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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 1714-1716

www.thepharmajournal.com
Received: 26-10-2022
Accepted: 29-11-2022

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Effect of dietary gamma aminobutyric acid on production performance and duodenal histomorphometry in commercial broilers

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Abstract
In the present study, the influence of dietary gamma aminobutyric acid (GABA) on production performance and duodenal villi height, crypt depth, and their ratios in commercial broilers were studied. A total of 192, day-old straight-run broiler chicks were divided into 4 treatment groups (T1, T2, T3, and T4) with four replicates of twelve chicks in each. T1 acted as the 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). Dietary GABA improved body weight in this study while there was no significant difference in FCR and carcass traits. A significant influence of GABA was observed only on the ratio of villi height to crypt depth.

Keywords: Broiler chickens, GABA, body weight, FCR, carcass traits, villi height, crypt depth

Introduction
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 were tested successfully as supplements to mitigate stress but 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). It is a four-carbon non-protein amino acid, a primary inhibitory neurotransmitter in the central nervous system with varied nutritional and pharmacological functions (Soltani, 2011; Jin *et al.*, 2013) [12, 9]. In view of the well-reported biological activities, GABA as an effective, functional, and safe feed additive (Takeshima *et al.*, 2014) [13] has been tried in the animal industry to improve growth performance (Zhong *et al.*, 2020) [14] and feed efficiency (Al Wakeel *et al.*, 2017) [1]. Hence, the present study was designed to evaluate the effects of dietary GABA on production and intestinal histomorphology in commercial broilers.

Material and Methods
The present study was carried out at Veterinary College and Research Institute (VCRI), Namakkal during November and December 2021. Day-old broiler chicks obtained from a commercial hatchery at Namakkal were reared in deep litter under uniform standard management practices up to five weeks of age. Chicks (192) were randomly allotted to four experimental groups with four replicates of twelve chicks in each replicate. A completely randomized design was followed. T1 served as control while T2, T3, and T4 groups were supplemented with GABA at 25, 50, and 75 mg/kg feed.
The ambient temperature inside the shed was recorded thrice daily (06:00, 14:00, and 20:00 h) using dry and wet bulb thermometers, and later the relative humidity and temperature-humidity index (THI) were calculated. The average relative humidity (%) and THI in the broiler shed were 86.67±0.64 and 77.47±0.35 respectively.
Individual body weight of broiler birds and feed intake (feed given – leftover feed) were recorded at weekly intervals. The mean daily feed consumption per bird and feed conversion ratio of each group were calculated.
At the end of five weeks of age, 12 birds from each group were randomly selected and humanely slaughtered. Segments from the duodenal loop were taken and fixed in 10% neutral buffered formalin and later embedded in paraffin wax. Histological studies were performed on 5 µm sections (4 cross-sections for each sample), stained with hematoxylin and eosin, and

examined under the microscope (Leica Microsystems) fixed with a camera. The images were analyzed by Leica image analyzing software.
Tissue processing and staining were carried out as per the protocol laid by Luna (1968) [10]. The villus height was measured from the villus-crypt junction to the villus tip, while crypt depth was measured as the depth of the invagination between 2 villi *i.e.*, downwards from the villus-crypt junction to the end of the mucosal layer (Awad *et al*., 2009) [2].

Results

Body weight and feed conversion ratio

The data on mean body weight are presented in Table 1. The average weight of the day-old chicks ranged from 50.25 to 50.88 g. The body weight at the end of first-, second-, third-, fourth- and fifth-week age ranged from 154.96 to 156.65 g, 471.20 to 481.90 g, 934.96 to 949.08 g, 1510.65 to 1540.71 g, and 2103.85 to 2180.35 g, respectively. Significant differences between the treatments were found only at the 4th and 5th week age, where GABA-supplemented groups displayed increased body weight values with the maximum being the T4 group. The data on FCR has been presented in Table 1. No significant difference was observed between the treatments except at the end of week 2. The FCR values ranged from 0.89 to 0.96, 1.27 to 1.40, 1.50 to 1.55, 1.48 to 1.54, and 1.81 to 1.88 at the end of weeks 1, 2, 3, 4, and 5 respectively.

Carcass traits

The weights of the live bird, eviscerated carcass, gizzard, liver, heart, and abdominal fat are presented in Table 2. In the present study, dietary supplementation of GABA did not influence the carcass traits significantly. The weights of the eviscerated carcass, gizzard, liver, heart, and abdominal fat ranged from 1304.48 to 1411.75 g, 39.29 to 41.14 g, 40.47 to 43.76 g, 9.27 to 10.67 g and 31.16 to 34.59 g, respectively.

Histomorphometry of duodenum

The mean villi height, crypt depth, and their ratios in the duodenum of broiler chickens are presented in Table 3. The villi height, crypt depth, and their ratio ranged from 1396.48 to 1480.56 µm, 179.19 to 216.11 µm, and 6.51 to 8.32, respectively.

Discussion

Body weight and feed conversion ratio

Supplementing GABA at different levels improved body weight in this study. Though published literature revealed that dietary GABA supplementation improved the body weight in broilers (Hu *et al.*, 2016a, 2016b; Chand *et al.*, 2016; Al Wakeel *et al*., 2017) [7, 8, 4, 1], most of the reports indicated that GABA supplementation improved body weights in heat-stressed birds and that the beneficial effects of GABA might have been achieved through stress alleviation mechanisms (Bhat *et al.*, 2010; Soltani *et al.*, 2011, Hu *et al.*, 2016a, 2016b) [7, 8]. During the present study, the average THI of the poultry house was 77.47, which might have not created stressful conditions. Lack of stress could have been one of the reasons for GABA not proving explicitly beneficial in the present study.
Mean cumulative FCR values were not influenced by dietary GABA supplementation at all levels. However, various studies showed positive results with regard to the influence of GABA on the FCR of broilers *i.e.*, GABA decreased the FCR by 8.37% (Chand *et al.*, 2016) [4] and by 13.51% (Zhong *et al.*, 2020) [14]. However, in all these studies the birds were subjected to stressful conditions. The absence of any known stressors could be the reason for the insignificant difference observed among different treatments with respect to FCR values in the present study.

