Indian Journal of Animal Sciences **90** (6): 912–916, June 2020/Article

Effect of glutamine on the blood biochemistry and the activity of serum enzymes in broilers challenged with *Salmonella enteritidis*

Q J WU*, S W LI, Z H LIU, C JIAO, D D ZHU, Y MA, Y Q WANG, Y WANG and X H WU

Henan University of Science and Technology, 263, Kaiyuan Avenue, Luoyang 471003, Henan, PR China

Received: 17 September 2019; Accepted: 14 October 2019

ABSTRACT

This study was conducted to investigate the effects of glutamine (Gln) on blood biochemistry and activity of serum enzymes in broilers challenged with *Salmonella enteritidis*. 240 one-day-old Arbor Acres broilers were distributed into four groups treated in a completely randomized design for 21 days. The groups included a non-infection control (CON); infected with *S. enteritidis* (SCC); infected +0.5% Gln (Gln 1), and infected +1.0% Gln (Gln 2). The plasma total protein, albumin, and globulin concentration, AST, LDH and CK activity in SCC group were lower than the CON overall except the albumin at 14, 21 d, and CK at 14 d. SCC group had lower RBC at 7 d; lower MCHC at 14 d; higher MCHC, and MCH at 7 d, or 21 d; higher WBC at d 7, 14, and 21; higher γ-GGT activity at d 14, and 21than the CON group. Gln group had higher plasma total protein, albumin, and globulin concentration, AST, LDH and CK activity than those in SCC overall except the albumin at 14, 21 d, and CK at 14 d. Gln group had higher RBC at 7 d; higher MCHC at 14 d; lower MCHC, and MCH at 7 d, or 21 d; lower WBC at d 7, 14, and 21, and lower γ-GGT activity at d 14, and 21 than the SCC group. These results suggest that Gln could effective improve the physiological responses and maintain the normal health status of *S. enteritidis* infection of broilers.

Keywords: Biochemical parameters, Broiler, Glutamine, Hematological parameters, *Salmonella enteritidis*

Generally, blood biochemical parameters can be used to assess the level of physiological disorders resulting from stressors and to monitor the health status of broiler chicks (Mohamed *et al*. 2012). According to published papers it has been demonstrated that *Salmonella* infected has a significant effect on the serum concentrations of proteins, albumin, globulin, and the activity of serum or plasma enzymes such as aspartate amino-transferase (AST) and alanine transaminase (ALT) (Abudabos *et al*. 2017), which would suggest damage to the internal organs of chickens. Therefore, it is critical to understand the blood biochemistry components and the activity of serum enzymes against *S.enteritidis* infection during the starter phase.

Glutamine (Gln) is the most abundant free amino acid in blood plasma, which plays important regulatory functions in the various organs and cell of animal body (Mustafa 2014). It has been reported that glutamine can improve nutrient metabolism, blood protein changes, growth performance, and immunity function (Wu *et al*. 2019). Even though many studies have focused on the function of Gln in broiler biochemical and mucosal barrier function, recent research due to the lack of the roles of glutamine on the blood protein concentration and the activity of serum enzymes in *S.enteritidis* challenged broilers. The objective of the present study was to investigate the effects of dietary supplementation with Gln on the blood biochemistry and the activity of serum enzymes in *S.enteritidis* challenged broilers.

*Corresponding author e-mail: wuqiujue@163.com

MATERIALS AND METHODS

All procedures involving the care, handling, and sampling of animals in the present study were approved by the Institutional Animal Care and Use Committee of Henan University of Science and Technology.

Experimental birds, diets and design: A total of 240 one-day-old Arbor Acres broiler chicks, were weighed and placed in two separate 3-tier battery cages each comprising 10 chicks and six replicates per treatment group following completely randomised design. Feed and water were given *ad lib*. The brooder temperature was maintained at about 34°C up to 7 days of age and gradually decreased to 22±1°C by 21st day of age. Twenty-three hours of light was provided from d 1 to d 7, and 18 h light was provided daily from d 8 to d 21.

All birds were fed on corn and soybean diet formulated to meet or exceed recommendations of the nutrient and energy requirements of the National Research Council (NRC, 1994) in starter (0–21 d) feeding periods, as shown in Table 1. Gln (99% purity) was added and thoroughly mixed into the basal feed.

