



Histomorphological and Histomorphometric Assessment of the Bursa of Fabricius in Rainbow Rooster Chickens Following Vaccination against Infectious Bursal Disease Virus

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ABSTRACT

Background: This study explores the histomorphological and histomorphometric features of the Bursa of Fabricius in Rainbow Rooster chickens, highlighting distinctions between vaccinated and non-vaccinated groups.

Methods: The study included 57 numbers of chickens of various sexes and developmental stages. These were divided into two groups: 30 in control group (C) and 27 to the treatment group (T). From each group, six chicks (C1 and T1) were retained for blood sample collection and three chickens (C2 and T2) were sacrificed to collect the samples of the Bursa at 4-week intervals from 0-28th week.

Result: The Bursa of Fabricius contains three layers: The tunica serosa, tunica muscularis and tunica mucosa. Two epithelial types were identified, Follicle-Associated Epithelium (FAE) and Inter-follicular Epithelium (IFE), both consist of principal columnar cells, basal cells and goblet cells. Histological assessments revealed an age-related rise in collagen and reticular fibers within the tunica muscularis and lamina propria. DNA and RNA in all follicular cells and weak positivity of PAS staining in the tunica mucosa. Morphometric analysis indicated that the number, length and breadth of plicae, epithelial height and follicular cortex and medulla dimensions peaked at 12th weeks, with higher values consistently observed in the vaccinated group. Secondary follicle development began earlier in vaccinated birds (4th week) indicating a stronger immune response due to vaccination. These findings highlight the role of vaccination in enhancing bursal development and immune function that can aid in improving poultry health and optimizing productivity.

Key words: Bursa of fabricius, Histomorphology, Histomorphometry, IBDV vaccination.

INTRODUCTION

The Rainbow Rooster is a low-maintenance, dual-purpose breed that matures between 4 to 6 months and lays approximately 160-180 eggs over 16 months (Singh *et al.*, 2021). Poultry farming, which includes both layer and broiler production, contributes to food security while also serving as a profitable source of income (Toor and Goel, 2024). Backyard poultry farming, especially in rural and hilly areas of India, holds promise for addressing nutritional deficiencies, poverty reduction, women's empowerment, and self-employment (Islam *et al.*, 2020). In Mizoram, a significant gap exists between the demand and supply of meat and eggs, with a shortage of 1540.559 tonnes of meat and 1992 lakh eggs (Economic Survey of Mizoram, 2021-2022). Bridging this gap requires farmer training in scientific poultry practices, including vaccination, feed management and housing (Singh *et al.*, 2021). Immunity is vital for poultry health, with the thymus and Bursa of Fabricius being primary lymphoid organs (Glick *et al.*, 1956). Bursa of Fabricius is closely linked to the cloaca and digestive system. It is generally recognized as the primary lymphoid organ in chickens and is a crucial organ for the immune system to respond to environmental antigens (Ifrah *et al.*, 2016). It appears by Day 5 of embryonic development, is essential for B-cell maturation and begins involution around 20 weeks (Fellah *et al.*, 2014). Bursa removal impairs B-cell function and antibody production (Glick *et al.*, 1956). Infectious Bursal

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Disease (IBD) is caused by the Infectious Bursal Disease Virus (IBDV), a double-stranded RNA virus belonging to the family Birnaviridae. The virus specifically targets bursal B cells, leading to extensive lymphocyte destruction and resulting in lymphopenia (Shinde *et al.*, 2021). It primarily affects 3 to 6-week-old chicks, damaging B cells and causing immunosuppression (Caston *et al.*, 2008; Dey *et al.*, 2019; Pandey

et al., 2021). The disease can lead to 100% morbidity and up to 60% mortality, depending on the strain (Jackwood, 2019). Vaccination is critical in controlling disease and boosting poultry productivity (Marangon and Busani, 2007).

