
Permix[1]	5.00
Calculated component (%)	
AME (MJ/kg)	11.55
CP	21.57
Lys	1.14
Met	0.35
Ca	0.96
Total P	0.57

[1]provide per kg of premix: Vitamin A, 154 300 IU; Vitamin B_1, 30 mg; Vitamin B_2, 160 mg; Vitamin B_6, 50 mg; Vitamin B_{12}, 0.22 mg; Vitamin D, 25 000 IU; Vitamin E, 176 IU; 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.

Performance measurements and organs collection

Two healthy chicks were randomly selected from each replicate on days 7 and 14 after 12 h fasting (without water), and then slaughtered for sampling. Blood was collected from the carotid artery of broilers in a 10 ml tube, centrifuged at 3000 r/min for 10 min, the serum was divided into 0.5 mL centrifuge tube and stored at -20 ℃ for later use. After the chicks were killed, the liver and the middle sections of small intestine were collected and stored at -80 ℃ for later use. Quantitative determination of catalase (CAT), glutathione peroxidase (GSH-PX), malonic dialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and protein concentration were purchased from Nanjing Jiancheng Bioengineering Institute.

Online First Article

Statistical analysis

Data was spread on Excel sheet to sort out the original data. The sorted data were analyzed by One-way ANOVA in SPSS 18.0 software under randomized block design. The results were expressed as mean ± standard error, and $P < 0.05$ was the difference significance standard.

RESULTS

Effect of Gln on serum antioxidant capacity of cold-stressed chicks

The data of serum SOD, CAT, GHS-PX, T-AOC and MDA are presented in the Table II. At day 7, compared with control group I, serum CAT and GSH-Px activities in group II were significantly increased ($P < 0.05$) by 59.26% and 15.09%, respectively. There were no significant differences in serum CAT and GSH-Px activities in group III and IV ($P > 0.05$); The serum MDA content in group IV was significantly decreased ($P < 0.05$) by 27.04%, and the serum MDA content in group II decreased by 19.54% (trend, $P= 0.057$) compared to the control group I, there was no significant difference in serum MDA content in group II and III ($P > 0.05$). There were no significant differences in the activities of SOD and T-AOC in serum of groups II, III and IV ($P > 0.05$).

At day 14, compared with control group I, serum GSH-Px activity in group III was significantly increased ($P < 0.05$) by 25.23%. There was no significant difference in serum GSH-Px activity between groups II and IV ($P > 0.05$). The content of MDA in serum of group II was significantly decreased ($P < 0.05$) by 28.28% compared to the control group I, but there was no significant difference in the content of MDA in serum groups III and IV ($P > 0.05$). There were no significant differences in the activities of CAT, SOD and T-AOC between the control and the treatment groups ($P > 0.05$).

Effect of Gln on liver antioxidant capacity of cold-stressed chicks

The data of liver SOD, CAT, GHS-PX, T-AOC and MDA are presented in the Table III. At day7, compared with control group I, the activity of CAT in liver of group II was increased by 11.15% ($P = 0.064$), and there was no significant difference in liver CAT activity between groups III and IV ($P > 0.05$). There were no significant differences in liver GSH-Px, SOD and T-AOC activities and MDA content in groups II, III and IV ($P > 0.05$). At day 14, compared with control group I, the activity of GSH-Px in liver of group II was increased by 13.23% ($P = 0.09$), but there was no significant difference in liver GSH-Px activity between groups III and IV ($P > 0.05$). There were no significant differences in the activities of CAT, SOD, T-AOC and MDA contents in groups II, III and IV ($P > 0.05$).

Effect of Gln on duodenum antioxidant capacity of cold-stressed chicks

The data of duodenum SOD, CAT, GHS-PX, T-AOC and MDA are presented in the Table IV. At day 7, compared with control group I, the activity of GSH-Px

Online First Article

Table II. Effects of Gln on serum antioxidant status of cold-stressed chicks on day 7 and 14[1].