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

Feed Ingredient (%)	1–21 d
Corn silage	54.60
Corn gluten meal	35.50
Soybean oil	3.50
Limestone	1.20
Dicalcium phosphate	1.50
Salt	3.00
50% Choline chloride	0.14
Premix[1]	0.24
L-Lysine	0.12
DL-Methionine	0.20
Total	100.00
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

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.

The experimental groups were as follows: the negative control (basal diet), positive control (SCC: *S. enteritidis* infection), 0.5% Gln + challenge (Gln 1), 1.0% Gln + challenge (Gln 2). On day 3, the chicks were infected with *S. enteritidis* at a dose rate of 2.0×10^4 orally. Each chick in the negative control group orally received an equivalent volume of sterile normal saline.

Sample collection and analysis: At days 4, 7, 14 and 21, six broilers from each treatment were randomly chosen and euthanized with cervical dislocation. 5 ml of blood samples were collected into disposable syringe from the wing vein and directly transferred into labeled test tube containing anticoagulant. Red Blood cell (RBC), White Blood cell (WBC), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were measured using a Sysmex Microcell Counter CL-180 (Tokyo, Japan).

Total protein (TP), globulin, albumin, ALT, AST, lactate dehydrogenase (LDH), creatine kinase (CK), and γ-Glutamyltransferase (γ-GGT) were measured using corresponding commercially available kits (Shenzhen Mindray Bio Medical, Shenzhen, China) with an automated biochemistry analyzer (Hitachi 7160, Hitachi Group, Japan).

Statistical analysis of the data: All analyses were performed using SPSS Statistics 21 for Windows statistical software package (SPSS Inc., Chicago, IL, USA, 2012). The one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to evaluate the significance of differences among individual means. The results are expressed as the mean±standard deviation. A value of 0.05 or less was defined as statistically significant.

RESULTS AND DISCUSSION

RBC count of birds in SCC were decreased at 7 d compared with that of CON, the addition of Gln increased the RBC count compared with that of the SCC group (Table 2). However, there were no significant differences in the RBC count between the SCC group and CON group at 7 d. RBC count were not affected after infection, and no significant differences were observed among the treatment groups at 4 d, 14 d, or 21 d. At d 7, 14, and 21, *S. enteritidis* infection increased the WBC count in the SCC group compared to the CON group. However, the Gln 1 and Gln 2 groups showed decreased the WBC count compared to the SCC group, but there were no differences when compared to the WBC count of the CON group. Compared with the control group, the SCC group decreased the MCHC, and MCH value at 7 d, or 21 d. The MCHC, and MCH value were increased in the Gln-treated groups compared with the *S. enteritidis* -challenged groups, but there was no diûerence when compared to these values of the CON group. *Salmonella* infection decreased the MCHC value at 14 d. Dietary Gln increased the MCHC value at 14 d compared with those of the SCC. However, there were no differences in the MCHC value between the CON and Gln group at 14 d. The WBC, MCHC, and MCH value at 4 d did not differ among groups. The RBC and MCH value at 14 d did not differ among groups. Throughout the evolution of the infection, *Salmonella* reduced the plasma total protein, albumin (with the exception of 14, 21 d), and globulin at 4, 7, 14, and 21 d in challenged chickens compared with that of the control group. However, Gln supplementation increased the concentrations of the plasma total protein, albumin (with the exception of 14, 21 d) and globulin compared with that of the non-supplemented SCC group. However, there were no differences in the concentration of plasma total protein, albumin (except day 14 and 21 d), and globulin between the CON and Gln group at 4, 7, 14, and 21 d.

S. enteritidis infection is one of the most important issues for poultry health in the world, which caused cumulative poisoning effects such as a remarkable disturbance in many metabolic processes, increased blood pressure, liver and kidney disorders. It is believed that the hematological parameters are closely associated with the status of systemic metabolic, physiological and health of the birds (Adeyemo 2013). The present experiment studied the impact of *S. enteritidis* on the starter broiler, which can result in reduced intensity of the overall metabolism. Vecerek *et al*. (2002) reported similar responses of broiler to unfavorable conditions. A change in the mentioned haematological parameters indicates worsening of the organism's health status (Adeyemo 2013). The adverse effect of *S. enteritidis* may be ascribable to destruct the hematopoietic organs.

