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Influence of Dietary Glutamine Supplementation on Growth Performance, Small Intestinal Morphology, Immune Response and Some Blood Parameters of Broiler Chickens

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Abstract: The objective of this experiment was to evaluate the influence of glutamine (Gln) supplementation in the diet of broiler chickens on the growth performance, immune response as well as some blood parameters. Two hundred fifty one day old broiler chickens were allotted into five equal groups (50 chicks per each) of mixed sex. Five experimental diets were formulated to be isonitrogenous and isocaloric with different levels of Gln, first group fed on basal diets without Gln supplementation (control group), while Gln included at 0.5, 1.0, 1.5 and 2.0% and fed to chick groups (2-5) respectively for continuous 6 weeks. The results revealed that 1% Gln supplementation significantly ($p \leq 0.05$) improved body weight. Weight gain, Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Efficiency of Energy Utilization (EEU) when compared with the control, while 0.5% Gln supplementation non significantly ($p>0.05$) improved broiler chick performance and the higher inclusion levels had negative effect on broiler growth performance. Moreover, 1% Gln supplementation significantly ($p \leq 0.05$) improved blood pictures, phagocytic activity, antibody production and increase immune organs relative weights, while the lower and higher Gln level had no effect. Chicks fed diet with Gln supplementation at different levels had heavier intestinal relative weights and longer intestinal villi ($p \leq 0.05$) as compared with the control. The results indicate that the addition of 1 % Gln to the broiler chick's diet improves growth performance and may stimulate development of the gastrointestinal tract and immune response, while higher level had negative effects.

Key words: Broiler chickens, glutamine, growth performance, immune response, intestinal morphology

INTRODUCTION

L-Glutamine (Gln) is the most prevalent amino acid in the blood stream, accounting for 30-50% of the amino acid Nitrogen (N) in the plasma and the free amino acid pool in the body (Newsholme *et al*., 1985). Because Gln contains 2 ammonia groups, one from its precursor, glutamate, and the other from free ammonia in the bloodstream, Gln acts as a "nitrogen shuttle" that helps protect the body from high levels of ammonia (Labow, 2001). Thus Gln can act as a buffer, accepting excess ammonia and then releasing it when needed to form other amino acids, amino sugars, glucose, proteins, nucleotides, glutathione and urea (Souba, 1993; Rennie, 2001). This capacity to accept and donate N makes Gln the major vehicle for nitrogen transfer between tissues. Glutamine is the principal metabolic fuel for small intestine enterocytes, lymphocytes, macrophages, and fibroblasts (Cynober, 1999; Andrews and Griffiths, 2002) and is considered an essential amino acid in some species under inflammatory conditions such as infection and injury (Newsholme, 2001).

Many benefits have been observed due to Gln supplementation in the diet of humans and rats; however, little research has been done with poultry. Yi *et al*. (2005) reported that supplementing the diet with 1% Gln improved weight gain and feed efficiency (weight gain : feed intake) of turkey poults during the first week posthatch as compared with poults fed a standard corn-Soybean Meal (SBM) diet. Glutamine supplementation increased intestinal villus height in poults (Yi *et al*., 2001). During stressful conditions, intestinal permeability increases allowing bacteria to enter the bloodstream, thus causing infection (Adjei *et al*., 1994), and Gln has also been shown to decrease the incidence of infection in surgery and trauma patients (Newsholme, 2001; Medina, 2001; Andrews and Griffiths, 2002).

Glutamine supplementation increased intestinal villus height in poults (Yi *et al*., 2001) and glutamine supplementation has been reported to stimulate gut mucosal proliferation in rats (INoue *et al*., 1993). It has also been observed that supplementing with 1.5% Gln in total parenteral nutrition diets maintains gut integrity, which is important in preventing bacterial infections and Gln has been shown to prevent intestinal hyper permeability and bacterial translocation in mice during an immunological challenge (Adjei *et al*., 1994). During stressful conditions, intestinal permeability increases allowing bacteria to enter the bloodstream, thus causing infection (Adjei, *et al*., 1994) and Gln has also been shown to decrease the incidence of infection in surgery and trauma patients (Newsholme, 2001; Medina, 2001; Andrews and Griffiths, 2002).

At hatching, the immune system of birds is already partially developed, and the primary organs-thymus and

bursa are present and populated with lymphoid cells. However, the secondary organs, such as spleen, cecal tonsils and lymphoid tissues scattered in the digestive and respiratory tract are still incomplete (Dibner and Richards, 2004). Gln is recognizes as a crucial energy substrate for rapidly dividing cells and may act on the humoral immune response, that is, in certain sites of mucosa membranes, such as the respiratory and gastrointestinal tracts, with increase in the number of lymph nodes in mammals (Newsholme, 2001).

Aiming at contributing to broiler nutrition research and considering the lack of information in literature to glutamine this experiment evaluated the influence of dietary Gln supplementation at different levels on the performance, small intestine development, immune response and some blood parameters of broiler chickens.

MATERIALS AND METHODS

This work was carried out to investigate the effect of different dietary additional levels of $_{L}$Glutamine (Gln) on growth performance, carcass traits, some blood constituents and immune response of broiler chickens.

Birds, Accommodation and management: A total of 250 Cob 500, one-day-old broiler chicks were used in this study. The broiler chicks were randomly allotted into 5 equal groups (50 per each) of mixed sex. The chicks were housed in a clean well ventilated room, previously disinfected with formalin. The room was provided with electric heaters to adjust the environmental temperature according to the age of the birds. Feeds and water were supplied ad-libitum. Prophylactic measures against the most common infectious diseases were carried out. The chicks were vaccinated against Newcastle disease with different types of Newcastle disease vaccine as presented in Table 1.

