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Effect of Dietary Supplementation of Glutamine on the Growth, Immunocompetence Traits, Blood Biochemical Attributes and Histopathology of Turkey Poults

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ABSTRACT

Background: Dietary glutamine (Gln) supplementation leads to beneficial morphological and physiological effects on the gastrointestinal tract of broilers, particularly during early development. The present study was designed to study the effect of different levels of Gln supplementation on growth, immunocompetence traits, blood biochemical attributes and histopathology of turkey poults.

Methods: 84 day old turkey poults were distributed into four dietary treatment groups, having three replicates of 7 birds each. Birds of control group (T1) were fed a basal diet, while T2 group was supplemented with 0.5% Gln along with basal diet, T3 group was supplemented with 1% Gln with basal diet and T4 group was supplemented with 1.5% Gln along with basal diet.

Result: T3 poults had significantly (P<0.05) better FCR than other treatment groups. HA titre and IgM response was significantly (P<0.05) higher in T3 as compared to other groups. Similarly, IgG response was comparatively better in T3 group as compared to other groups after 8 weeks of age. Serum IgG, IgM and cortisol level was significantly (P<0.05) higher in T3 as compared to T1 and comparatively higher than other treatment groups. Total plasma protein and ALP values was significantly (P<0.05) higher in T2 as compared to T1 and comparatively higher than T3 and T4. Serum SOD value was significantly higher (P<0.05) in T2 than other treatment groups. LPO value was significantly (P<0.05) higher in control group as compared to glutamine supplemented groups. Supplementation of Gln at different levels showed normal histoarchitecture of both liver and spleen in all treatment groups.

Key words: Cell mediated immunity, Glutamine, Growth, Humoral immunity.

INTRODUCTION

Glutamine (Gln) is traditionally considered as a non-essential amino acid. However, several studies have shown that Gln may be a conditionally essential amino acid in maintaining gut integrity and reducing inflammation (Reeds and Burrin, 2000). Glutamine enriched diets have been linked with favorable intestinal effects including maintenance of gut barrier function, enterocyte differentiation and increases height of villi (Yi *et al.,* 2001).

Sell *et al.* (1991) pointed out that at the time of hatching, the weight of the small intestine represents 1.2 to 2.6% of the body weight of the bird and 6.2 to 6.6% at maximum development between day 5 and 7 after hatch (Murakami *et al.,* 2007). Hence the immaturity of the GIT in the first week after hatch is a limiting factor, since major gut transitions like increase in absorption capacity with a relative increase in the area of absorption through the longitudinal growth of the intestine and increase in the height of the villi, proper secretion of enzymes are events yet to happen. Thus, stimulation of the GIT by different substrates, soon after hatching, can accelerate its development.

Feed cost occupies the major portion of recurring investment in poultry production. Dietary supplementation of glutamine favours proper utilization of feed at bird level and thereby reduces required nutrient density and feed cost. Strengthening the gut integrity of birds is one of the ways for better assimilation of nutrients in feed. Gln is gradually emerging as one of the potential feed additives because of their ability in enhancing the villous growth of intestine. In situations of stress, Gln can be decisive for maintenance of intestinal structure and function, being

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employed by isolated cells of the immune system for lymphocyte proliferation and cytokine production (Newsholme, 2001).Gln supplementation improves oxidative damage and immunity (Bai *et al.,* 2023; Ayazi, 2014).

Dietary Gln supplementation has been previously investigated, revealing beneficial morphological and physiological effects on the gastrointestinal tract of broilers, particularly during early development. However, despite the positive effects of Gln supplementation on intestinal quality in poultry, its benefits regarding performance are not consistently evident when birds reach the age of slaughter (Maiorka *et al.,* 2000; Zavarize *et al.,* 2011; Martinez *et al.,* 2012; Kishawy *et al.,* 2024). Further, studies on dietary supplementation of Gln in turkeys are lacking. Hence, the current study was designed to study the effect of different levels of Gln supplementation on thegrowth, immuno-competence traits, histology and blood biochemical attributes of turkey poults.