MATERIALS AND METHODS

Collection and preparation of samples

Chickens from each group were sacrificed by the cervical dislocation method (Jaksch, 1981). The food and water were withheld for two hours prior to their slaughter. After the chickens were slaughtered, the feathers were physically removed and the bursa of Fabricius was retrieved *via* ventral abdominal dissection. The samples were cleaned with normal saline and blotted using blotting paper. The organs were observed critically. After collecting gross data, the bursa was preserved in 10% neutral buffered formalin and prepared for paraffin wax embedding. The samples were dehydrated in increasing alcohol concentrations for two hours. The tissue was then embedded in paraffin wax after clearing. Sections were cut to a thickness of 5-6 μ m using a semi-motorized rotary microtome. Subsequently, the samples were treated with various stains to facilitate histological analysis (Bancroft and Cook, 1994).

Location of experiments

The present research work was conducted from April 2022-April 2023 in the Department of Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry,

Central Agricultural University, Selesih, Aizawl, Mizoram, India.

Experimental design

This study involved 57 chickens representing different genders and stages of development. The chickens were divided into two groups: 30 in the control group (C) and 27 in the treatment group (T). The treatment group received an oral dose of a commercially available live IBDV vaccine on days 14 and 21. Each bird was given one drop containing at least 10^3 PFU of the intermediate strain of IBD virus per dose. Six chicks from each group (C1 and T1) were maintained for blood sample collection, while three birds from each group (C2 and T2) were sacrificed at 4-week intervals from 0-28th weeks for collection of the Bursa. The experiment was ethically approved by the Institutional Animal Ethics Committee (IAEC), vide Approval Reference Number CVSC/CAU/IAEC/21-22/P-4 dated 16.11.2022.

RESULTS AND DISCUSSION

Histomorphology

The Bursa of Fabricius comprises of three distinct layers: the tunica serosa, tunica muscularis and tunica mucosa (Fig 1). The tunica serosa includes mesothelium, subserosal connective tissue, blood vessels and nerve endings. The tunica muscularis contains major blood vessels, nerves, and two layers of smooth muscle fibers. The innermost tunica mucosa features the lamina epithelialis and lamina propria, the latter forming bursal folds that enclose lymphoid

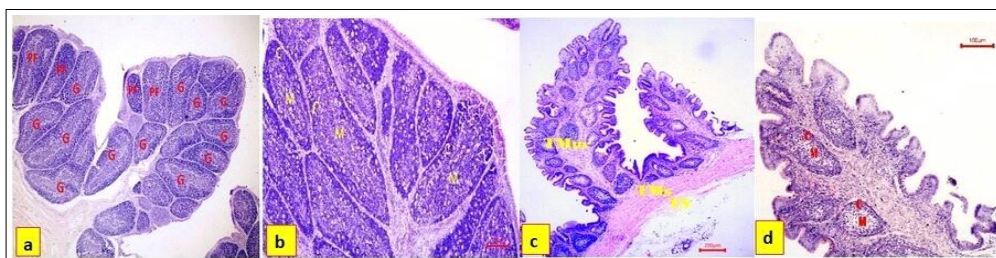


Fig 1: (a) 4th weeks old treatment group showing PF: Primary follicles, G: Germinal centers (H and E, $\times 40$); (b) Cortex (C) And medulla (M) Of bursal follicles in 4th weeks old treatment group (H and E, $\times 100$); (c) Tunica serosa (TS), Tunica mucosa (TMuc), Tunica muscularis (TMs) in 8th weeks old control group (H and E, $\times 100$); (d) cortex (C) and medulla (M) of bursal follicles of 8th weeks old control group.

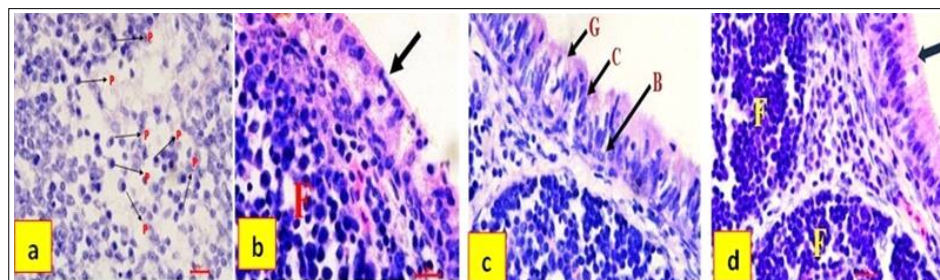


Fig 2: (a) Plasma cells in the medulla of bursal follicles in 8th weeks old treatment group (H and E $\times 1000$); (b) Follicle associated epithelium (arrow), F: Follicle (H and E, $\times 1000$); (c) Three cell types in the epithelium of bursa of Fabricius: Principal columnar cells (C); Basal cells (B); Goblet cells (G) (H and E, $\times 1000$); (d) Inter-follicular epithelium (arrow), F: Follicle (H and E, $\times 1000$).