Experimental design and feeding program: The broiler chicks were randomly allotted into 5 groups; each group of (50 per group in two replicate) received one out of the different experimental diets during the experimental period (6 weeks experiment). The treatment diets were formulated to meet the requirements of broiler chickens according to NRC, 1994. The experimental diets were formulated to be isonitrogenous and isocaloric with different levels of Gln supplementation (0.0, 0.5, 1.0, 1.5 and 2.0%) for control and treatment groups (2-5) respectively. The ingredient composition and chemical analysis (according to AOAC, 1985) of the experimental diets are presented in Table, 2.

Measurements: Body weight development, body weight gain and feed intake of broiler chicks in different groups were weekly recorded. Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Efficiency of Energy Utilization (EEU) were calculated according to Lambert *et al*., 1936; McDonald *et al*., 1987 and North (1981) respectively.

Table 1: Vaccination program of broiler chicks during the experimental period

Age (days)	Vaccine	Route of vaccination
7	Hitchner[1]	Eye Drop
12	Gumboro[2]	"
14	Killed ND[3]	I/M injection
17	Lasota[4]	Eye Drop
20	Gumboro	"
26	Lasota	"
30	Gumboro	"
35	Lasota	"

1- B1 Hitchner (Izovac), 2- Izovac Gumboro Batch No. 7125, 3- Killed N.D. (Intervet), 4- ND vaccine Lasota (Vet. Ser. And Vacc., Research Institute Cairo, Egypt)

Immune response measurements

Haemagglutination Inhibition test: Four sets of blood samples were collected from the experimental birds of each group at 14, 24, 34 and 42 days of age. Blood samples was collected without anticoagulant for separation of sera to detect the titer of antibodies against Newcastle disease vaccine using Haemaglutination Inhibition Test (HI) as an indicative of the bird's immune response in the different experimental groups. Micro technique of HI test was done according to Takatasy (1955). Geometric Mean Titre (GMT) was calculated according to Brugh (1978).

Phagocytic activity and index: Phagocytic activity was determined according to Kawadara *et al*. (1991). Fifty micrograms of Candida albicans culture were added to 1 ml of citrated blood, collected at the end of experiment by slaughtering five birds from each group .Treated blood samples were put in shaker water bath at 23-25°C for 3-5 h. Smears of blood were made and then stained with Geimsa stain. Phagocytosis was estimated by determining the proportion of macrophages which contain intracellular yeast cells in a random sample of 300 macrophages and expressed as percentage of Phagocytic Activity (PA). The number of phagocytized Candida cells was counted in the phagocytic cells to calculate the phagocytic index according to the following equations: Phagocytic activity = (Macrophages containing yeast/Total number of macrophages) X 100. While, Phagocytic index = (Number of cell phagocytized / Number of phagocytic cell) X 100.

Differential leucocytic count: This test was done at the end of experimental period as blood film was prepared according to the method described by Lucky (1977). Ten drops from May-Grunwald stain stock solution were added to equal amount of distilled water on a dry unfixed smear then mixed and left for 1 min

for staining. The dye was decanted without rinsing. Diluted Geimsa stain was poured over the film as counter stain and left for 20 min then rinsed in water current and examined by oil emersion lens. The percentage and absolute value for each type of cells were calculated according to Maxine and Benijamin (1985).

Estimated blood parameters: At the end of the experimental period, blood samples were taken from five birds of each replicate in different groups. The blood samples were left to drop on the side of the tube to prevent destruction of RBCs. Each blood sample was left to coagulate at room temp. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 10 min. The clear serum was transferred carefully to clean and dry vials and kept in deep freezer until analysis for determination of total serum protein, albumin and globulin according to Doumas *et al*. (1981); Reinhold (1953) and Coles (1974) respectively.

Carcass characteristic: At the end of the experimental period, 5 chicks from each replicate of different groups were randomly selected and scarified to calculated the carcass and dressing percentages, also collect the spleen, the liver, the thymus and the bursa and relative weight of each organ was calculated as follows: Relative weight = (organ weight/Live body weight) X 100.

Intestinal Morphology: At the end of the experimental period (day 42 of the chick's age), 5 birds from each replicate were killed to collect small intestine. The intestinal was removed and divided into three segments: duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileocecal junction). Because morphologic analysis of the duodenum and jejunum was to be determined, these segments were flashed with 20 ml of physiological saline solution and the empty weight was recorded.

For morphologic analysis, approximately 5 cm of the middle portion of the duodenum and jejunum (the apex of the duodenum and the midway between the point of entry the bile ducts and Meckel's diverticulum of the jejunum) was excised and fixed in 10% formalin. Six cross sections of 70% ethanol preserved segments for each duodenal and jejunal sample were then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures (Uni *et al*., 1995). Villus Height (VH) and Crypt Depth (CD) were measured using the Image-Pro Plus as described in details by (Touchette *et al*., 2002) and VH:CD Ratio (VCR) were calculated.

Statistical analysis: The analysis of variance for the obtained data was performed using Statistical Analysis System (SAS, 1996) to assess significant differences.