MATERIALS AND METHODS

A total of eighty four straight run turkey poults were randomly distributed into four dietary treatments having three replicates each with seven turkey poults. The birds of the control group (T1) were fed a basal diet (turkey starter ration; National Research Council, 1994), while T2 group was supplemented with 0.5% glutamine along with the basal diet, T3 group was supplemented with 1% glutamine with the basal diet and T4 group was supplemented with 1.5% glutamine along with basal diet from day old to 8 weeks of age. The poults were housed in deep litter system. Water was offered *ad lib*. Glutamine was procured from Sisco Research Laboratories Pvt. Ltd. (SRL), India. The experiment was conductedafter due approval of IAEC.

Growth performance parameters

Weekly body weight and group feed consumption were recorded. Thereafter, body weight gain, feed consumption and feed conversion ratio (FCR) (feed consumption: weight gain) during 0-4 weeks, 4-8 weeks and 0-8 weeks of age were calculated.

Immunocompetence traits

The general innate immune-competence status of turkey poults was assayed by measuring two important immunocompetence traits, as antibody response to SRBC. The micro-titre plate haemagglutination procedure as described by Siegel and Gross (1980) with slight modifications was followed to measure total HA antibody titres in turkey poults on day zero and day 5th post injection. The antibody titre was determined by HA methods (Vander Zijpp, 1983; Siegel and Gross, 1980).

2-Mercaptoethanol resistant antibodies (MER or IgG) against 1% SRBCwere determined by means of a mercaptoethanol (ME) HA test as per the method described by Martin *et al.* (1989). The reduction of total titre due to 2-ME treatment was called 2-ME sensitive antibody (MES or IgM) and the titre was expressed as log 2 value (Total HA titre-MER=MES). The cell mediated immune response to PHA-P (Phytohaemagglutinin, lectin from *Phaseolus vulgaris*) after 8 weeks of age (Corrier and De Loach, 1990) was determined by Foot web index using the formula:

Foot web index= (thickness after 24 hrs injection of PHA-P of right foot - thickness before injection of the same foot) - (thickness after 24 hrs of injection of PBS of left foot - thickness before 24 hrs of injection of PBS of the same foot).

Serum IgG and IgM concentration

Serum IgG and IgM concentration of turkeys were determined by Chicken Immunoglobulin ELISA Test kit (Bioassay Technology Laboratory, Shanghai, China).

Blood biochemical parameters

After 8 weeks of age, serum prepared from the blood of 6 turkey poults were analyzed for the estimation of protein, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), cholesterol and HDL Cholesterol by using standard biochemical kits (Span Cogent Diagnostics product).

Superoxide dismutase (SOD) activity in serum was measured using the method as described by Madesh and Balasubramanian (1998) with some modifications.

$$\text{SOD activity (units/ml)} = 2 \times 100 \text{ X AT/AB}$$

Where,
AT= Absorbance for test.
AB= Absorbance for blank.

Lipid peroxidation/MDA (Malondialdehyde assay) estimation was done by the TBARS (Thio Barbituric Acid Reactive Substance) method of Ohkawa *et al.* (1979). Serum cortisol concentration of turkeys was determined by Chicken Immunoglobulin ELISA Test kit (Bioassay Technology Laboratory, Shanghai, China).

Histopathology

After 8 weeks of age, tissue samples of liver and spleen of 4 birds from each group were collected in 10% buffered formalin saline for histological examination. 4-5 micron thick tissue sections were cut from the paraffin embedded tissues and stained with haematoxylin and eosin stain (H and E) for routine histopathology (Luna 1968).