Item[3]	Dietary treatment[2]				SEM
	Group I	Group II	Group III	Group IV	
Day 7					
CAT(U/ml)	0.81^{b}	1.29^{a}	0.94^{ab}	0.88^{ab}	0.07
GSH-PX(U/ml)	1070.60^{b}	1232.14^{a}	1097.80^{b}	1156.32^{ab}	23.76
MDA(nmol/ml)	3.07^{a}	2.47^{ab}	2.80^{ab}	2.24^{b}	0.12
SOD(U/ml)	86.95	90.51	93.84	88.47	1.86
T-AOC(U/ml)	0.65	0.67	0.70	0.68	0.01
Day 14					
CAT(U/ml)	1.35	1.53	1.52	1.58	0.05
GSH-PX(U/ml)	1153.02^{b}	1323.63^{ab}	1443.96^{a}	1216.48^{b}	40.58
MDA(nmol/ml)	2.97^{a}	2.13^{b}	2.70^{ab}	2.40^{ab}	0.13
SOD(U/ml)	69.43	78.79	71.98	72.02	2.82
T-AOC(U/ml)	0.65	0.68	0.66	0.72	0.02

$^{a-b}$Means with different superscripts in each row are significantly different ($P < 0.05$). [1]Each group had 4 replicates; Two chicks were randomly selected from each replicate for sampling (n=8). [2]Group I, control group (basal diet); Group II, basal diet + 1.0% Gln; Group III, basal diet + 1.5% Gln; Group IV, basal diet + 2.0% Gln. [3]CAT, catalase; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

Table III. Effects of Gln on liver antioxidant capacity of cold-stressed chicks[1].

Item[3]	Dietary treatment[2]				SEM	P-value
	Group I	Group II	Group III	Group IV		
Day 7						
CAT(U/mg prot)	9.78	10.87	9.90	9.93	0.20	0.211
GSH-PX(U/mg prot)	23.06	24.85	23.37	23.38	0.44	0.558
MDA(nmol/mg prot)	0.61	0.52	0.56	0.55	0.04	0.916
SOD(U/mg prot)	145.85	155.85	147.21	146.34	2.42	0.432
T-AOC(U/mgprot)	0.51	0.53	0.51	0.51	0.01	0.858
Day 14						
CAT(U/mg prot)	10.20	11.50	11.40	10.91	0.27	0.334
GSH-PX(U/mg prot	20.18	22.85	22.17	22.38	0.57	0.332
MDA(nmol/mg prot)	0.69	0.67	0.62	0.56	0.03	0.603
SOD(U/mg prot)	142.21	156.17	151.59	144.32	3.17	0.389
T-AOC(U/mg prot)	0.48	0.50	0.51	0.49	0.01	0.714

For statistical details of the dietary treatment groups and abbreviations, see Table II.

Table IV. Effects of Gln on duodenal antioxidant capacity of cold-stressed chicks[1].

Item[3]	Dietary treatment[2]				SEM	P-value
	Group I	Group II	Group III	Group IV		
Day 7						
CAT (U/mg prot)	0.64	0.68	0.61	0.62	0.03	0.882
GSH-PX (U/mg prot)	37.11^{b}	51.63^{a}	42.17^{ab}	44.10^{ab}	2.39	0.192
MDA (nmol/mg prot)	0.91	0.90	0.88	0.90	0.05	0.999
SOD (U/mg prot)	84.14	96.76	83.76	93.05	4.23	0.641
T-AOC (U/mg prot)	0.078	0.103	0.078	0.089	0.005	0.211
Day 14						
CAT (U/mg prot)	0.76	0.84	0.80	0.90	0.06	0.879
GSH-PX (U/mg prot)	20.87	23.95	23.89	24.14	1.22	0.761
MDA (nmol/mg prot)	1.39	1.29	1.37	1.14	0.06	0.518
SOD (U/mg prot)	105.51	112.44	103.00	105.53	3.29	0.785
T-AOC (U/mg prot)	0.091	0.096	0.089	0.099	0.003	0.724

For statistical details of the dietary treatment groups and abbreviations, see Table II.