RESULTS

Body weight development and growth performance: The effect of dietary Gln supplementation on Body Weight (BW) development of broiler chicks is presented in Table, 3. The analysis of variance of the data at the start of the experiment showed that there was no significant difference in BW between different experimental groups, while there were differences between the broiler fed different levels of Gln supplementation began in the third week and more appeared at the end of the experiment. It was observed that broiler chick groups which fed on the diets containing 1% of Gln (groups, 3) showed significantly ($p \leq 0.05$) higher BW by about 8.3% when compared with the control, while chicks groups which fed on diet containing 0.5 Gln (group, 2) exhibited non significant ($p>0.05$) increased of BW by about 2.99% when compared with the control one, on the other hand the higher inclusion levels of Gln (1.5 and 2%) in broiler chicks diets (groups 4 and 5) recorded non significant ($p>0.05$) reduction in the body weight by about 0.82% and 2.2% respectively when compared with the control. The highest body weight was recorded in broiler group No. 3 (2273.34 g) which fed on 1% Gln containing diets, followed by group No. 2 (2162.11 g) which fed on 05% Gln containing diets, followed by group No. 1 (2099.35 g) which fed on the control diets and the lowest body weight was recorded in broiler chicks in group No. 5 (2053.92 g) which fed on the highest inclusion levels of Gln (2%).

Analysis of variance of the obtained data revealed that 1% Gln supplementation in broiler chickens diets significantly ($p \leq 0.05$) improved daily body gain by about 9.3% when compared with the control while lower and higher inclusion levels of Gln had no effect ($p>0.05$). Regarding feed intake, it was clear that 0.5 and 1% inclusion levels of Gln non significantly ($p>0.05$) increased daily feed intake and in contrast the intake reduced with increasing the level of Gln in broiler chickens diets. On the other hand Gln supplementation non significantly improved FCR and PER when compared with the control while 1% Gln addition significantly improved EEU by about 5.8%.

Blood picture: The effect of dietary Gln supplementation on some blood pictures are presented in Table, 4. Analysis of variance indicated that Gln supplementation up to 1% improved RBCs, WBCs counts, HB% and PCV% by about 18.9%, 14.4%, 11.0%, 12.4% respectively, when compared with control while higher inclusion levels of Gln l broiler chick diets non significantly ($p>0.05$) negatively affecting blood picture parameters.

Table 2: Ingredient composition and chemical analysis of the basal diet

	Starter Diet					Grower Diet					Finisher Diet				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Ingredients															
Yellow Corn	61.05	60.89	6.89	60.78	60.78	65.95	65.84	65.84	65.68	65.68	68.10	67.84	67.84	67.58	67.58
Soy meal (48%)	33.0	32.5	32.0	31.5	31.0	28.5	28.0	27.5	27.0	26.5	26.75	26.25	25.75	25.25	24.75
Fish meal (65%)	1.5	1.5	1.5	1.5	1.5	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5	0.5	0.5
Vegetable Oil	0.5	0.65	0.65	0.75	0.75	0.75	0.85	0.85	1.00	1.00	1.0	1.25	1.25	1.5	1.5
Limestone	1.5	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.3	1.3
Dicalcium phosphate	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Salt (NaCl)	0.25	0.25	0.25	0.2	50.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[1]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
mineral premix[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DL-methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-Lysine	0.05	0.06	0.06	0.07	0.07	0.05	0.06	0.06	0.07	0.07	0.05	0.06	0.06	0.07	0.07
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostate[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.0	0.0	0.0	0.0	0.0
¿Glutamine	0.00	0.5	1.0	1.5	2.0	0.0	0.5	1.0	1.5	2.0	0.0	0.5	1.0	1.5	2.0
Chemical Analysis															
Moisture%	11.0	11.1	11.2	11.2	11.0	10.8	10.6	11.4	11.3	11.1	11.2	11.0	10.7	10.99	11.3
Crude protein%	22.0	22.1	22.1	22.2	22.2	20.1	20.1	20.2	20.2	20.3	19.0	19.1	19.2	19.2	19.2
Ether extract%	3.12	3.19	3.18	3.24	3.26	3.52	3.59	3.58	3.71	3.69	3.88	3.89	4.02	4.26	4.21
Ash%	6.53	6.61	6.72	6.59	6.58	6.33	6.72	6.56	6.42	6.38	6.29	6.38	6.56	6.33	6.61
ME Kcal/Kg[4]	2984	2982	2986	2988	2992	3057	3053	3053	3061	3065	3086	3090	3090	3102	3102
C/P ratio	136	135	135	135	135	153	152	152	152	151	162	162	161	162	162
DL- Methionie%[4]	0.50	0.49	0.49	0.49	0.49	0.47	0.46	0.46	0.46	0.46	0.44	0.43	0.43	0.43	0.43
L- Lysine%[4]	1.17	1.15	1.15	1.14	1.14	1.01	1.00	1.00	0.98	0.98	0.94	0.93	0.93	0.92	0.92

[1]Vitamin premix produced by Centraly's Co (France) and contain the following vitamins per Kg premix (vitamin A, 12000000, IU; Vitamin D, 2000000 IU; Vitamin E, 20000 mg; Vitamin K, 2000 mg; vitamin B12, 10 mg; Biotin, 200 mg; Folacin, 1000 mg; Niacin, 30000 mg; pantothenic acid, 10000 mg; pyridoxine, 4000 mg; ribroflvin, 5000 mg; thiamin, 2000 mg and proper dose of antitoxidant).

[2]Mineral premix produced by Centraly's Co. (contain the following minerals per Kg, Cobalt, 100 mg; Copper, 10000 mg; Iron, 50000 mg; Iodine, 500 mg; manganese, 85000 mg; zinc, 65000 mg and selenium, 200 mg).

[3]Coccidiostate (Salinomycin 12%, produced by Pfizer Co., USA and used at 0.5 Kg per ton diet during starter and grower periods).

[4]Metabolizable energy (ME), DL-Methionie and lysine content were calculated according the NRC (1994).