Statistical analysis

The data pertaining to various parameters were analyzed statistically as per the standard procedure (Snedecor and Cochran, 1989) and difference between the treatment means were obtained by using Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance parameters

Poults fed with T3 diet had a comparatively higher body weight gain than other treatment groupsduring 0-4, 4-8 and 0-8 weeks of age (Table 1). The results are in agreement with the

findings of Bartell and Batal (2007); Avellaneda *et al.* (2008) and Dai *et al.* (2009) who reported a significant improvement in body weight gain in broiler chickens supplemented with Gln than non supplemented group. On the contrary, Maiorka *et al.* (2000), Sakamoto *et al.* (2006) and Soltan (2009) found that Gln did not increase weight gain in poultry.

Data on phase wise feed consumption revealed that T1 poults had a comparatively higher feedconsumption than othertreatment groups at 0-4 weeks of age (Table 2). Further, T1 poults had asignificantly (P<0.05) higher feed consumption than T2 and comparatively higher feed consumption than other treatment groups during 4-8 and 0-8 weeks of experiment. The results of the present experiment are in agreement with results of other studies (Murakami *et al.,* 2007; Sakamato *et al.,* 2006), who observed no significant increase in feed intake in broilers fed diets with 1% dietary Gln.

Table 1: Effect of glutamine supplementation on body weight gain (g) of turkey poults at different phases of growth during 0-8 weeks of age.

Treatment	0 to 4 weeks	4 to 8 weeks	0 to 8 weeks
T_1	229.23	621.33	850.57
T_2	238.66	633.52	872.19
T_3	250.00	671.61	921.61
T_4	229.52	631.04	860.57
Pooled SEM	5.49	10.95	15.26
Sig. level	NS	NS	NS

Means bearing different superscripts within a column differ significantly (P<0.05).

NS: Not significant (P>0.05); SEM: Standard error of means.

Table 2: Effect of glutamine supplementation on feed consumption (g) of turkey poults at different phases of growth during 0-8 weeks of age.

Treatment	0 to 4 weeks	4 to 8 weeks	0 to 8 weeks
T_1	479.42	1739.60^{a}	2219.10^{a}
T_2	450.69	1637.90^{b}	2088.60^{b}
T_3	466.98	1701.00^{ab}	2168.00^{ab}
T_4	461.99	1706.60^{ab}	2168.60^{ab}
Pooled SEM	6.39	14.97	19.77
Sig. level	NS	P<0.05	P<0.05

Means bearing different superscripts within a column differ significantly (P<0.05).

NS: Not significant (P>0.05); SEM: Standard error of means.

Table 3: Effect of glutamine supplementation on feed conversion ratio of turkey poults at different phases of growth during 0-8 weeks of age.

Treatment	0 to 4 weeks	4 to 8 weeks	0 to 8 weeks
T_1	2.09^{a}	2.81^{a}	2.61^{a}
T_2	1.89^{b}	2.58^{b}	2.39^{b}
T_3	1.87^{b}	2.53^{b}	2.35^{b}
T4	2.01^{ab}	2.70^{ab}	2.52^{ab}
Pooled SEM	0.037	0.041	0.039
Sig. level	P<0.05	P<0.05	P<0.05

Means bearing different superscripts within a column differ significantly (P<0.05).

NS: Non significant (P>0.05); SEM: Standard error of means.

T3 group poults had a significantly (P<0.05) better FCR at 0-4, 4-8 and 0-8 weeks of growth phase than T1 group (Table 3). Our results are in agreement with Yi *et al.* (2001), who observed improvement in FCR in turkey poults on a diet supplemented with 1% Gln in broilers. However, many other studies reported that there was no effect of dietary Gln on broiler FCR (Murakami *et al.,* 2007; Sakamato *et al.,* 2006; Bartell and Batal, 2007).