Online First Article

in duodenum of group II was significantly increased ($P < 0.05$) by 39.13% while T-AOC activity increased by 32.05% (trend, $P = 0.069$). There were no significant differences in the activities of GSH-Px and T-AOC in duodenum of groups III and IV ($P > 0.05$). There were no significant differences in CAT, SOD activity and MDA content in duodenum of groups II, III and IV ($P > 0.05$). At day 14, compared with control group I, there were no significant differences in the activities of CAT, GSH-Px, SOD and T-AOC and the content of MDA in duodenum of groups II, III and IV ($P > 0.05$).

Effect of Gln on jejunum antioxidant capacity of cold-stressed chicks

The data of jejunum SOD, CAT, GHS-PX, T-AOC and MDA are presented in the Table V. At day 7, compared with control group I, jejunum CAT activity in group II was significantly increased ($P < 0.05$) 43.21%. The activity of GSH-Px in jejunum in groups II and IV was significantly increased ($P < 0.05$) by 23.46% and 24.63%, respectively. Jejunum T-AOC activity in group IV was significantly increased ($P < 0.05$) by 24.68%. There were no significant differences in MDA content and SOD activity in jejunum

Table V. Effects of Gln on jejunum antioxidant capacity of cold-stressed chicks[1].

Item[3]	Dietary treatment[2]				SEM	P value
	Group I	Group II	Group III	Group IV		
Day 7						
CAT (U/mg prot)	0.81[b]	1.16[a]	0.89[ab]	0.92[ab]	0.05	0.080
GSH-PX (U/mg prot)	44.54[b]	54.99[a]	52.23[ab]	55.51[a]	1.73	0.086
MDA (nmol/mg prot)	0.94	0.89	0.93	0.90	0.04	0.964
SOD (U/mg prot)	77.58	91.45	86.55	85.69	3.66	0.609
T-AOC (U/mg prot)	0.077[b]	0.090[ab]	0.082[ab]	0.096[a]	0.003	0.168
Day 14						
CAT (U/mg prot)	0.73[b]	1.15[a]	1.10[a]	0.98[ab]	0.07	0.101
GSH-PX (U/mg prot)	16.03[b]	19.51[ab]	20.57[a]	20.89[a]	0.79	0.106
MDA (nmol/mg prot)	0.51	0.49	0.46	0.49	0.04	0.975
SOD (U/mg prot)	82.48[b]	101.29[a]	89.24[ab]	98.36[ab]	3.20	0.140
T-AOC (U/mg prot)	0.064	0.074	0.070	0.071	0.003	0.760

For statistical details of the dietary treatment groups and abbreviations, see Table II.

Table VI. Effects of Gln on ileal antioxidant capacity of cold-stressed chicks.[1]

Item[3]	Dietary treatment[2]				SEM	P-value
	Group I	Group II	Group III	Group IV		
Day 7						
CAT (U/mg prot)	0.76[b]	0.85[ab]	1.12[a]	0.82[ab]	0.06	0.189
GSH-Px (U/mg prot)	34.44[b]	42.67[ab]	48.85[a]	40.09[ab]	2.25	0.149
MDA (nmol/mg prot)	0.52	0.48	0.49	0.45	0.03	0.913
SOD (U/mg prot)	72.78	83.41	87.09	78.80	3.76	0.575
T-AOC (U/mg prot)	0.066	0.083	0.078	0.079	0.004	0.525
Day 14						
CAT (U/mg prot)	0.84[bc]	0.75[c]	1.13[a]	1.04[ab]	0.05	0.039
GSH-Px (U/mg prot)	17.46	17.67	20.64	20.56	0.91	0.445
MDA (nmol/mg prot)	0.44	0.33	0.44	0.43	0.03	0.509
SOD (U/mg prot)	66.01	65.78	78.41	65.40	2.96	0.374
T-AOC (U/mg prot)	0.063	0.063	0.066	0.067	0.002	0.922

For statistical details of the dietary treatment groups and abbreviations, see Table II.

Online First Article

in groups II, III and IV ($P > 0.05$). At day 14, compared with control group I, the activity of CAT in jejunum in groups II and III was significantly increased ($P < 0.05$) by 57.53% and 50.68%, respectively. The activity of GSH-Px in jejunum in groups III and IV was significantly increased ($P < 0.05$) by 28.32% and 30.32%, respectively. The SOD activity of jejunum in group II was significantly increased ($P < 0.05$) by 22.81% while the SOD activity of jejunum in group IV was increased by 19.25% (trend, $P = 0.077$). There were no significant differences in MDA content and T-AOC activity in jejunum in groups II, III and IV ($P > 0.05$).