Table 2. Effect of dietary Gln on the haematological indices and blood chemistry of broilers infected with *S. enteritidis*

Item	Diet treatment			
	CON	SCC	Gln1	Gln2
4 d				
RBC (10^{12}/L)	1.68±0.08	1.66±0.05	1.70±0.14	1.97±0.08
WBC (10^{9}/L)	189.27±1.68	216.25±0.45	201.75±24.15	186.70±21.40
MCHC (g/L)	357.67±1.20	350.50±3.50	353.00±13.00	355.00±2.00
MCH (pg)	54.17±0.50	52.60±2.40	54.95±0.75	54.60±0.40
Total protein (g/L)	47.86±0.51^{a}	36.01±0.29^{b}	45.41±0.61^{a}	46.83±0.49^{a}
Albumin (g/L)	14.87±0.30^{a}	11.91±0.12^{b}	14.19±0.15^{a}	14.72±0.19^{a}
Globulin (g/L)	31.91±0.21^{a}	23.27±0.91^{b}	30.24±0.71^{a}	31.15±0.40^{a}
7 d				
RBC (10^{12}/L)	2.51±0.12^{a}	2.00±0.18^{b}	2.42±0.09^{a}	2.48±0.12^{a}
WBC (10^{9}/L)	223.10±12.40	229.73±26.14	210.17±11.37	203.63±14.71
MCHC (g/L)	377.67±3.84^{a}	301.33±5.67^{b}	369.67±4.06^{a}	375.33±10.76^{a}
MCH (pg)	55.77±0.09^{a}	51.13±0.07^{b}	54.37±0.08^{a}	54.07±0.10^{a}
Total protein (g/L)	50.19±0.37^{a}	39.54±1.07^{b}	48.67±0.73^{a}	49.96±0.64^{a}
Albumin (g/L)	15.05±0.25^{a}	12.10±0.16^{b}	14.75±0.13^{a}	14.97±0.20^{a}
Globulin (g/L)	34.29±0.21^{a}	24.91±0.42^{b}	33.27±0.31^{a}	33.85±0.12^{a}
14 d				
RBC (10^{12}/L)	2.45±0.03	2.40±0.18	2.25±0.11	2.42±0.26
WBC (10^{9}/L)	239.7±5.38^{b}	292.97±12.20^{a}	248.0±9.02^{b}	238.2±8.59^{b}
MCHC (g/L)	400.33±1.86^{a}	316.00±6.03^{b}	398.00±3.21^{a}	402.33±0.67^{a}
MCH (pg)	63.93±2.05	60.40±2.72	59.73±4.56	62.40±3.03
Total protein (g/L)	52.46±0.63^{a}	40.01±0.82^{b}	50.14±0.48^{a}	51.21±0.50^{a}
Albumin (g/L)	17.22±0.29	16.83±0.21	16.97±0.37	17.14±0.28
Globulin (g/L)	36.36±0.11^{a}	27.17±1.07^{b}	35.07±0.53^{a}	35.47±0.20^{a}
21 d				
RBC (10^{12}/L)	2.89±0.03	2.76±0.70	2.95±0.23	3.03±0.03
WBC (10^{9}/L)	310.40±10.31^{b}	375.87±9.68^{a}	323.50±7.50^{b}	314.40±5.85^{b}
MCHC (g/L)	426.33±5.00^{a}	324.67±9.00^{b}	418.67±8.00^{a}	428.60±7.86^{a}
MCH (pg)	61.67±1.18^{a}	55.10±1.53^{b}	60.17±1.47^{a}	60.93±1.34^{a}
Total protein (g/L)	54.95±0.75^{a}	42.00±1.80^{b}	52.40±0.80^{a}	53.15±0.45^{a}
Albumin (g/L)	17.35±0.55	17.10±0.20	17.15±0.25	17.30±0.20
Globulin (g/L)	37.90±0.20^{a}	29.90±2.00^{b}	35.25±0.55^{a}	36.15±0.25^{a}

CON, non-infected control group; SCC, *S. enteritidis* infected control group received the basal diet; Gln1, *S. enteritidis* infected control group received the basal diet plus 0.5% Gln; Gln2, *S. enteritidis* infected control group received the basal diet plus 1.0% Gln. ^{a,b}Values within the same row that do not share a common superscript are significantly different at $P<0.05$; n = 8. RBC, Red blood cell; WBC, White blood cell; MCH, Mean cell haemoglobin; MCHC, Mean cell haemoglobin concentration.