The beneficial effect of Gln supplementation on growth performance of chickens has been associated with better development of the intestinal mucosa (Yi *et al.,* 2005; Bartell and Batal, 2007). Glutamine is responsible for maintenance of the mucosal structure (Khan *et al.,* 1999) and reconstitution after damage (Newsholme, 2001). Bartell and Batal (2007) reported that birds fed diets with Gln had significantly longer intestinal villi than those fed a control diet. Increase in intestinal villi height early in the chicks' life may allow a more efficient utilization of nutrients and consequently improved growth performance. Lilja (1983) reported that avian species with a high growth rate capacity were characterized by a rapid early development of the digestive organs and liver. Birds with faster growth rates were reported by Nitsan *et al.* (1991) to secrete high levels of digestive enzymes, implying that initial growth is only limited by the early development of the digestive organs. By reducing the time for development of the digestive organs, growth improvements could be achieved.

Immunocompetence traits

HA titre was significantly (P<0.05) higher in poults of T3 as compared to other treatment groups (Table 4). Further IgM response was significantly (P<0.05) higher in T3 group as compared to other groups. Similarly, IgG response was comparatively better in T3 group as compared to the other treatment groups after 8 weeks of age.Serum IgG level was significantly higher (P<0.05) in T3 as compared to T1 and comparatively higher than other treatment groups (Table 5). However, there was no significant difference among treatment groups in serum IgM values although apparently higher values were recorded in T3 as compared to other treatment groups. This is in agreement with the results obtained by Sakamoto *et al.* (2006) who stated that birds treated with 1% Gln during the first week of age produced more antibodies. Bartell and Batal (2007) also reported that the birds fed diets supplemented with 1% Gln for 7 days or more had significantly higher IgG concentrations in the serum. Similarly, Wu *et al.* (2022) reported that dietary supplementation of 1% glutamine in broilers subjected to heat stress resulted in increased IgG levels. In another study, Wu *et al.* (2025) observed that glutamine supplementation resulted in increased antibody concentration in broilers subjected to cold stress.

Glutamine supplementation has been shown to increase the proportion of T- helper: T-cytotoxic/suppressor cells (Kew *et al.,* 1999; Yeh, 2001), which suggests that the supplementation of Gln stimulates the proliferation of T-helper cells in preference to T-suppressor cells. The IgG expression is T-helper cell dependent (Singh, 1996) and is indicative of T-helper cell response (Mathers and Cuff, 2004). Because IgG levels did increase in birds fed diets supplemented with Gln, this may indicate that Gln is important for the synthesis of the IgG antibodies or perhaps required for thymus derived T-cell helper function and response.

Table 4: Effect of glutamine supplementation on the humoral immune response [antibody titre (log 2) values] to 1% SRBC and cell mediated immune response (response to PHAP-P) (foot web index) in turkey poults at 8 weeks of age.

Treatment	Total immunoglobulins	IgG	IgM	Foot web index
T_1	5.28^{b}	2.00	3.42^{b}	0.15
T_2	6.14^{b}	2.28	3.85^{b}	0.17
T_3	7.71^{a}	2.42	5.28^{a}	0.16
T_4	5.42^{b}	2.14	3.14^{b}	0.15
Pooled SEM	0.283	0.110	0.232	0.007
Sig level	P<0.05	NS	P<0.05	NS

Means bearing different superscripts within a column differ significantly (P<0.05).

NS: Not significant (P>0.05); SEM: Standard error of means.

Table 5: Effect of glutamine supplementation on concentration of IgG and IgM antibodies and serum cortisol of turkey poults at 8 weeks of age.

Treatment	IgG (mg/ml)	IgM (mg/ml)	Cortisol (mg/ml)
T_1	5.27^{b}	2.12	2.75
T_2	6.65^{ab}	2.62	3.24
T_3	8.20^{a}	2.96	3.51
T_4	7.00^{ab}	2.57	3.38
Pooled SEM	0.353	0.146	0.15
Sig. level	P<0.05	NS	NS

Means bearing different superscripts within a column differ significantly (P<0.05).

NS: Not significant (P>0.05); SEM: Standard error of means.