follicles. These findings align with Ebru *et al.* (2015), Tamilselvan *et al.* (2017) and Dahariya *et al.* (2020), while Jain *et al.* (2010), Fang and Peng (2014), and Penchev. (2020) reported four tunics, indicating possible breed differences. These follicles are organized into a cortex and medulla, separated by a clearly defined corticomedullary border visible by the 8th week in the control group and by the 4th week in the treatment group similar to findings by Kempashi *et al.* (2017), Ayman *et al.* (2020) and Wu *et al.* (2021).

The cortex contains lymphocytes, lymphoblasts, macrophages and plasma cells, while the medulla comprises reticular cells, macrophages and plasma cells (Fig 2). The cortex stains more intensely due to the higher density of small lymphocytes consistent with Sultana *et al.* (2011), Yadav *et al.* (2020) and He *et al.* (2015). Medullary plasma cells exhibit prominent rough endoplasmic reticulum and large Golgi regions as also noted by Yadav *et al.* (2020). Secondary follicle or germinal center formation began at 4th

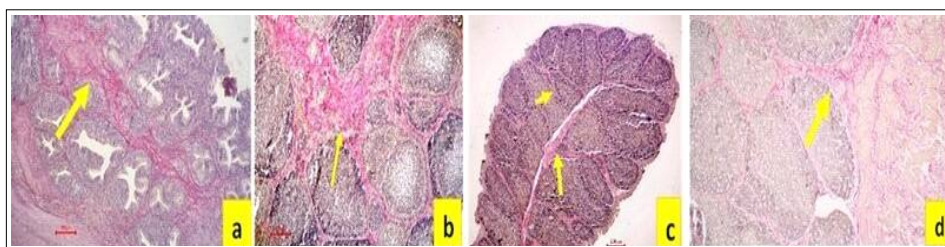


Fig 3: Collagen fibers (arrow), in (a) 8th weeks control group (VanGieson, × 40), (b) 12th weeks control group (VanGieson, × 100), (c) 12th weeks treatment group (VanGieson, × 40) and (d) 24th weeks treatment group (VanGieson, × 100).

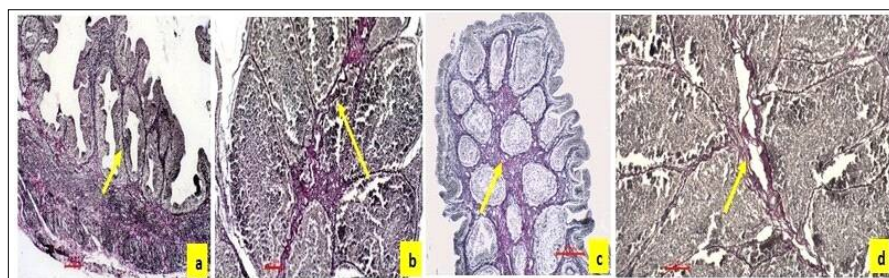


Fig 4: Reticular fibers (arrow) in (a) 8th weeks control group (Gomori's, × 40), (b) 8th weeks treatment group (Gomori's, × 100), (c) 12th weeks control group (Gomori's, × 40), (d) 12th weeks treatment group (Gomori's, × 100).