These disturbed effects of SCC group were treated by Gln treatments (Gln 1 and Gln 2), which indicates Gln ameliorates these harmful toxic effects of *S. enteritidis* endotoxin. It was indicated that disturbed blood parameters under the stress condition were improved by treatment with the addition of Gln, which is in accord with Rodrigues' *et al*. (2007) study. They suggested that this was achieved because Gln is very important for suppressing the pathogenic bacteria and viruses, and for the activities of phagocytosis and secretion by the macrophages (El-Sheikh and Khalil 2011).

These values decreased in the *S. enteritidis* infected broilers compared with control group, this indicated that there was physiological stress caused by *S. enteritidis* orally at starter stages of broilers. Nakajothi *et al*. (2011) reported similar regularities within the change of biochemical profile of plasma in broiler chickens under cold stress condition. These decreases of plasma protein by *S. enteritidis* orally may be attributed to *S. enteritidis* on the intestinal wall. In the present study, it is clear that a number of biochemical changes occur in the natural defense against toxicity. Wu *et al*. (2020) who reported that intestinal mucosal damage by *Salmonella* infections led to intestinal colonization, mucosal damage, and inflammation responses, as well as invasion of internal organs, then resulting in the poor digestion and absorption of nutrients, inhibition of protein biosynthesis. The achieved results support the conclusion published by Al-Baadani *et al*. (2018). Moreover, total protein and albumin (no inclusion of days 14 and 21) levels were improved and alleviated by the treatment with Gln in our present study, and thus clearly reduced liver injury and enhanced the detoxification function of the liver. These changes have been mentioned already by El-Sheikh and Khalil (2011) in his work.

Table 3. Effect of dietary Gln on the catalytic activity of enzymes in the serum of broilers infected with *S. enteritidis* (U/L)

Item	Diet treatment			
	CON	SCC	Gln1	Gln2
4 d				
ALT	1.65±0.37	2.50±0.64	1.50±0.53	2.20±0.71
AST	354.00±13.86^{b}	493.00±31.53^{a}	282±38.51^{b}	399.50±15.55^{b}
LDH	778.00±54.39^{b}	1174.00±45.00^{a}	780.50±32.50^{b}	756.5±41.5^{b}
CK	1997.6±167.06^{b}	2402.5±215.5^{a}	1938.5±159.5^{b}	1764.5±173.5^{b}
γ-GGT	13.67±1.20	13.50±0.50	14.00±2.00	13.50±0.50
7 d				
ALT	1.52±0.53	2.16±0.81	1.56±0.57	1.69±0.34
AST	305.00±26.12^{b}	414.50±35.52^{a}	290.00±6.43^{b}	302.500±6.51^{b}
LDH	521.00±40.58^{c}	1072.00±106.50^{a}	716.33±39.17^{b}	498.67±28.83^{c}
CK	3498.00±123.01^{ab}	3539.08±149.80^{a}	3425.00±161.74^{ab}	3121.00±194.86^{b}
γ-GGT	12.00±0.58	16.67±1.86	12.67±1.86	17.33±2.73
14 d				
ALT	1.52±0.51	2.05±0.56	1.57±0.88	1.68±0.32
AST	352.31±18.04^{b}	564.01±48.11^{a}	333.21±31.08^{b}	372.54±27.52^{b}
LDH	576.67±52.39^{b}	651.33±48.94^{a}	551.67±35.83^{b}	569.00±40.95^{b}
CK	7678.33±132.00	7700.00±496.61	6921.00±399.09	6799.00±389.78
γ-GGT	19.67±1.33^{b}	29.67±1.37^{a}	21.00±1.15^{b}	20.33±1.76^{b}
21 d				
ALT	3.00±0.80	3.50±0.25	2.85±1.01	3.25±0.36
AST	349.81±35.22^{b}	415.0±23.54^{a}	241.5±16.53^{b}	354.5±34.51^{ab}
LDH	205.33±12.90^{b}	804.33±33.93^{a}	247.67±74.53^{b}	212.67±46.40^{b}
CK	3008.00±132.51^{b}	3368.00±135.95^{a}	3087.33±124.55^{b}	3064.67±101.25^{b}
γ-GGT	26.67±5.49^{b}	37.00±5.58^{a}	28.27±1.45^{b}	27.67±3.71^{b}

CON, non-infect control group; SCC, *S. enteritidis* infect control group received the basal diet; Gln1, *S. enteritidis* infected control group received the basal diet plus 0.5% Gln; Gln2, *S. enteritidis* infected 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. ALT, Alanine transaminase; AST, Aspartate aminotransferase; LDH, Lactate Dehydrogenase; CK, Creatine Kinase; γ-GGT, γ-Glutamyltransferase.