Results indicated that Gln @ 0.5 mg/kg and 1mg/kg diet supplemented poults had a comparatively better cell mediated immune status as compared to the control group. Bartell and Batal (2007) also stated that birds diet supplemented with 1% Gln (P<0.05) during first week of age or more, had significantly higher IgA concentration in bile and serum than control diet while birds feds more Gln concentration, instead serum having lower concentration. It means dietary supplemented 1% Gln resulted in better and more resistance to infection.

Blood biochemical parameters

Total serum protein was significantly(P<0.05) higher in T2 as compared to the other treatment groups (Table 6). Further, serum ALP values were significantly higher in T2 as compared to T1 and numerically higher than T3 and T4. Our findings are similar to Martinez-Tome *et al.* (2001) who reported that dietary glutamine supplementation decreased ALT and AST levels, while increased ALP levels in 28- and 35-day-old chickens. However, Sandercock *et al.* (2006) and Melesse *et al.* (2011) observed increase in ALT and AST activities and decrease in ALP activity in glutamine supplemented chickens. Serum SOD value was significantly higher (P<0.05) in T2 than T1 and T3 and comparatively higher than T4. LPO value was significantly (P<0.05) higher in the control (T1) group as compared to the glutamine supplemented groups (T2, T3 and T4). Kishawy *et al.* (2024) observed that supplementing with 1% glutamine resulted in lower levels of reactive oxygen species, H_2O_2 and malondialdehyde while increasing the overall antioxidant capacity and the activity of the enzymes glutathione peroxidase and superoxide dismutase. The mechanism how glutamine affect serum enzyme activities of broilers under cycling high temperature is not clear. However, it may be caused by antioxidant function of glutamine (Martinez-Tome *et al.,* 2001). Our results could not be compared with other studies as there are limited studies pertaining to effect of dietary glutamine supplementation on cortisol level in serum of birds.

Histopathology

Effect of different level of glutamine supplementation on turkeys on the histology of liver and spleen has been portrayed

Table 6: Effect of glutamine supplementation on blood biochemical parameters of turkey poults at 8 weeks of age.

Treatment	Total protein (g/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	Uric acid (mg/dL)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	SOD (units/mL)	LPO (nM/mL)
T_1	4.82^{b}	109.00	58.85	5.72	252.76^{b}	3.93	4.16	391.86^{b}	0.0482^{a}
T_2	6.76^{a}	108.83	57.94	7.98	261.57^{a}	3.41	3.67	468.70^{a}	0.0420^{b}
T_3	4.95^{b}	105.45	60.80	6.15	258.00^{ab}	3.25	3.07	382.08^{b}	0.0422^{b}
T_4	4.68^{b}	108.31	57.43	7.09	256.33^{ab}	3.45	3.40	423.63^{ab}	0.0428^{b}
Pooled SEM	0.237	0.983	0.927	0.307	1.15	0.194	0.414	12.30	0.0009
Sig. level	P<0.05	NS	NS	NS	P<0.05	NS	NS	P<0.05	P<0.05

Means bearing different superscripts within a column differ significantly (P<0.05).

NS: Not significant (P>0.05); SEM: Standard error of means.

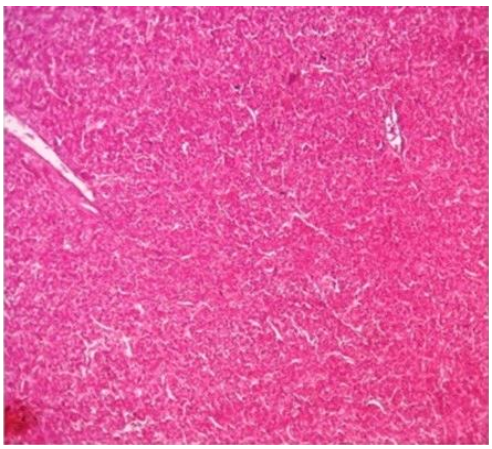


Fig 1a: Liver section of T1 turkey poultshowing normal histo-architecture of hepatocytes with arrangements of hepatic cords.

