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Effect of Dietary Supplementation of GABA and Nano GABA on the Gross morphology of Bursa of Fabricius in Post-hatch coloured Chicken

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ABSTRACT

The bursa of Fabricius is a primary lymphoid organ unique to birds and plays an important role in the development of B lymphocytes and humoral immunity. The present study was conducted to evaluate the gross morphology of the bursa of Fabricius in post-hatch coloured chicken and to determine the effect of dietary GABA and Nano GABA supplementation. Thirty healthy chicks were reared up to 60 days of age and divided into three groups: Group I (control), Group II (GABA at 5 mg/ml), and Group III (Nano GABA at 2 mg/ml) supplemented through drinking water. At the end of the experimental period, six birds from each group were sacrificed for detailed examination. In all groups, the bursa appeared as an oval to spherical blind sac with a yellowish-white to pale pink colour and contained 12–14 internal plicae. The mean bursal weight and length showed a numerical increase in the supplemented groups; however, these differences were not statistically significant ($p > 0.05$). In contrast, a significant increase ($p < 0.05$) in bursal width was observed in both GABA and Nano GABA treated groups compared to the control. The findings indicate that dietary GABA and Nano GABA supplementation may support bursal growth, particularly in terms of width, without affecting its normal anatomical features.

Keywords: Bursa of Fabricius, GABA, Humoral immunity and Nano GABA

INTRODUCTION

The bursa of Fabricius (also known as bursa cloacalis) is a lymphoepithelial organ that was first identified and described in the 17th century by Hieronymus Fabricius and is unique to birds (Ratchliffe, 2002). It is connected to the proctodeal part of the cloaca by a short stalk. The bursa forms as a blind, sac-shaped projection from the dorsal wall of the cloaca (Hodges, 1974). It plays a vital role in immune development. The bursa of Fabricius produces and matures B lymphocytes without antigen stimulation, which later migrate to peripheral tissues and contribute to improved humoral immune response (Song *et al.*, 2021). Gamma-aminobutyric acid (GABA) is a non-proteinamino acid that acts as a principal inhibitory neurotransmitter in the central nervous system of animals (Kinnersley and Turano, 2000). GABA also exhibits immunomodulatory, antioxidant properties and enhances immune cell function which indirectly improves productivity (Jeong *et al.*, 2020). Nano-encapsulation of GABA improves its absorption, bioavailability, stability, targeted delivery, and transport to the intestinal mucosa after oral intake (Xie *et al.*, 2020). There is a lack of published literature on the effects of dietary GABA and Nano GABA supplementation on the morphology of the bursa of Fabricius in post-hatch coloured chicken, therefore, this study was aimed to examine normal morphology and to assess the influence of these supplements on the bursa of Fabricius.

MATERIALS AND METHODS

For the present study thirty apparently healthy post-hatch-coloured chicks were procured and reared up to 60 days of age under standard husbandry conditions at the Department of Poultry Science and the Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh India. The coloured chicken strain employed in the present study was developed by cross between Barred Plymouth Rock and Red

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Cornish breeds. The birds were divided into three experimental groups each with 10 birds. Group I (control/basal diet), Group II (basal diet + GABA at 5 mg/ml in drinking water) and Group III (basal diet + Nano GABA at 2 mg/ml in drinking water). At the end of the experimental period (60 day), six birds from each group (n = 18) were randomly selected and kept off feed for 12 hours before sacrifice. The birds were ethically slaughtered by cervical subluxation. Subsequently, the bursa of Fabricius was carefully removed through abdominal dissection, to examine the topographic location, colour and shape. Weight, length and width of bursa of Fabricius were recorded with the help of digital weighing balance and digital vernier caliper. The data generated were statistical analysed using analysis of variance with the help of statistical package SPSS for window V 20.0.

RESULTS AND DISCUSSION

Body weight

In this study, the mean live body weight of coloured chickens showed a numerical increase in the GABA-supplemented group (Group II: 1251.80 ± 37.81 g) compared to the control (Group I: 1168.00 ± 63.85 g), indicating a positive trend in growth performance. Although (Group III: 1232.80 ± 60.03 g) showed a slight reduction compared to Group II, the difference was statistically non-significant ($p > 0.05$) (Table 1).