The activity of AST enzymes in SCC were increased at these time points compared with that of CON (Table 3). Compared with SCC group, Gln-treated group can decrease the activity of AST enzymes. However, there were no differences in the activity of AST between the CON and Gln group at these time points. The ALT activity in serum at these time points did not differ among groups. Compared with that of the CON group, the activity of LDH and CK (except day 14 d) at 4, 7, 14, and 21 d was significantly increased in the SCC group. The activity of LDH and CK (except day 14 d) of the serum were decreased in the Gln-treated groups compared with the *S. enteritidis* -challenged groups, but there were no differences when compared to the activity of serum LDH and CK of the CON group. Neither *S. enteritidis* infection nor Gln supplementation had an effect on the activity of γ-GGT at 4, and 7 d. At d 14, and 21, *S. enteritidis* infection significantly increased the activity of γ-GGT in the serum in the SCC group compared to the CON group. The Gln1 and Gln 2 groups showed significantly decreased the activity of γ-GGT in the serum compared to the SCC group, but there were no differences in the activity of γ-GGT between the CON group and the Gln groups.

The activities of hepatic AST and ALT enzymes in plasma can reflect the liver damage and disruption of the liver function and metabolic status. *S. enteritidis* produced the significant increase in the activity of plasma AST, which indicated that toxic induced damage and dysfunction to liver tissues in broilers. These results also supported that administration of gentamicin toxicity leads to increase in the concentration of serum enzymes AST (Nayma *et al*. 2012). The adverse effect indicated that *S. enteritidis* did toxic damage to the liver cell membrane, which may be responsible for liver tissue injury and hepatocellular death, ultimately may influence various metabolic pathway of liver.

Addition of Gln can reduce the activities of the two above parameters compared to SCC group, which showed liver damage of broilers was improved by Gln intake. It has been proved that Gln can improve the AST levels in the mycotoxin deoxynivalenol infected pigs (Wu *et al*. 2019), and impaired the liver function and morphology. These evidence showed that Gln have hepato-protective effects against *S. enteritidis* infected by attenuating enzyme release from the liver cells (Carlos *et al*. 2015). All these effects have been shown that Gln can protect the structure and function of liver by participating in energy metabolism of cell, which are probably attributed to Gln which might mobilize gluconeogenesis and glycogenosis to meet the increasing demand of energy from broiler under the stress condition.

Serum levels of CK, GGT and LDH are used as diagnostic marker for the destruction of hepatocytes and dysfunction of animal liver (Jiang *et al*. 2014). In present study, *S. enteritidis* infected broilers had increased LDH, CK and GGT values. These changes have been mentioned already by Mahmoud *et al*. (2015) in his stock broiler model, which showed that stress can cause the disruption of liver. The results of CK, GGT and LDH enzyme activity which could be due to hepatic degeneration (Jiang *et al*. 2014), and triggering of the oxidative stress process. Treatments with Gln alleviated the toxicity of *S. enteritidis* on the LDH and CK activity, which showed that Gln exerted a protective effect to the tissue injuries against *S. enteritidis* infection. Report revealed a reduction in LDH and CK activity in tissues varies with Gln application (Zhang *et al*. 2013). These results showed that Gln improved the LDH and CK activity in the serum, reduced the liver structural damage, which may be due to the antioxidant function of glutamine (Hu *et al*. 2016).

In conclusion, it can be stated that *S. enteritidis* infected can significantly influence hematological parameters and indices, bio-chemical constituent, and the activity of serum enzymes of chickens. Administration of Gln could effective reduce the physiological parameters and tissue damages in *S. enteritidis* infected broilers.

ACKNOWLEDGEMENTS

This research was supported by a project supported by the National Natural Science Foundation of China (Grant No. 31601971).

REFERENCES

Abudabos A M, Alyemni A H, Dafalla Y M and Khan R U. 2017. Effect of organic acid blend and Bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidant status in broilers exposed to *Salmonella typhimurium* challenge during the starter phase. *Journal of Applied Animal Research* **45**: 538–42.