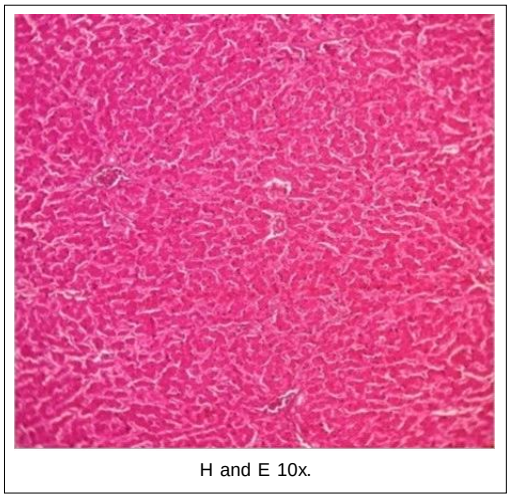


Fig 1b: Liver section of T2 turkey poult showing prominent normal large hepatocytes and central vein with normal arrangements of card.

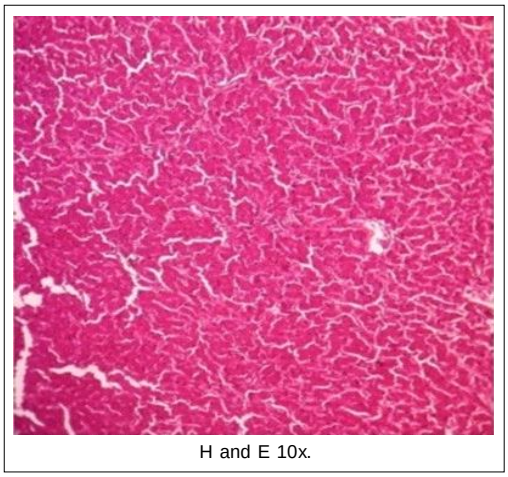


Fig 1c: Liver section of T3 turkey poult showing normal histo-architecture of hepatocytes with arrangements of hepatic cords.

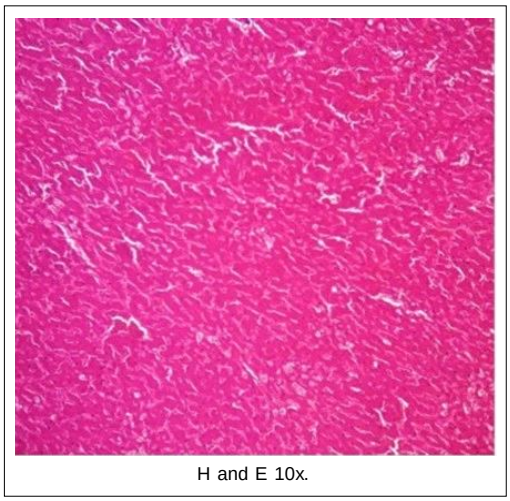


Fig 1d: Liver section of T4 turkey poult showing normal histoarchitecture of hepatocytes and central vein with hepatic cords and sinusoids.

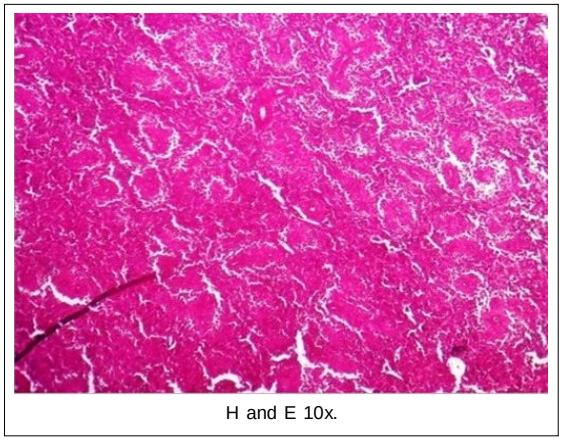


Fig 1e: Spleen section of T1 turkey poult showing normal architectural details with many small germinal centres and red pulp.