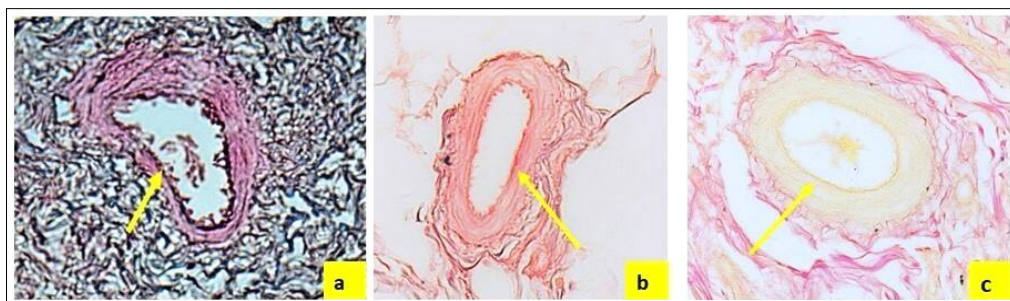


Fig 5: Elastic fibers (arrow) at (a) 0-day old, (b) 24th weeks control group and (c) 24th weeks treatment group. (Hart's method, × 1000).

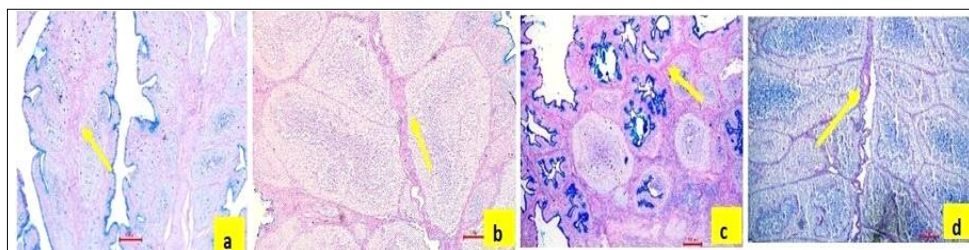


Fig 6: Mucopolysaccharides (arrow) at (a) 8th weeks control group, (b) 8th weeks treatment group, (c) 12th weeks control group and (d) 12th weeks treatment group. (PAS-Alcian blue, × 400).

weeks in the treatment group and at 8th weeks in the control group. The bursal folds are lined with two epithelial types: Follicle-associated epithelium (FAE), made of simple columnar cells and Inter-follicular epithelium (IFE), composed of pseudostratified columnar cells (Fig 2), supporting findings by Romano *et al.* (1996), Kanasiya *et al.* (2017) and Kadam *et al.* (2020), Ward and Middleton (1971) and Ebru *et al.* (2015) observed variations in cell types across species. Intraepithelial lymphocytes (IELs) were unevenly distributed, with higher density in the treatment group, consistent with Ayman *et al.* (2020). The epithelium contained columnar, basal and goblet cells, as described by Onyeausi *et al.* (1993) and Kadam *et al.* (2020), while Kanasiya *et al.* (2017) and Yadav *et al.* (2020) noted four cell types in Kadaknath chickens, suggesting breed-specific variation.

In this study, collagen fibers (red) were identified in the connective tissue of the tunica muscularis and the

lamina propria around the follicles (Fig 3) using the van Gieson's staining (van Gieson, 1889). These findings align with Kanasiya *et al.* (2017) in Kadaknath chickens, Dahariya *et al.* (2020) in Hansli and Vencoob broiler chicken and Udoumoh *et al.* (2022) in broilers. At day 0, these fibers were sparse but increased with age in both control and treatment groups. Although the distribution pattern remained similar across groups, collagen fiber presence became more prominent by the 24th week, showing greater abundance compared to the 0-day group. In contrary, Jain *et al.* (2010) noted higher fiber density in Vanaraja chicks compared to CARL Shyama growers, indicating possible breed-specific differences. Reticular fibers (black) were observed alongside collagen fibers using the Gomori's staining method (Gomori, 1937) in the loose connective tissue of the tunica muscularis and around the follicles in the lamina propria (Fig 4). These finding were consistent with findings by Kanasiya *et al.* (2017) in Kadaknath birds and Deka *et al.* (2020) in

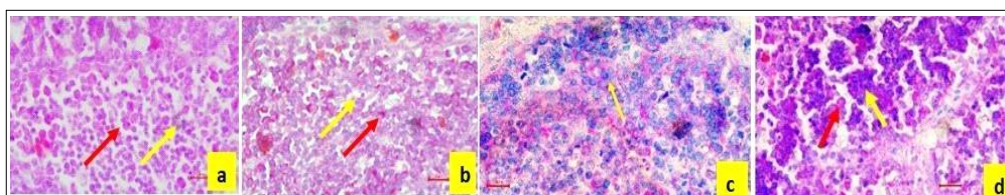


Fig 7: DNA (yellow arrow) and RNA (red arrow) at (a) 4th weeks control group, (b) 4th weeks treatment group, (c) 20th weeks control group and (d) 20th weeks treatment group (Methyl green pyronin method, $\times 1000$).