Carcass traits

In the present study, dietary supplementation of GABA did not influence the carcass traits significantly except for the eviscerated weight ($p \leq 0.05$) which ranged from 1304.48 to 1411.75 g. However, the dressing percentage values did not show any statistical significance. Literature on the effect of dietary supplementation of GABA on carcass traits is scanty. Dai *et al.* (2011) [6] reported that dietary GABA offered a potential nutritional strategy to prevent heat stress-related depression in the performance and carcass characteristics of the broiler. The findings of the present study showed that dietary GABA did not yield any changes in the ready-to-cook meat of broiler chickens.

Histomorphometry of duodenum

Dietary supplementation of GABA did not influence the histomorphometry of the duodenal villi and crypts in broilers at 5 weeks of age in the present study. However, there was a significant difference between the groups with respect to the villi height-crypt depth ratio, where T3 and T4 recorded the highest ratio of villi height and crypt depth. A higher ratio of villus height and crypt depth recorded in the current study indicates a greater capacity for nutrient digestibility and absorption in chickens (Silva *et al.*, 2009) [11]. Published literature revealed the protective effects of GABA in alleviating the heat stress-induced damages of the intestinal mucosa in chickens such as increasing villi height and thereby improving the growth performance of broilers exposed to high temperature (Chen *et al.*, 2015; Al Wakeel *et al.*, 2017; Zhong *et al.*, 2020) [5, 1, 14]. However, in the present study, the birds were not exposed to heat stress as indicated by lower THI measured during the study. But still, the intestinal villi and crypt measurements increased though non-significantly. This might be due to the effect of GABA on intestinal development which has been reflected as improved body weight as shown in Table1.

Table 1: Mean±SE body weight (g) and feed conversion ratio in broiler chickens fed gamma-aminobutyric acid at different levels from 0 to 5 weeks of age

	Body weight						FCR				
	Day old chick	Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
T1	50.53±0.29	156.48±0.96	471.92±1.04	934.96±3.22	1510.65^{c}±5.84	2103.85^{b}±6.86	0.96±0.03	1.38^{a}±0.01	1.51±0.02	1.54±0.02	1.86±0.02
T2	50.88±0.35	156.65±0.61	471.20±1.29	936.54±3.90	1520.72^{bc}±5.48	2113.85^{ab}±6.97	0.89±0.02	1.40^{a}±0.01	1.50±0.01	1.51±0.01	1.84±0.02
T3	50.38±0.36	154.96±0.61	475.75±2.18	946.92±5.30	1532.73^{ab}±5.90	2120.77^{ab}±6.32	0.91±0.01	1.27^{b}±0.01	1.55±0.02	1.48±0.02	1.88±0.02
T4	50.25±0.26	155.48±0.51	481.90±2.54	949.08±6.21	1540.71^{a}±5.95	2180.35^{a}±4.60	0.94±0.01	1.29^{b}±0.01	1.54±0.02	1.49±0.02	1.81±0.02
P value	0.54	0.26	0.10	0.09	< 0.01	0.02	0.10	< 0.01	0.16	0.15	0.21

* Means with same superscripts within each column do not differ significantly; (n = 48); $p \leq 0.05$

Table 2: Mean±SE of carcass traits (g) at 5 weeks of age in broiler chicken fed gamma-aminobutyric acid at different levels

Treatment	Live weight	Eviscerated carcass weight	% Eviscerated carcass	Gizzard weight	Liver weight	Heart weight	Abdominal fat weight
T1	2052.92±32.83	1304.48 ^{b}±22.16	63.55±0.52	39.29±1.73	41.25±1.85	10.67±0.45	34.59±1.98
T2	2082.50±44.98	1344.49 ^{ab}±30.67	64.56±0.54	39.29±1.60	41.69±1.29	9.45±0.52	34.07±1.36
T3	2143.08±29.85	1384.37 ^{ab}±22.31	64.60±0.50	41.14±1.47	43.76±1.11	9.27±0.90	31.16±1.44
T4	2166.50±11.94	1411.75 ^{a}±31.23	65.20±1.54	40.45±2.45	40.47±1.28	9.72±0.48	34.02±1.54
P value	0.06	0.04	0.63	0.87	0.41	0.39	0.43

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

Table 3: Mean±SE villi height, crypt depth and their ratios in duodenum of broiler chickens supplemented with GABA

Treatment	Villi Height (VH) µm	Crypt Depth (CD) µm	V:C
T1	1396.48±25.94	194.52±8.54	7.26^{b}±0.37
T2	1400.95±20.97	216.11±7.82	6.51^{c}±0.17
T3	1464.57±21.14	179.19±10.65	8.32^{a}±0.52
T4	1480.56±33.04	184.46±12.35	8.19^{a}±0.51
P value	0.07	0.08	0.02

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

Conclusion

In the present study, an increase in the ratio of villi height to crypt depth was observed concomitantly with increased dose of GABA in the feed. This might have resulted in the increased body weights. In light of published literature and the results of the present study, the beneficial effect of GABA would be more in birds subjected to stress rather than in normal birds. Thus, GABA can be a potential stress-mitigating supplement.

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