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Influence of gamma aminobutyric acid on blood biochemical and antioxidant parameters in commercial broilers

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Abstract
In the present study, the influence of dietary gamma aminobutyric acid on the blood biochemical and antioxidant parameters of commercial broilers was studied. A total of 192, day-old straight-run broiler chicks (Vencobb) were divided into 4 treatment groups (T1, T2, T3 and T4) with four replicates of twelve chicks in each viz., T1 as control. T2, T3 and T4 groups were supplemented with dietary GABA at 25, 50 and 75 mg per kg feed, respectively. The birds were reared under standard management conditions for 5 weeks (mean THI=75.0). Blood was collected twice (3rd and 5th week) during the study for biochemical and antioxidant analysis. It was concluded that GABA could not exert its influence on any of the parameters of biochemical and antioxidant profile studied except calcium and albumin which were significantly higher in group T4 (75 mg) after 5 weeks age.

Keywords: Gamma aminobutyric acid, broilers, antioxidants, blood biochemistry

Introduction
Gamma aminobutyric acid (GABA) is a four-carbon non-protein amino acid, a primary inhibitory neurotransmitter in the central nervous system of animals. The market demand for poultry products has been increasing at a steady pace and the pressure is on the industry to increase production. Various anti-stress agents tested successfully as supplements to mitigate stress and most of them had little to moderate reactions in the body. Very few endogenously derived supplements are tested in broiler chickens and one such substance is gamma-aminobutyric acid (GABA), which exhibits various nutritional and pharmacological functions (Soltani *et al.*, 2011; Jin *et al.*, 2013) [11, 9]. In view of the well-reported biological activities, GABA as an effective, functional and safe feed additive (Takeshima *et al.*, 2014) [15] has been tried in the animal industry to improve growth performance (Zhong *et al.*, 2020) [18] and prevent stress-related signs by reducing the excitability of nervous system in farm animals (Al Wakeel *et al.*, 2017) [1].
Hence, the present study was designed to evaluate the effects of dietary GABA on the blood biochemistry and antioxidative parameters in commercial broilers.

Materials and Methods
One hundred and ninety-two, day-old straight-run Vencobb broiler chicks obtained from a commercial hatchery were utilized in this biological trial. The chicks were wing-banded, weighed, and randomly allotted to four experimental groups (treatments T1 to T4) each with four replicates and twelve chicks in each replicate. T1 acted as control, while T2, T3 and T4 groups were supplemented with dietary GABA at 25, 50 and 75 mg per kg feed, respectively. Feed and water were provided *ad libitum*. A completely randomized design was followed. The broiler chicks were reared in deep litter pens under uniform standard management practices upto five weeks of age.

Sample collection and analysis
Blood was collected twice (3rd and 5th week) during the study for biochemical and antioxidant analysis.
The blood biochemical parameters viz., serum glucose, total protein, total cholesterol, triglycerides, albumin, calcium, and phosphorus were estimated using a biochemical auto analyzer (A15 Bio systems®).

Antioxidant parameters like superoxide dismutase was assayed in the plasma samples as per the method of Marklund and Marklund (1974) [10] as modified by Nandi and Chatterjee (1988) [11].
Glutathione peroxidase activity (Rotruck *et al*., 1973) [13] and extent of lipid peroxidation (Hsieh *et al*., 2006) [6] was measured.

Results

Total protein

Dietary supplementation with GABA did not significantly influence the total protein concentrations at 3 and 5 weeks of age. It ranged from 3.03 to 3.38 g/dL at 3 weeks and from 3.27 to 3.89 g/dL at 5 weeks of age. Further, higher total protein content was noticed in broiler chickens as age advanced.

Total cholesterol

Significant difference ($p \leq 0.05$) among the treatment groups and control was revealed by the analysis of variance at three weeks of age only. However, the T4 treatment group did not differ significantly from control group at 5 weeks. The total serum cholesterol levels in the present study ranged from 125.33 to 138.67 mg/dL at three weeks and from 132.33 to 156.50 mg/dL at five weeks of age.

Triglycerides

In the present study, dietary supplementation of GABA did not influence the serum triglycerides levels at both the ages studied (3rd and 5th week). However, T4 recorded substantially lower triglyceride value at 3 and 5 weeks of age compared with control and other treatment groups. The mean triglycerides levels ranged from 49.00 to 79.17 mg/dL at 3 weeks and from 50.67 to 70.00 at 5 weeks of age.

Calcium and phosphorus

Dietary supplementation of GABA significantly ($p \leq 0.05$) increased the serum calcium levels at 5 weeks of age but not at three weeks of age (12.86 to 15.25 mg/dL). At 5 weeks age, T3 and T4 treatment groups have recorded significantly ($p \leq 0.05$) higher calcium levels (T3: 15.963, T4: 16.73mg/dL) in comparison with the other treatment groups. The mean serum calcium levels ranged from 12.86 to 15.25 mg/dL at three weeks age, however, the differences among the groups were insignificant.
Serum phosphorus levels ranged from 6.83 (T1) to 8.27 mg/dL (T2) at 3 weeks and from 7.85 (T3) to 10.39 mg/dL (T2) at 5 weeks of age and it had non-significant difference at both the ages studied.