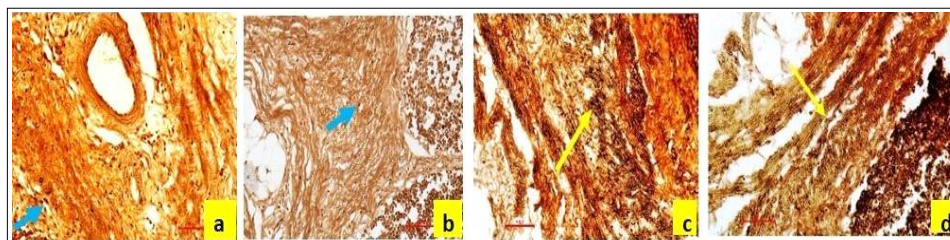


Fig 8: Nerve fibers (arrow) in bursa of Fabricius at (a) 8th weeks control group (b) 8th weeks treatment group, (c) 16th weeks control group, (d) 16th weeks treatment group (Bielchowsky's method, $\times 1000$).

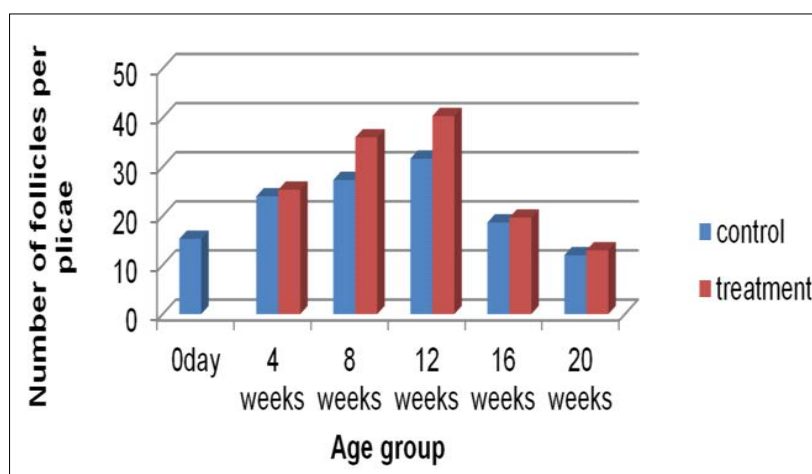


Fig 9: Number of follicles per plicae in bursa of Fabricius of Rainbow Rooster chickens from 0-day to 20th weeks of age.

Pati ducks. Although less dense than collagen fibers, reticular fibers increased with age in both control and treatment groups. However, no variation in their distribution was noted within the same age group, indicating no influence from IBDV vaccination. Hart's staining (Mallory, 1961) revealed elastic fibers (blue-black to black) in the bursa of Fabricius, primarily around blood vessels in the tunica mucosa (Fig 5) consistent with findings by Kanasiya *et al.* (2017) in Kadaknath chickens, Dahariya *et al.* (2020) in Hansli and Vencoob broilers. No differences were observed between

control and treatment groups of the same age, suggesting the IBDV vaccine had no effect on tissue fibers. PAS-Alcian Blue staining at pH 2.5 (Mowry and McManus, 1963) revealed weak magenta staining, indicating low mucopolysaccharide presence. Both the epithelium and tunica mucosa showed weak PAS positivity across all age groups in both control and treatment birds (Fig 6). These findings were similar with Sugimura *et al.* (1975) and Kempashi *et al.* (2017). Methyl Green Pyronin staining revealed positive detection of DNA (green-blue) and RNA (red) in the follicular cells