Effect of Gln on ileal antioxidant capacity of cold-stressed chicks

The data of ileal SOD, CAT, GHS-PX, T-AOC and MDA are presented in the Table VI. At day 7, compared with control group I, the activities of CAT and GSH-Px in ileum of group III were significantly increased ($P < 0.05$), 47.37 and 41.84%, respectively. There were no significant differences in the activities of CAT and GSH-Px in ileum between groups II and IV ($P > 0.05$). There were no significant differences in ileum MDA content and activities of SOD and T-AOC in groups II, III and IV (P

> 0.05). At day 14, compared with control group I, ileum CAT activity in group III was significantly increased ($P < 0.05$) 34.52%, but there was no significant difference in ileum CAT activity in groups II and IV ($P > 0.05$). There were no significant differences in GSH-PX, SOD, T-AOC activities and MDA content in groups II, III and IV ($P > 0.05$).

DISCUSSION

The animal body has a free radical scavenging system to resist the damage of free radicals to the body. This system is mainly composed of enzyme and non-enzyme system, among which the enzyme system includes CAT, GSH-Px, SOD and other enzymes, which are directly or indirectly involved in scavenging free radicals or inhibiting the generation of free radicals. SOD and GSH-Px play an important role in maintaining the dynamic balance between oxidation and anti-oxidation (Emami *et al*., 2020). And MDA can be used as general biomarker for biological oxidative stress (Kadiiska *et al*., 2005). The amount of MDA in animals can reflect the degree of lipid peroxidation to a certain extent, and indirectly reflect the degree of cell damage. Under normal physiological conditions, the ability of generating free radicals and scavenging free radicals of broilers kept a certain balance state. However, under low temperature environment, broilers need to consume a lot of energy to maintain body temperature, so the energy used for other physiological activities must be reduced, leading to the reduction of the ability of broiler body to clear free radicals, resulting in excessive free radicals, lipid peroxidation and oxidative damage of the body (Wang *et al*., 2019; Yang *et al*., 2014).

Yang *et al*. (2016) and Yang *et al*. (2014) found that cold stress can reduce the activity of GSH-Px and increase the content of MDA in serum and liver of broilers. Gln is a precursor of glutathione synthesis in the body, which can maintain glutathione reserves in the body, protect or reduce the body from free radicals, stabilize the structure of cell membranes and proteins, and contribute to the repair and function recovery of damaged cells, thus improving the body's antioxidant capacity. In this study, adding a certain level of Gln to the diet under cold stress could significantly improve the activities of GSH PX and CAT in serum, duodenum, jejunum and ileum of broilers; At the same time, it also significantly increased the activity of jejunal SOD and the content of jejunal T-AOC. It was found that the addition of Gln in diet could improve the oxidative stress and increase serum antioxidant capacity of chickens (Liu *et al*., 2021). Denno *et al*. (1996) reported that Gln supplementation could significantly increase the level of GSH in rat serum. In this study, 1% Gln can significantly increase the activity of CAT and GSH-Px in serum, and reduce the content of MDA. This is consistent with previous studies. This indicates that Gln can effectively alleviate the decrease of antioxidant capacity caused by cold stress. We hypothesized that Gln improved the antioxidant capacity of cold-stressed chicks, which was beneficial to improve the performance of chicks. Xiao *et al*. (2019) previous study showed that dietary 1% Gln can increase the average daily feed intake by 14.91% and average daily gain by 14.94% of cold-stressed chicks aged from 1 to 7 days; Add 2. 0% Gln could significantly increase thymus index and bursa of Fabricius index of cold-stressed chicks at 14 days of age.