Albumin

Serum albumin level was significantly influenced by dietary supplementation of GABA at both three and five weeks of age. There is no significant difference between control and T3, T4 and also between T2 and T3 at 3 weeks of age. Similarly, there is no significant difference between control and T2, T3 at 5 weeks of age. Among the treatment groups, T4 differs significantly from T2 and T3 at 3 weeks age and differs significantly from all others at 5 weeks of age. The mean serum albumin levels ranged from 1.74 to 1.98 g/dL at three weeks and from 1.9 to 2.26 g/dL at five weeks of age.

Glucose

Dietary supplementation of GABA increased the serum glucose levels in the present study. However, the differences were significant at five weeks of age only but not at 3 weeks. The treatment groups recorded significantly ($p \leq 0.01$) higher serum glucose values of 235.00, 249.17, and 265.17 mg/dL in T2, T3, and T4 respectively compared to 177.00 of control group at 5 weeks of age.

Antioxidant parameters

The antioxidant assays viz. glutathione peroxidase and superoxide dismutase and lipid peroxidation assay (MDA), were performed to assess the antioxidant status of dietary GABA supplementation in broiler chickens. These assays were performed twice during the trial (at the end of 3 and 5 weeks) and their concentrations are presented in Table 2.
The present antioxidant assay results showed no significant difference between the control and treatment groups throughout the study period
The MDA concentrations (nmol/mL) ranged from 0.41 to 0.51 at 3rd week and 0.39 to 0.69 at 5th week.
Glutathione peroxidase concentrations (µmol/L) ranged from 1266.42 to 1334.66 in the 3rd week and 1076.76 to 1136.69 in the 5th week.
Superoxide dismutase concentrations (U/mL) ranged from 248.00 to 260.37 in the 3rd week and 146.28 to 151.35 in the 5th week.

Discussion

Total protein

There was no specific trend at 3 weeks of age but at 5 weeks of age, the GABA supplemented groups showed elevated levels of total protein though it was non-significant statistically. However, there was a general trend of higher total protein level in treatment groups. Zhang *et al*. (2012) [17], Hu *et al*. (2016) [7] and Ncho *et al*. (2021) [12] reported that dietary supplementation of GABA increased the levels of total protein significantly in heat stressed birds. Perusal of literature suggests that GABA may act on the hepatocytes to increase the serum total proteins under heat stress conditions.

Total cholesterol

Although the data is statistically significant ($p \leq 0.05$) in the third week, no significant difference exists at 5 weeks of age. Zhong *et al*. (2020) [18] reported significantly lowered serum cholesterol levels (21.05% decrease) in stressed birds supplemented with dietary GABA. On the contrary, the chickens raised under low stocking density and supplemented with dietary GABA (100mg/kg diet) showed a 14.52% increase in serum total cholesterol levels compared to the control group (Jeong *et al*., 2020) [8]. In another study, El-Naggar *et al*. (2019) [5] reported that GABA increased the serum total cholesterol levels by 10.02% in Cobb birds reared under heat-stress conditions.

Triglycerides

Present study revealed a non-significant influence of GABA supplementation on serum triglyceride levels. Literature on the influence of GABA supplementation on serum triglycerides in broilers is scanty. Hu *et al*. (2016) [7] reported GABA-supplemented diet significantly ($p<0.05$) reduced the serum triglyceride concentrations in broilers exposed to hot environment by 6.1%. In rats, Ullah *et al*. (2017) [16] reported a 10.78% reduction in the serum triglyceride concentration compared to induced polycystic ovarian syndrome group, when GABA was supplemented at 500mg/kg body weight.

Table 1: Mean (±SE) blood biochemical parameters in broiler chickens supplemented with GABA at 3 and 5 weeks of age