Adeyemo G O. 2013. Influence of Varying Crude Protein Levels and Balanced Amino Acids on the Performance and Haematological Characteristics of Laying Hens at the second phase of production. *Food and Nutrition Sciences* **4**: 11–5.

Al-Baadani H H, Abudabos A M, Al-Mufarrej S I, Al-Baadani A A and Alhidary I A. 2018. Dietary supplementation of Bacillus subtilis, Saccharomyces cerevisiae and their symbiotic effect on serum biochemical parameters in broilers challenged with Clostridium perfringens. *Journal of Applied Animal Research* **46**(1): 1064–72.

Hu H, Bai X, Shah A A, Wen A Y, Hua J L, Che C Y, He S J, Jiang J P, Cai Z H and Dai S F. 2016. Dietary supplementation with glutamine and c-aminobutyric acid improves growth performance and serum parameters in 22- to 35-day-old broilers exposed to hot environment. *Journal of Animal Physiology and Animal Nutrition* **100**: 361–70.

Mahmoud T S, Reza B, Gholam-Reza M and Reza B. 2015. Effects of Cinnamon extract on biochemical enzymes, TNF-α and NF-κB gene expression levels in liver of broiler chickens inoculated with *Escherichia coli*. *Pesquisa Veterinária Brasileira* **35**(9): 781–87.

Mohamed E A A, Ali O H A, Huwaida M E E and Yousif I A. 2012. Effect of season and dietary protein level on some haematological parameters and blood biochemical compositions of three broiler strains. *International Journal of Poultry Science* **11**(12): 787–93.

Mustafa A. 2014. The effect of dietary glutamine supplementation on performance and blood parameter, carcass characteristics, quality and characteristics meat of broiler chickens under continuous heat stress condition. *International Journal of Farming and Allied Sciences* **3**(12): 1234–42.

Nakajothi N, Nanjappan K, Selvaraj P, Jayachandran S and Visha P. 2011. Production performance and blood biochemical changes in broiler chickens fed amia during induced-stress conditions. *Indian Journal of Animal Sciences* **79**(11): 1124–26.

National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th ed. National Academy of Sciences, Washington, DC.

Nayma S, Choudhury S S, Hossain P M T and Akhtar J. 2012. Effects of Ashwagandha (*Withania somnifera*) root extract on some serum liver marker enzymes (AST, ALT) in gentamicin intoxicated rats. *Journal of Bangladesh Society of Physiologist* **7**(1): 1–7.

Rodrigues N C L, Nunes L A, Horie L M, Torrinhas R and Waitzberg D L. 2007. Glutamine supplementation effect in hematological parameters and nutritional status of rats submitted to malnutrition protocol. *ABCD Arq Bras Cir Dig* **20**(4): 270–73.

Vecerek V, Strakova E, Suchy P and Voslarova E. 2002. Influence of high environmental temperature on production and haematological and biochemical indexes in broiler chickens. *Czech Journal of Animal Science* **47**(5): 176–82.

Wu Q J, Jiao C, Liu Z H, Li S W, Zhu D D, Ma W F, Wang Y Q, Wang Y and Wu X H. 2019. Effect of glutamine on the intestinal function and health of broilers challenged with *Salmonella pullorum*. *Indian Journal of Animal Research* **53**(9): 1210–16.

Wu Q J, Liu Z H, Jiao C, Cheng B Y, Zhu D D, Ma Y, Wang Y Q, Ma W F and Wang Y. 2020. Effects of Glutamine on Growth Performance, Intestinal Morphology and Intestinal Barrier Function of Broilers. *Indian Journal of Animal Research* 10.18805/ijar.B-1207.

Wu Q J, Liu Z H, Li S W, Jiao C, Wang Y Q and Wang Y. 2019. Effects of glutamine on digestive function and redox regulation in the intestines of broiler chickens challenged with *Salmonella enteritidis*. *Brazilian Journal of Poultry Science* **21**(4): eRBCA-2019-1123.

Zhang Y, Yan H, Lv S G., Wang L, Liang G P, Wan Q X and Peng X. 2013. Effects of glycyl-glutamine dipeptide supplementation on myocardial damage and cardiac function in rats after severe burn injury. *International Journal of Clinical and Experimental Pathology* **6**(5): 821–30.