Phagocytic activity and differential leucocytes counts: Effect of dietary Gln supplementation on phagocytic activity and index as well as on differential leucocytes counts are presented in Table, 6. The data revealed that Gln supplementation at 1% of broiler chick diets significantly ($p \leq 0.05$) improved phagocytic activity and index by about 21.3 and 13.1% respectively, when compared with the control, while the lower and higher inclusion levels of Gln had no significant ($p>0.05$) effect on phagocytic parameters. Moreover, 1% of Gln significantly ($p \leq 0.05$) increased lymphocytes counts compared with the control, but had no significant effect on other types of WBC.

Hemagglutination Inhibition test (HI) to Newcastle disease vaccine: Table 7, illustrates the effects of dietary Gln supplementation on the results of HI antibody titer to Newcastle disease vaccine of broiler chickens. The analysis of variance of the obtained data showed non significant ($p>0.05$) variations in HI titer at 14th day of broiler chickens fed on the basal diet or supplemented with Gln at different levels. Moreover, the data revealed that 1% of Gln supplementation significantly ($p \leq 0.05$) increased HI at 24th, 34th and 42nd days when compared with the control, while 0.5% Gln supplementation non significantly ($p>0.05$) improved HI and 1.5 or 2% Gln supplementation non significantly ($p>0.05$) decreased HI when compared with the control.

Effect of Gln supplementation on serum protein, albumin and globulin levels: The effects of dietary Gln supplementation on serum total protein, albumin, globulin and Albumin/globulin (A/G) ratio of broiler chickens in different groups are illustrated in Table, 8. Analysis of variance of the data revealed that Gln supplementation at 1% (Group 3) significantly ($p \leq 0.05$) increased serum total protein and albumin concentration by about 16.1 and 20.4% respectively when compared with the control, while the other inclusion levels of Gln had no significant ($p>0.05$) effect on the mentioned parameters. However, Gln supplementation at different levels had no significant effect on serum globulin and A/G ratio.

Carcass characteristics: The effects of dietary Gln supplementation on dressing percent and some organs (liver, spleen, bursa, thymus gland, duodenum and jejunum) weights relative to the live body weight of broiler chicks in different groups at the end of experimental period are summarized in Table, 9. Analysis of variance of the data revealed that Gln supplementation had no effect on dressing percent, liver and thymus gland weights relative to the live body weight. While, 1% of Gln supplementation significantly ($p \leq 0.05$) increased spleen index and bursa index by about 30.8 and 27.8% respectively when compared with control. However, 0.5% of Gln supplementation non significantly ($p>0.05$) improved of spleen index and bursa index by about 15.4 and 11.1% respectively when compared with control. On the other hand higher inclusion levels of Gln supplementation (1.5 and 2%) exhibited a non significant ($p>0.05$) reduction of immune organs relative weights when compared with the control.

Table 3: Effect of dietary L-Glutamine supplementation on body weight development (g/bird) of broiler chickens in different experimental groups

	Glutamine supplementation %				
	0	0.5	1.0	1.5	2.0
	No. of observations				
Age (Week)	50	50	50	50	50
W0	41.99±0.45^{a}	41.54±0.92^{a}	42.11±0.44^{a}	41.81±0.81^{a}	41.69±0.52^{a}
W1	155.21±2.87^{a}	162.11±2.95^{a}	168.21±3.39^{a}	154.50±3.96^{a}	156.31±3.56^{a}
W2	408.92±5.98^{a}	410.21±7.83^{a}	428.30±8.12^{a}	407.50±6.92^{a}	406.31±7.52^{a}
W3	757.68±12.93^{b}	770.32±13.08^{b}	799.62±12.08^{a}	750.34±12.09^{b}	741.43±16.32^{b}
W4	1180.97±20.04^{ab}	1198.98±25.23^{ab}	1260.19±26.87^{a}	1165.20±31.65^{b}	1115.99±27.65^{b}
W5	1587.31±25.87^{b}	1661.22±36.21^{ab}	1715.33±32.45^{a}	1590.02±31.6^{b}	1540.29±28.56^{b}
W6	2099.35±45.22^{b}	2162.11±41.67^{b}	2273.34±42.76^{a}	2082.15±41.98^{b}	2053.92±39.54^{b}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Table 4: Effect of dietary L-Glutamine supplementation on growth performance parameters of broiler chickens in different experimental groups

	Glutamine supplementation %				
Items	0	0.5	1.0	1.5	2.0
T. body gain (TBG g/bird)	2056.36±27.56^{b}	2120.07±29.92^{b}	2230.65±30.11^{a}	2041.06±33.12^{b}	2012.76±29.65^{b}
TBG (RTC)*	100	+ 3.1	+ 8.5	- 0.74	- 2.12
Daily body gain (g/bird)	48.86±0.92^{b}	50.48±0.87^{b}	53.42±0.93^{a}	48.68±1.15^{b}	47.92±0.76^{b}
Daily feed intake (DFI g/bird)	90.35±2.72^{a}	91.95±2.86^{a}	92.84±4.40^{a}	89.15±4.25^{a}	87.75±4.25^{a}
DFI (RTC)	100	+ 1.77	+ 2.76	- 1.3	- 2.88
Feed conversion ratio (FCR)	1.85±0.03^{a}	1.82±0.04^{a}	1.75±0.05^{a}	1.83±0.04^{a}	1.83±0.03^{a}
FCR (RTC)	100	- 1.6	- 5.4	- 1.1	- 1.1
Protein efficiency ratio (PER)	2.74±0.13^{a}	2.77±0.14^{a}	2.89±0.15^{a}	2.74±0.14^{a}	2.73±0.13^{a}
EEU **	5.66±0.43^{b}	5.58±0.54^{ab}	5.33±0.35^{a}	5.63±0.24^{b}	5.63±0.36^{b}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$). * RTC = Relative to control. ** EEU = Efficiency of energy utilization.