Treatment	Total protein (g/dL)		Total Cholesterol (mg/dL)		Triglycerides (mg/dL)		Calcium (mg/dL)		Phosphorus (mg/dL)		Albumin (g/dL)		Glucose (mg/dL)	
	3rd week	5th week	3rd week	5th week	3rd week	5th week	3rd week	5th week	3rd week	5th week	3rd week	5th week	3rd week	5th week
T1	3.21±0.07	3.27±0.18	135.33^{ab}±4.46	132.33±7.75	71.67±11.27	61.33±6.84	10.86±1.58	11.83 ^{c}±1.68	5.83±0.41	7.98±0.35	1.74^{b}±0.07	1.92^{b}±0.05	221.50±14.21	177.00^{b}±19.97
T2	3.06±0.11	3.59±0.20	125.50^{b}±4.06	153.00±3.20	71.33±8.19	70.00±15.91	12.27±1.44	13.29^{bc}±1.33	7.27±0.88	7.85±0.53	1.92^{a}±0.04	1.97^{b}±0.06	240.00±6.88	235.00^{a}±7.39
T3	3.03±0.12	3.35±0.13	125.33^{b}±2.16	152.17±14.45	79.17±7.30	60.33±11.28	13.25±0.74	15.963 ^{ab}±0.12	7.21±0.11	10.39±0.28	1.86^{ab}±0.04	1.93^{b}±0.08	249.50±7.08	249.17^{a}±5.17
T4	3.38±0.08	3.89±0.16	138.67^{a}±2.19	156.50±5.17	49.00±13.23	50.67±2.97	11.39±0.74	16.73 ^{a}±0.26	6.10±0.37	10.12±0.41	1.98^{a}±0.03	2.26^{a}±0.07	256.00±6.29	265.17^{a}±10.81
P- value	0.072	0.072	0.021	0.229	0.219	0.639	0.522	0.015	0.143	0.377	0.014	0.003	0.076	0.000

* Means with similar superscripts within each column do not differ significantly ($p \leq 0.05$); (n=12)

Table 2: Mean (±SE) serum antioxidant enzyme concentrations in broiler chickens fed gamma-aminobutyric acid at different levels at the 3rd and 5th week of age

Treatment	**MDA** (nmol/ mL)		**GPx** (µmol/L)		**SOD** (U/mL)	
	3rd week	**5th week**	**3rd week**	**5th week**	**3rd week**	**5th week**
T1	0.41±0.10	0.39±0.09	1266.42±43.95	1076.76±29.54	260.37±12.39	146.28±1.94
T2	0.51±0.03	0.43±0.09	1281.79±29.79	1129.00±12.39	248.00±9.73	148.91±2.16
`T3	0.48±0.02	0.46±0.10	1292.28±23.39	1131.74±15.65	250.76±13.04	148.54±2.52
T4	0.45±0.04	0.69±0.13	1334.66±26.52	1136.69±20.60	251.17±11.64	151.35±2.05
P- value	0.680	0.210	0.490	0.170	0.890	0.450

* Means with similar superscripts within each column do not differ significantly ($p \leq 0.05$); (n=6)

Calcium and phosphorus

Serum calcium level was significantly increased in GABA supplemented groups at five weeks of age in the present study while there was no significant influence on phosphorous levels. Feeding freeze-dried GABA-producer organisms improved serum levels of calcium and phosphorus which was related to intestinal absorption (Chen *et al.*, 2013) [2]. Zhu *et al.* (2015) [19] found that serum calcium increased quadratically ($p = 0.040$), while the concentration of phosphorus showed linear effect after feeding the hens with freeze-dried GABA-producer organisms. Increased concentration of serum calcium in the treatment groups may be due to GABA-driven benefits in transportation and absorption of calcium (Zhu *et al.*, 2015) [19].

Glucose

The treatment groups recorded significantly higher serum glucose values compared to control group at 5 weeks of age. Zhang *et al.* (2012) [17], Zhu *et al.* (2015) [19] and El-Naggar *et al.* (2019) [5] also reported a significant increase in glucose levels of birds supplemented with GABA. This could be due to increased serum concentration of GABA, which in turn stimulates the fat and muscle protein reserves to release free fatty acids and/ or amino acids, which are later converted into glucose (Dai *et al.*, 2011) [3].

Serum antioxidant enzymes

Dietary supplementation of GABA did not reveal any significant influence on all the three antioxidant parameters studied. Chen *et al.* (2013) [2] reported that GABA at 50 mg/kg body weight successfully reversed the heat-stress associated decreases in antioxidant enzyme activities in broiler chickens while Elbadawy and Aboubakr (2019) [4] observed a non-significant increase in superoxide dismutase and glutathione peroxidase and reduced MDA levels in GABA-supplemented birds. The minimal insignificant increase in MDA was attributed to faster growth rate in later phases with energy rich diet and protective nature of glutathione peroxidase and superoxide dismutase.

Conclusions

Present investigation was carried out to study the effect of dietary supplementation of GABA on certain blood biochemical and antioxidant parameters in broiler chickens under normal conditions (THI= 75.0). There was significant influence of GABA on total cholesterol and albumin at three weeks of age and on calcium, albumin and glucose at five weeks of age. Non-significant differences were observed in total protein, triglycerides and phosphorous levels and also on antioxidant parameters at both the ages. Birds reared under hot environment had decreased serum glucose, total protein, albumin, total cholesterol levels and increased triglyceride levels compared with the birds reared under stress-free conditions. As the present study recorded mean THI of 75.0, which indicates comfortable environment as evidenced by the results obtained showing metabolic consistency under the study period.

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