Gross morphology of bursa of Fabricius

In this study, the bursa of Fabricius in post hatch coloured chicken appeared as an oval or spherical blind sac, yellowish-white to pale pink in colour, located on the dorsal aspect of the cloaca and connected to the proctodeal region by a short, thick stalk (Fig. 1). The internal surface exhibited 12–14 plicae projecting toward the lumen. No significant differences were observed in the number of bursal plicae among the groups. Gross morphological features were consistent across all treatment groups, and all groups exhibited the characteristic anatomical features of the bursa. However, the bursa in Group III (Nano GABA) appeared slightly larger than those in Groups I and II. This observation suggests that Nano GABA supplementation may have exerted a subtle stimulatory effect on bursal growth, as indicated by the numerical increase in organ size. Despite this, no marked morphological changes were observed in any of the treatment groups. These observations were in agreement with classical anatomical descriptions of the bursa. Romanoff (1960) and Glick (1960) described it as a unique avian organ near the cloacal region, often termed the "cloacal Thymus," while Hodges (1974) defined it as a dorsal diverticulum arising from the proctodeal wall. Similarly blind sac-like structures were reported by Mishra (2002) and Islam *et al*. (2019) in domestic and broiler chicken. Singh *et al.* (2019) in Chabro chicken and Dahariya *et al.* (2020) in Hansli chicken and Vencobb broiler chickens confirmed its attachment to the cloaca by a short stalk and the presence of internal plicae projecting into the lumen. With respect to its shape, bursa has been variably described as pear-shaped (Glick, 1960), globular or spherical (Hodges, 1974 and Akter *et al.*, 2006) in domestic fowl, or oval with luminal folds by Dahariya *et al.* (2020) in Hansli chicken and Vencobb broiler chickens. Nickel *et al.* (1977) noted that its shape changed with age, becoming circular to pear-shaped by 8 weeks. The coloration in early life is reported as pinkish or rose (Glick, 1960), yellow to milky white (Ayman *et al.*, 2020) in Sonali chicken, gradually transitioning to pale white with age, which aligns with the current observations. The plicae count in this study (12–14) was within the range reported in previous research. Jain *et al*. (2010) reported 8–16 plicae in CARI Shyama and Vanraja breeds, while Singh *et al*. (2019) in Chabro chicken and Dahariya *et al*. (2020) in Hansli chicken and Vencobb broiler chicken confirmed multiple small and large plicae. Ayman *et al.* (2020) in Sonali chicken refined this range to 15–20 plicae of varying sizes, suggesting variability based on breed, age, and developmental stage.

Weight of bursa (g)

In this study, the mean bursal weight was numerically higher in Group III 4.17 ± 0.13 g than Group II 3.86 ± 0.42 g and Group I 3.53 ± 0.36 g, although the differences were not statistically significant ($p > 0.05$) (Table 1). This suggests that GABA supplementation may provide a mild enhancement in bursal growth without causing major anatomical changes. Compared to the control, both GABA and Nano GABA groups showed a numerical increase, indicating that supplementation may support bursal development, but gross organ weight alone may not fully capture its functional immunomodulatory effects.

Length and width of Bursa (cm)

In the present study, the mean length of the bursa increased slightly from 2.33 ± 0.04 cm in (Group I) control group to 2.40 ± 0.06 cm in Group II and 2.43 ± 0.07 cm in Group III , but this difference was not statistically significant ($p > 0.05$) (Table 1). In contrast, the mean width of the bursa showed a significant increase ($p < 0.05$) from 1.92 ± 0.04 cm in the control (Group I) to 2.34 ± 0.08 cm in Group II and 2.42 ± 0.07 cm in Group III, indicating that both GABA and Nano GABA supplementation positively influenced bursal width, while the effect on length remained modest. There was no significant difference in bursal width between groups II and III, suggesting similar efficacy of both forms of supplementation in enhancing this dimension. The significant increase in bursal width in GABA and Nano GABA supplemented groups may indicate enhanced lymphoid tissue development, potentially reflecting improved immune organ maturation. The lack of significant change in length suggests that supplementation may preferentially influence lateral expansion of the bursa rather than elongation, which could be due to structural constraints or genetic programming of organ shape. The effect of GABA and Nano GABA on gross morphology of bursa of Fabricius could not be discussed because of lack of literature.

Table 1 : Showing weight and morphometric parameter of Bursa of Fabricius Post-hatched coloured Chicken.

Parameters	Group I Mean±SE (Range)	Group II Mean±SE (Range)	Group III Mean±SE (Range)
Live body weight (g)	1168.00 ± 63.85 (956-1288)	1251.80 ± 37.81 (1140-1367)	1232.80 ± 60.03 (1038-1372)
Weight of bursa (g)	3.53 ± 0.36 (2.22-4.66)	3.86 ± 0.42 (2.90-5.55)	4.17 ± 0.13 (3.77-4.55)
Length of bursa (cm)	2.33 ± 0.04 (2.24-2.52)	2.40 ± 0.06 (2.09-2.55)	2.43 ± 0.07 (2.12-2.64)
Width of bursa (cm)	$1.92^{a} \pm 0.47$ (1.74-2.06)	$2.34^{b} \pm 0.08$ (1.93-2.52)	$2.42^{b} \pm 0.07$ (2.07-2.59)

Note- figures in parenthesis a, b, superscript showed the significant ($p < 0.05$) difference between the groups; similar superscript showed no significant ($p > 0.05$) difference between the groups.

Fig. 1. Showing (a) bursa of Fabricius and (b) Cloaca **Fig. 2.** Showing bursa of Fabricius (a) group I (b) group II and (c) group III **Fig. 3.** Showing (a) Large plicae and (b) Small plicae.

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