Table 5: Effect of dietary L-Glutamine supplementation on blood picture {erythrocyte count (RBCs), leucocytes counts (WBCs), Hemoglobin (Hb) and packed cell volume (PCV) %} of broiler chickens

	Glutamine supplementation %				
	0	0.5	1.0	1.5	2.0
	No. of observations				
Parameters	5	5	5	5	5
RBCs Count(10^{6})	1.85±0.18^{b}	2.01±0.2^{ab}	2.20±0.2^{a}	1.95±0.41^{b}	1.88±0.43^{b}
WBCs count(10^{3})	20.71±1.23^{b}	22.50±1.13^{ab}	23.70±1.21^{a}	20.11±0.98^{b}	21.33±1.03^{b}
HB%	11.01±0.85^{b}	11.33±0.67^{ab}	12.22±0.87^{a}	11.51±0.72^{ab}	9.75±0.78^{b}
PCV%	31.25±1.32^{b}	32.11±.25^{b}1	35.11±1.11^{a}	27.97±0.87^{c}	28.22±1.25^{bc}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Intestinal morphology: Effect of Gln supplementation on intestinal tract morphology of broiler chicks is presented in Table, 10. It was clear that chicks fed on diets supplemented by Gln on at different levels (groups 2-5) had heavier duodenum and jejunum relative weight by about (15.3, 23.7, 27.5 and 34.5%) and (5.2, 16.7, 20.6 and 22.3%) respectively when compared with control.

The morphometric parameters are presented in Table, 10. The result indicated the chicks fed on GLn supplemented diet (groups 2-5) had longer duodenum and jejunum VH by about (11.5, 14.9, 17.6 and 22.3%) and (35.9, 60.0, 63.5 and 72.0%) respectively when compared with the control while, in contrast to the VH Gln supplementation decreased CD and consequently increased VH: CD ratio when compared with the control.

DISCUSSION

Significant improvements in body weight gain were observed when 1% Gln was supplemented in the broiler chick diets as compared with the birds fed control diets (an average 8.3% increase). These finding in agreement with those obtained by (Yi *et al*., 2005) reported better feed efficiency, weight gain of broilers fed 1% Gln and (Barelee and Batal, 2007) who concluded that 1% Gln supplementation in the broiler feed significantly improved body weight gain by about 11%. The data are

Table 6: Effect of dietary $_{L}$-Glutamine supplementation on phagocytic activity, phagocytic index and differential leucocytes count % of broiler chick groups

	Glutamine supplementation %				
	0	0.5	1.0	1.5	2.0
	No. of observations				
Parameters	10	10	10	10	10
Phagocytic activity	18.23±0.54^{b}	19.23±0.49^{b}	22.11±0.76^{a}	19.75±0.65^{ab}	17.75±0.65^{b}
Phagocytic index	1.53±0.19^{bc}	1.57±0.16^{bc}	1.73±0.17^{a}	1.62±0.14^{b}	1.43±0.12^{c}
Lymphocytes	42.11±0.89^{b}	42.10±0.96^{b}	46.21±1.02^{a}	43.22±0.82^{ab}	42.22±0.88^{b}
Monocytes	1.22±0.20^{a}	1.42±0.19^{a}	1.53±0.38^{a}	1.20±0.06^{a}	1.11±0.15^{a}
Basophile	8.30±0.51^{a}	7.51±0.35^{a}	7.34±0.66^{a}	7.50±0.52^{a}	7.35±0.68^{a}
Eosinophil	10.20±0.93^{a}	9.22±0.49^{a}	8.50±0.75^{a}	8.33±0.90^{a}	8.75±0.78^{a}
Neutrophil	38.17±1.45^{ab}	39.75±1.23^{a}	36.42±1.03^{b}	39.75±0.88^{a}	40.57±1.41^{a}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Table 7: Effect of dietary $_{L}$-Glutamine supplementation on hemagglutination inhibition (HI), Geometric mean antibody titer (log_2) against ND virus vaccine of broiler chickens

	Glutamine supplementation %				
	0	0.5	1.0	1.5	2.0
	No. of observations				
Age/day	10	10	10	10	10
14	1.53±0.23^{a}	1.61±0.31^{a}	1.37±0.31^{a}	1.43±0.25^{a}	1.71±0.34^{a}
24	2.35±0.05^{b}	2.42±0.11^{b}	3.10±0.07^{a}	2.11±0.05^{bc}	1.43±0.09^{c}
34	3.02±0.12^{b}	3.52±0.23^{ab}	3.72±0.15^{a}	3.51±0.24^{ab}	2.98±0.32^{b}
42	4.35±0.07^{b}	4.62±0.09^{b}	5.34±0.14^{a}	4.42±0.09^{b}	3.98±0.11^{b}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

disagreement with those obtained by (Maiorka *et al*., 2000; Kitt *et al*., 2002 and Sakamoto *et al*., 2006) who indicated that there was no effect (p>0.5) of 1% Gln on broiler performance during the studied rearing periods. An improvement in FCR, PER and EEU were observed by 1% Gln supplementation, these results are in agreement with (Yi *et al*., 2001) when they fed turkey poults on a diet supplemented with 1% Gln. The weight depression observed in chicks fed diets supplemented with 1.5 and 2.0% Gln may indicate a toxic effect when supplemented at higher levels in the feed. The weight depression in chicks which fed on higher levels of Gln may also indicate that the high levels of Gln have an effect and depress feed intake. Because there was no difference in feed efficiency but an effect on weight gain, it is clear that there was a decrease in feed intake.