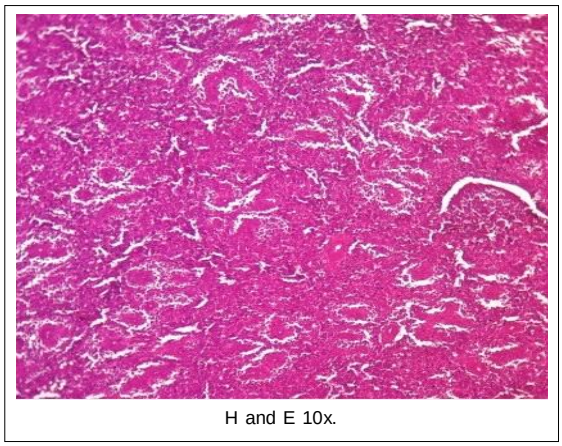
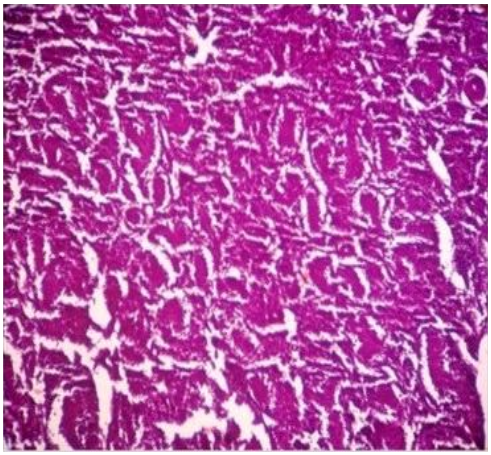
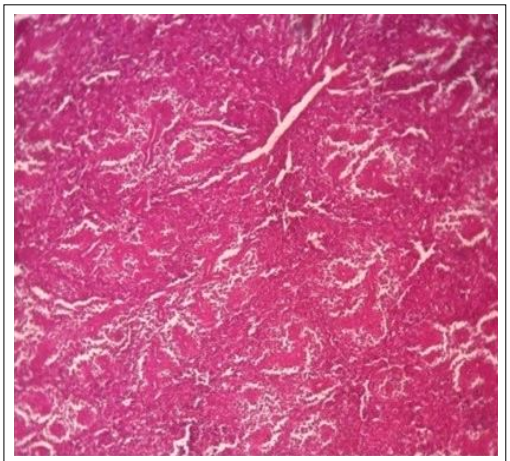


Fig 1f: Spleen section of T2 turkey poult showing normal architectural details with many germinal centres having central artery, compact red pulp.



H and E 10x.

Fig 1g: Spleen section of T3 showing normal architectural details with separated germinal centres, red pulp.



H and E 10x.

Fig 1h: Spleen section of T4 showing normal architectural details with many small germinal centres, normal red pulp.

in Fig 1 (a to h). Supplementation of glutamine at different levels showed normal histoarchitecture of both liver and spleen in all the groups. The changes in group T2 and T4 showedmore prominent structures in tissue sections in both liver and spleen such as hepatocytes in liver and germinal centers with red pulp in spleen as compared to control group. The increase in the body weight gain and improvement in FCR in turkey poults along with blood biochemical attributes correlates with histological studies as better structural arrangement was observed in liver and spleen of T2 and T4. Further, studies on effect of supplementation of glutamine on histology of organs of poultry are limited in the literature.

CONCLUSION

It was concluded that Gln supplementation @ 1% elicited the growth performance and immunocompetence traits of turkey poults. Further, supplementation Gln @ 1% resulted in significantly lower LPO values in the serum which reflected better antioxidative status of the turkey poults.

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Conflict of interest

All authors declare that they have no conflict of interest.

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