Some research showed that cold stress decreased SOD and T-AOC activities and increased MDA content in duodenum, jejunum and ileum of broilers (Xu *et al*., 2012; Zhang *et al*., 2011). In this study, 1% Gln increased the activity of GSH-Px in duodenum and the activities of GSH-Px, CAT and SOD in jejunum. And 1.5% Gln increased the activities of GSH-PX and CAT in jejunum and ileum. It can be seen that the addition of Gln in the diet under cold stress can enhance the protective effect on the intestine by increasing the activity of antioxidant enzymes in the serum and intestine of broilers, and then alleviate the damage of low temperature stress to the broiler body. The antioxidant effect of Gln is achieved by its participation in the synthesis of GSH, which prevents damaging oxidative effects of oxygen free radicals on bio-film (Cruzat, 2019).

CONCLUSION

At low temperature (25 ± 2 ℃), adding 1%, 1.5% and 2% Gln to the diet could significantly improve the activities of GSH PX and CAT in serum, duodenum, jejunum and ileum; At the same time, it also significantly increased the activity of jejunal SOD and the content of jejunal T-AOC. It has no effect on liver antioxidation. It is suggested that glutamine can enhance the antioxidant capacity of the body by increasing the activity of antioxidant enzyme GSH-PX and inhibiting lipid peroxidation.

ACKNOWLEDGMENTS

The authors appreciated all the help from our colleagues and collaborators.

Financial support

This study was supported by the National Natural Science Foundation of China (32160799), the Key R and D program of Jiangxi Province (20203BBF63021), and Jiangxi Provincial Natural Science Foundation (20224ACB205010).

Online First Article

Ethical approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval (JXAULL-20190015) has been received. The authors confirm that animals were cared for under guidelines comparable to those of the guide to the care and use of experimental animals.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdulkarimi, R., Shahir, M.H., and Daneshyar, M., 2019. Effects of dietary glutamine and arginine supplementation on performance, intestinal morphology and ascites mortality in broiler chickens reared under cold environment. *Asian-Australas. J. Anim. Sci.,* **32**: 110–117. https://doi.org/10.5713/ajas.17.0150

Coqueiro, A.Y., Rogero, M.M., and Tirapegui, J., 2019. Glutamine as an anti-fatigue amino acid in sports nutrition. *Nutrients,* **11**: 863. https://doi.org/10.3390/nu11040863

Cruzat, V.F., 2019. Glutamine and Skeletal Muscle. In: *Nutrition and skeletal muscle*. Humana Press, New York, USA. pp. 299-313. https://doi.org/10.1016/B978-0-12-810422-4.00017-8

Dai, S.F., Bai, X., Zhang, D., Hu, H., Wu, X.Z., Wen, A.Y., He S.J., and Zhao, L., 2018. Dietary glutamine improves meat quality, skeletal muscle antioxidant capacity and glutamine metabolism in broilers under acute heat stress. *J. appl. Anim. Res*., **46**: 1412-1417. https://doi.org/10.1080/09712119.2018.1520113

Denno, R., Rounds, J.D., Faris, R., Holejko, L.B., and Wilmore, D.W., 1996. Glutamine-enriched total parenteral nutrition enhances plasma glutathione in the resting state. *J. Surg. Res.,* **61**: 35–38. https://doi.org/10.1006/jsre.1996.0077

Emami, N.K., Jung, U., Voy, B., and Dridi, S., 2020. Radical response: Effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. *Antioxidants* (Basel), **10**: 35. https://doi.org/10.3390/antiox10010035

Hao, Q., Yadav, R., Basse, A.L., Petersen, S., Sonne, S.B., Rasmussen, S., Zhu, Q., Lu, Z., Wang, J., Audouze, K., Gupta, R., Madsen, L., Kristiansen, K., and Hansen, J.B., 2015. Transcriptome profiling of brown adipose tissue during cold exposure reveals extensive regulation of glucose metabolism. *Am. J. Physiol. Endocrinol. Metab.,* **308**: E380–E392. https://doi.org/10.1152/ajpendo.00277.2014

Hassan, N.M., and Amir, H.A., 2013. Improved performance and small intestinal development of broiler chickens by dietary L-glutamine supplementation. *J. appl. Anim. Res.,* **41**: 1-7. https://doi.org/10.1080/09712119.2012.738214