In regards to blood picture parameters, the present experiment indicated that 1% Gln supplementation significantly ($p \leq 0.05$) increased RBCs and WBCs counts and improved HB%, increased the lymphocyte counts and improved the phagocytic function in the broiler chicks. Those indicate that Gln is important for lymphocyte proliferations, cytokine production as well as for the activities of phagocytosis and secretion by the macrophages (Newsholme, 2001; Sakamoto *et al*., 2006). These reasons could therefore justify the increase in lymphoid organs relative weights observed in the present experiment. The observed immune response of broiler chickens fed on 1% Gln supplemented diets was supported by recent study which revealed that the Gln intake increases the function of peritoneal macrophages and hemopoiesis in early weaned mice through increased leuckocyes and lymphocyte counts in the peripheral blood and granulocyte and lymphocyte counts in the bone marrow and spleen (Rogero *et al*., 2008).

In terms of humoral immune response, the results indicated that birds fed on diets containing1% Gln significantly ($p \leq 0.05$) more produced antibodies, while the higher levels had negative effect on humoral immune response of broiler chickens. These data are in agreement with those obtained by (Sakamoto *et al*., 2006) who stated that birds treated wit 1% Gln during the first week of age produced more antibodies and (Barelee and Batal, 2007) reported that the birds fed diets supplemented with 1% Gln for 7 days or more had significantly higher IgA concentrations in the serum and bile than the control, while the birds fed diets supplemented with 4% Gln had significantly lower IgA concentrations in the serum. This may indicate that the birds fed on diets supplemented with 1% Gln had better health and thus may be more resistant to infection.

Regarding some blood serum parameters, it was observed that 1% Gln supplementation significantly increased serum total protein and albumin concentration, while other inclusion levels of Gln in

Table 8: Effect of dietary L-Glutamine supplementation on some blood parameters of broiler chickens

	Glutamine supplementation %				
	0	0.5	1.0	1.5	2.0
	No. of observations				
Parameters	10	10	10	10	10
Total protein (g/dl)	3.67±0.12^{b}	3.89±0.17^{ab}	4.26±0.18^{a}	3.98±0.21^{ab}	3.83±0.19^{b}
Albumin (g/dl)	2.01±0.08^{b}	2.12±0.10^{b}	2.42±0.06^{a}	2.18±0.15^{ab}	2.09±0.10^{b}
Globulin (g/dl)	1.67±0.16^{a}	1.77±0.11^{a}	1.84±0.16^{a}	1.80±0.18^{a}	1.74±0.18^{a}
A/G Ratio	1.20	1.20	1.32	1.21	1.20

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Table 9: Effect of dietary L-Glutamine supplementation on some carcass traits of broiler chickens

	Glutamine supplementation %				
	0	0.5	1.0	1.5	2.0
	No. of observations				
Parameters	10	10	10	10	10
Dressing %	71.92±1.32^{a}	72.31±1.45^{a}	72.89±1.64^{a}	69.99±1.45^{a}	70.13±1.65^{a}
Liver relative weight	2.19±0.24^{a}	2.21±0.32^{a}	2.31±0.16^{a}	2.11±0.24^{a}	2.08±0.33^{a}
Spleen index	0.13±0.02^{b}	0.15±0.04^{ab}	0.17±0.02^{a}	0.11±0.01^{b}	0.10±0.01^{b}
Bursa index	0.18±0.06^{b}	0.20±0.01^{ab}	0.23±0.01^{a}	0.16±0.02^{b}	0.14±0.01^{b}
Thymus gland index	0.44±0.01^{a}	0.45±0.0^{a}	0.46±0.01^{a}	0.43±0.01^{a}	0.43±0.02^{a}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

Table 10: Effect of dietary L-Glutamine supplementation on intestinal morphology of broiler chickens

	Glutamine supplementation %				
	0	0.5	1.0	1.5	2.0
	No. of observations				
Parameters	10	10	10	10	10
Duodenum relative weight	1.31±0.02^{b}	1.51±0.01^{ab}	1.62±0.01^{a}	1.67±0.02^{a}	1.76±0.02^{a}
Jejunum relative weight	2.33±0.21^{b}	2.45±0.22^{ab}	2.72±0.23^{a}	2.81±0.15^{a}	2.85±0.13^{a}
Duodenal Villi Height (μm) (VH)	890.7±76.8^{b}	992.7±65.2^{ab}	1023.1±66.2^{a}	1047.1±71.3^{a}	1089.5±69.3^{a}
Duodenal Crypt Depth (μm) (CD)	173.4±5.9^{a}	150.3±6.2^{ab}	141.4±4.7^{ab}	134.9±5.7^{b}	129.2±4.9^{b}
Duodenal VH:CD ratio	5.2	6.6	7.2	7.8	8.4
Jejunual Villi Height (μm) (VH)	456.5±43.9^{b}	620.5±46.1^{b}	730.4±67.2^{a}	746.6±57.3^{a}	785.4±68.9^{a}
Jejunual Crypt Depth (μm) (CD)	101.2±7.4^{a}	95.4±5.6^{a}	91.5±5.1^{a}	90.2±4.9^{a}	87.4±6.3^{a}
Jejunual VH:CD ratio	4.5	6.5	8.0	8.3	8.9

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$)

broiler diets had no effect on those parameters. These data indicated that Gln supplementation improve protein synthesis. However, this statement must be further studied and evaluated.

In regards to carcass traits, the data indicated the Gln supplementation had no effect on dressing percent and liver weight %. While immune tissue development is the basis of immune functionally. The supplementation of 1% Gln in diets fed to chicks significantly improved the growth of spleen and bursa but non significantly improved the thymus growth. Te increase in immune tissue weight resulting from 1% Gln supplementation correlated with the antibody production and phagocytosis activity and also confirmed that reduction of immune organs relative weights in birds fed on higher levels of Gln related with lower antibody production and negative effect on chicken immune response. The results of this experiment are supported by previous studies which reported that improvement of spleen and thymus weights % in broiler chicks fed on diets supplemented with 1% Gln when compared with control (Sakamoto *et al*., 2006; Barelee and Batal, 2007). As regards the intestinal morphology of broiler chickens, independent (Uni, 1999; Kondo, 2003; Murakami *et al*., 2007), also observed greater development of the duodenum in relation to other intestinal segments. This greater development can be attributed to the fact that it is the segment with the fastest cell renewal and is also the first segment of the small intestine to receive physical, chemical and hormonal stimuli provoked by the presence of the diet in the lumen. The present study indicated that birds fed on diets supplemented by different levels of Gln had an heavier duodenum and jejunum relative weights and longer intestinal villi than

the results recorded in chicks fed on the basal diets without Gln supplementation and the results supported by many previous studies showed the same effect of Gln supplementation in broiler chicks diets (Barelee and Batal, 2007; Murakami *et al*., 2007). The heavier intestinal segments weights may be reflected on longer intestinal villi than the intestinal villi of birds fed the control diets and then the chick may be able to utilize nutrients more efficiently earlier in life and thus have improved growth performance (Lilja, 1983; Nitsam *et al*., 1991). The longer villi and heavier intestinal relative weights may be also combined with increasing surface area and consequently greater nutrient absorption and improved weight gain that were observed due to Gln supplementation. Although villus height is correlated positively with BW gain and feed intake (Kelly *et al*., 2001), the same can not be observed in this experiment because the performance parameters (BW gain, feed intake and FCR) did not present significant effect in relation to the treatments and the birds fed diets supplemented with 1.5 or 2.0% Gln had increased intestinal weight in comparison with the control or with the lower inclusion levels of Gln, but they had the lowest growth performance. This may be suggest that in fact increased intestinal weight and surface area does not necessarily lead to increased nutrint utilization and then increased performance or may be due to highly decreased intestinal Crypt Depth (CD) with higher levels of Gln supplementation.

The results give in sights into a potential dietary method to modulate chicken immunity responses toward improving chicken performance. For example, the inflammatory response is the first line of defense against novel pathogens, but cells and mediators of the inflammatory responses have been implicated in the pathology of many poultry diseases, including coccidiosis (Trout and Lillehoj, 1993). Modification of antibody production and activity by dietary Gln supplementation may provide an avenue to strengthen the chick's immunity and protection against various pathogens. However, long term effects of immunomoduation induced by Gln supplementation on the chick resistance and performance remain to be needed further investigation.

Conclusion: In conclusion, the results of this study suggest that 1% of dietary Gln supplementation improved growth performance, humoral, cellular immune response and may stimulate development of the gastrointestinal tract in broiler chickens while higher level had negative effects.

REFERENCES

Adjei, A.A., Y. Matsumoto, T. Oku, Y. Hiroi and Y. Yamamoto, 1994. Dietary arginine and glutamine combination improves survival in spetic mice. Nutr. Res., 14: 1591-1599.

Andrews, F.J. and R.D. Griffiths, 2002. Glutamine: Essential for immune nutrition in the critically ill. Br. J. Nutr., 51: 3-8.

AOAC (Official methods of analysis. Association of Official Analytical Chemists), 1985. 14th Ed. Washington, D.C.

Barelee, S.M. and A.B. Batal, 2007. The effect of supplemental glutamine on growth performace, development of the gastrointestinal tract and immune response of broilers. Poult. Sci., 86: 1940-1947.

Brugh, M.G.A., 1978. Simple method for recording and analyzing serological data. Avian Disease, 22: 362-365.

Coles, E.H., 1974. Veterinary Clinical Pathology. Pp. 211-213. W.B. Saunders, Company, Philadelphia, London, Toronto.

Cynober, L.A., 1999. Glutamine metabolism in stressed patients (Abstract). Page 5 in Proc. Int. Congr. Amino Acids (Germany). Springer-Verlag, Vienna, Austria.

Dibner, J.J. and J.D. Richards, 2004. The digestive system: challenges and opportunities. J. Appl. Poult. Res., 13: 86-93.

Doumas, B.T., D.D. Bayso, R.J. Carter, T. Peters and R. Schaffer, 1981. Determination of total serum protein. Clin. Chem., 27: 1642-1643.

Inoue, Y. Grant J.P. and M.H. Snyder, 1993. Effect of glutamine supplemented intravenous nutrition on survival after E. coli induced peritonitis. J. Parenter. Enteral Nutr., 17: 41-46.

Kawadara, E., T. Ueda and S. Nomura, 1991. In vitro phagocytic activity of white spotted shark cells after injection with Aeromoas salmonicida extraceular products. Gyobyo, Kenkyu, Japan , 26: 213-214.

Kelly, D., J.A. Smith and K.J. McCraken, 2001. Digestive development of the early weaned pig. 1. Effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. Br. J. Nutr., 65: 169-180.