Hu, H., Dai, S.F., Li, J.Q., Wen, A.Y., and Bai, X., 2020. Glutamine improves heat stress-induced oxidative damage in the broiler thigh muscle by activating the nuclear factor erythroid 2-related 2/Kelch-like ECH-associated protein 1 signaling pathway. *Poult. Sci.,* **99**: 1454–1461. https://doi.org/10.1016/j.psj.2019.11.001

Kadiiska, M.B., Gladen, B.C., Baird, D.D., Germolec, D., Graham, L.B., Parker, C.E., Nyska, A., Wachsman, J.T., Ames, B.N., Basu, S., Brot, N., Fitzgerald, G.A., Floyd, R.A., George, M., Heinecke, J.W., Hatch, G.E., Hensley, K., Lawson, J.A., Marnett, L.J., Morrow, J.D., Murray, D.M., Plastaras, J., Roberts, L.J., Rokach. J., Shigenaga, M.K., Sohal, R.S., Sun, J., Tice, R.R., Van Thiel, D.H., Wellner, D., Walter, P.B., Tomer, K.B., Mason, R.P., and Barrett, J.C., 2005. Biomarkers of oxidative stress study II: Are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? *Free Radic. Biol. Med.,* **38**: 698–710. https://doi.org/10.1016/j.freeradbiomed.2004.09.017

Li, L., Meng, Y., Li, Z.Y., Dai, W.H., Xu, X., Bi, X.L., and Bian J.L., 2019. Discovery and development of small molecule modulators targeting glutamine metabolism. *Eur. J. med. Chem.,* **163**: 215–242. https://doi.org/10.1016/j.ejmech.2018.11.066

Liu, Y., Yang, Y.Y., Yao, R.Z., Hu, Y.J., Liu, P., Lian, S., Lv, H., Xu, B., and Li, S., 2021. Dietary supplementary glutamine and L-carnitine enhanced the anti-cold stress of Arbor Acres broilers. *Anim. Breed.,* **64**: 231-243. https://doi.org/10.5194/aab-64-231-2021

NRC (National Research Council), 1994. *Nutrient requirements of poultry*. 9th rev. Natl. Acad. Press, Washtington, DC.

Nguyen, P., Greene, E., Ishola, P., Huff, G., Donoghue, A., Bottje, W., and Dridi, S., 2015. Chronic mild cold conditioning modulates the expression of hypothalamic neuropeptide and intermediary metabolic-related genes and improves growth performances in young chicks. *PLoS One,* **10**: e0142319. https://doi.org/10.1371/journal.pone.0142319

Oh, M.H., Sun, I.H., Zhao, L., Leone, R.D., Sun, I.M., Xu, W., Collins, S.L., Tam, A.J., Blosser, R.L., Patel,

Online First Article

C.H., Englert, J.M., Arwood, M.L., Wen, J., Chan-Li, Y., Tenora, L., Majer, P., Rais, R., Slusher, B.S., Horton, M.R., and Powell, J.D., 2020. Targeting glutamine metabolism enhances tumor-specific immunity by modulating suppressive myeloid cells. *J. clin. Invest.,* **130**: 3865–3884. https://doi.org/10.1172/JCI131859

Stehle, P., and Kuhn, K.S., 2015. Glutamine: An obligatory parenteral nutrition substrate in critical care therapy. *BioMed. Res. Int.,* **2015**: 545467. https://doi.org/10.1155/2015/545467

Tsiouris, V., Georgopoulou, I., Batzios, C., Pappaioannou, N., Ducatelle, R., and Fortomaris, P., 2015. The effect of cold stress on the pathogenesis of necrotic enteritis in broiler chicks. *Avian. Pathol.,* **44**: 430–435. https://doi.org/10.1080/03079457.2015.1083094

Wang, J.P., Yang, G.L., Zhang, K.Y., Ding, X.M., Bai, S.P., and Zeng, Q.F., 2019. Effects of dietary supplementation of DL-2-hydroxy-4 (methylthio) butanoic acid on antioxidant capacity and its related gene expression in lung and liver of broilers exposed to low temperature. *Poult. Sci.,* **98**: 341–349. https://doi.org/10.3382/ps/pey371