Kitt, J., P.S. Miller, A.J. Lewis and R.L. Fischer, 2002. Effects of glutamine on growth performance and small intestine villus height in weanling pigs.Pages 29-32 in Nebraska Swine Rep. Univ. Nebraska, London.

Kondo, N., 2003. Esrudo das carateristicas morfometricas de diferentes regioes do intestine Delgado e indices zootecnicos em quarto linhagens de frangos de corte.tese (Doutorado) Fac. Med. Vet. Zoot. Univ. Estadual Paulista, Botucatu, Brazil.

Labow, B.L., 2001. Mechanisms governing th expression of the enzymes of glutamine metabolism-glutaminase and glutamine synthetase. J. Nutr., 131: 2467-2474.

Lambert, W.V., N.R. Ellis, W.H. Block and H.W. Titus, 1936. The role of nutrition in genetics. Am. Res. Sco. Anim. Prod., 29: 236.

Lilja, C., 1983. A comparative study of postnatal growth and organ development in some species of birds. Growth, 47: 317-339.

Lucky, Z., 1975. Methods for diagnosis of fish disease. Ameruno publishing Co, PVT, Ltd New Delhi, Bomby, New York.

Maiorka, A., A.V.F. Slila, E. Santin, S.A. Borges, I.C. Boleli and M. Macari, 2000. Influencia da suplementacao de glutamina sobre o desempenho e o desenvolvimento de vilos e criptas do intestino delgado de frangos. Arq. Bras. Med. Vet. Zoot., 52: 487-490.

Maxine, M. and B.S. Benijamin, 1985. Outline of veterinary clinical pathology 3rd Ed., Colorado State University.

McDonald, P., R.A. Edwards and J.F.D. Greenhalgh, 1987. Animal Nutrition Text Book. English Language Book Socity/Longman.

Medina, M.A., 2001. Glutamine and cancer. J. Nutr., 131: 2539-2542.

Murakami, A.E., M.I. Sakamoto, M.R.M. Natali, L.M.G. Souza L.M.G. and J.R.G. Farnco, 2007. Supplementation of glutamine and vitamin E on the morphometry of the intestinal mucosa in broiler chickens. Poult. Sci., 86: 488-495.

Newsholme, P., B. Crabtree and M.S. Andrawi, 1985. Glutamine metabolism in lymphocytes: Its biochemical physiological and clinical importance. Q. J. Experimental Physiol., 70: 473-489.

Newsholme, P., 2001. Why is L-glutamine metabolism important to cells of the immune system in health post-immune, surgery or infection? J. Nutr., 131: 2515-2522.

Nitsam, Z.G., B. Avraham, Z. Zoref and I. Nir, 1991. Organ growth and digestive enzymes levels to fifteen days of age in lines of chickens differing in body weight. Poult. Sci., 70: 2040-2048.

North, M.O., 1981. Commercial chicken production. Annual 2th edition, AV., Publication Company I.N.C., Westpost Connecticut, USA.

NRC, 1994. National Research Council: Nutrient requirements of poultry, 9th Ed National Academy press, Washington, D.

Reinhold, R.R., 1953. Determination of serum albumin. Clin. Chem., 21: 1370-1372.

Rennie, M.J., 2001. Interaction between glutamine availability and metabolism of glycogen, tricarboxylic acid cycle intermediates and glutathione. J. Nutr., 131: 2488-2490.

Rogero, M.M., J. Tirapegui,M.A. Vinolo, M.C. Borges,I.A. De Castro, I.S. Pries and P. Borelli, 2008. Dietary glutamine supplementation increases the activity of peritoneal macrophages and hemopoiesis in early-weaned mice inoculated with Mycobacterium bovis bacillus Calmette-Guerin. J. Nutr., 38: 1343-1348.

Sakamoto, M.I., A.E. Murakami, T.G.V. Silveira, J.I.M. Fernandes and C.A.L. Oliveira, 2006. Influence of glutamine and vitamin E on the performance and the immune response of broiler chickens. Brazilian J. Poult. Sci., 8: 243-249.

SAS, 1996. Statistical Analysis System. Users Guide Statistics. As. Institute Cary, North Carolina.

Souba, W.W., 1993. Glutamine and cancer. Ann. Surg., 218: 715-728.

Takatasy, G.Y., 1955. The use of 100 M in serological and virological micro methods.

Touchette, K.J., J.A. Carroll,G.L. Allee, R.L. Matteri, C.J. Duer, L.A. Beausang and M.E. Zamelli, 2002. Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. J. Anim. Sci., 80: 494-501.

Trout, J.M. and H.S. Lillehoj, 1993. Evidence of a role of intestinal CD8+ lymphocytes and macrophages in transport of Eimeria acervulina sporozoites. J. Parisitol., 74: 1622-1629.

Uni, Z., Y. Noy and D. Sklan, 1995. Posthatch changes in morphology and function of the small intestines in heavy and light strain chicks. Poult. Sci., 74: 1622-1629.

Uni, Z., 1999. Functional development of the small intestine in domestic birds: Cellular and molecular aspects. Poul. Avian Biol. Rev., 10: 167-179.

Yi, G.F., G.L. Allee, J.W. Frank, J.D. Spencer and K.J. Touchette, 2001. Impact of glutamine, menhadan fish mea land spray-dried plasma on the growth performance and intestinal morphology of broilers. Poult. Sci., 80 (supp. 1) : 201. (Abstr.).

Yi, G.F., G.L. Allee, C.D. Knight and J.J. Dibner, 2005. Impact of glutamine and oasis hatching supplement on growth performance, small intestinal morphology ans immune response of broiler vaccinated and challenged with Eimeria maxima. Poult. Sci., 84: 283-293.