Wischmeyer, P.E., 2007. Glutamine: Mode of action in critical illness. *Crit. Care Med.,* **35**: S541–S544. https://doi.org/10.1097/01.CCM.0000278064.32780.D3

Wu, Q.J., Jiao, C., Liu, Z.H., Cheng, B.Y., Liao, J.H., Zhu, D.D., Ma, Y., Li, Y.X., and Li, W., 2021. Effect of glutamine on the growth performance, digestive enzyme activity, absorption function, and mRNA expression of intestinal transporters in heat-stressed chickens. *Res. Vet. Sci.,* **134**: 51–57. https://doi.org/10.1016/j.rvsc.2020.12.002

Xiao, S.S., Ye, M.L., Li, J.Q., Kong, L., Hu, H., Wu, X.Z., Bai, X., and Ma S.T., 2019. Effects of glutamine on growth performance and immune organ index of cold-stressed chicks. *J. Anhui Univ. Sci. Technol.,* **33**: 4.

Xu, M., Yu, X.Y., Li, J.L., Han, Y.H., Shu, L., and Xu, S.W., 2012. Effects of acute and chronic cold stress on antioxidant function in intestinal tracts of chickens. *J. Northeast Agric. Univ.* (Eng. Ed.), **19**: 8. https://doi.org/10.1016/S1006-8104(13)60038-0

Xue, G.D., Barekatain, R., Wu, S.B., Choct, M., and Swick, R.A., 2018. Dietary L-glutamine supplementation improves growth performance, gut morphology, and serum biochemical indices of broiler chickens during necrotic enteritis challenge. *Poult. Sci.,* **97**: 1334–1341. https://doi.org/10.3382/ps/pex444

Yang, G.L., Zhang, K.Y., Ding, X.M., Zheng, P., Luo, Y.H., Bai, S.P., Wang, J.P., Xuan, Y., Su, Z.W., and Zeng, Q.F., 2016. Effects of dietary DL-2-hydroxy-4(methylthio)butanoic acid supplementation on growth performance, indices of ascites syndrome, and antioxidant capacity of broilers reared at low ambient temperature. *Int. J. Biometeorol.,* **60**: 1193–1203. https://doi.org/10.1007/s00484-015-1114-7

Yang, X., Luo, Y.H., Zeng, Q.F., Zhang, K.Y., Ding, X.M., Bai, S.P., and Wang, J.P., 2014. Effects of low ambient temperatures and dietary vitamin C supplement on growth performance, blood parameters, and antioxidant capacity of 21 days old broilers. *Poult. Sci.,* **93**: 898–905. https://doi.org/10.3382/ps.2013-03438

Zhang, Z.W., Bi, M.Y., Yao, H.D., Fu, J., Li, S., and Xu, S.W., 2014. Effect of cold stress on expression of AMPKalpha-PPARalpha pathway and inflammation genes. *Avian Dis.,* **58**: 415–426. https://doi.org/10.1637/10763-010814-Reg.1

Zhang, Z.W., Lv, Z.H., Li, J.L., Li, S., Xu, S.W., and Wang, X.L., 2011. Effects of cold stress on nitric oxide in duodenum of chicks. *Poult. Sci*., **90**: 1555–1561. https://doi.org/10.3382/ps.2010-01333

Zhao, F.Q., Zhang, Z.W., Yao, H.D., Wang, L.L., Liu, T., Yu, X.Y., Li, S., and Xu, S.W., 2013. Effects of cold stress on mRNA expression of immunoglobulin and cytokine in the small intestine of broilers. *Res. Vet. Sci.,* **95**: 146–155. https://doi.org/10.1016/j.rvsc.2013.01.021

Zhou, H.J., Kong, L.L., Zhu, L.X., Hu, X.Y., Busye, J., and Song, Z.G., 2021. Effects of cold stress on growth performance, serum biochemistry, intestinal barrier molecules, and adenosine monophosphate-activated protein kinase in broilers. *Animal,* **15**: 100138. https://doi.org/10.1016/j.animal.2020.100138

Online First Article