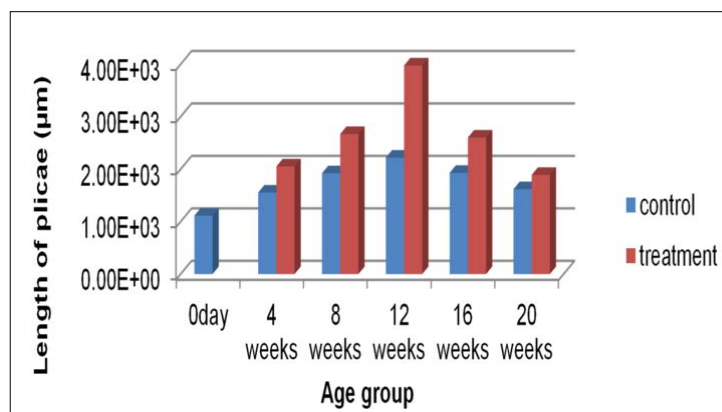


Fig 10: Plical length of bursa of Fabricius in Rainbow Rooster chickens from 0-day to 20th weeks of age (µm).

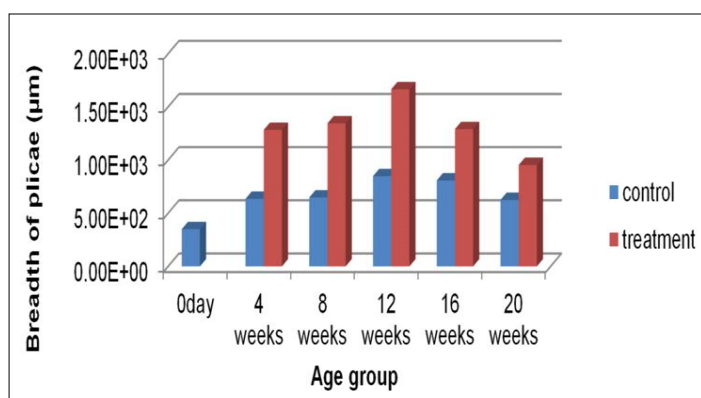


Fig 11: Plical breadth of bursa of Fabricius in Rainbow Rooster chickens from 0-day to 20th weeks of age (µm).

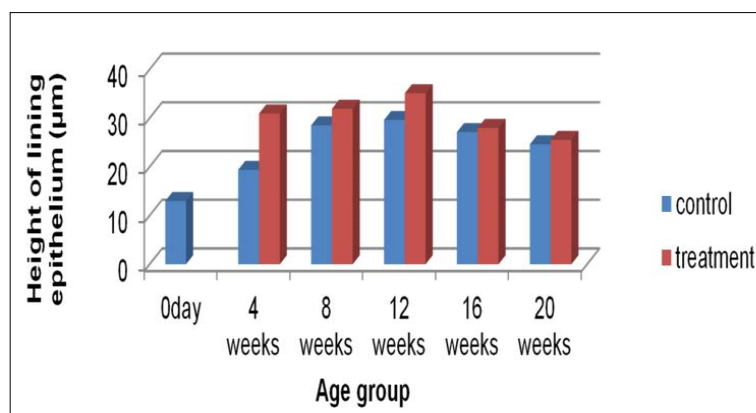


Fig 12: Height of epithelium of bursa of Fabricius in Rainbow Rooster chickens from 0-day to 20th weeks of age (µm).

of all experimental birds (Fig 7). Both control and treatment groups showed consistent DNA and RNA positivity across all age groups. These findings align with Gulmez and Aslan, (1999) in native geese. Bielschowsky's staining identified black-stained nerve fibers, including axis cylinders and dendrites, in the tunica muscularis of the bursa of Fabricius (Fig 8). These fibers were present in all experimental birds from both control and treatment groups, regardless of age, consistent with Deka *et al.* (2020) in Pati ducks.

Histomorphometry

The number of follicles per plica increased with age until the 12th week, then declined in both groups (Fig 9). The treatment group showed a significantly higher follicle count at 8th and 12th weeks, with the highest number observed at 12th weeks. Jain *et al.* (2010) observed a higher number of follicles in CARI Shyama chicks and Vanaraja growers. Similarly, Kanasiya *et al.* (2017) reported the highest follicle count at 8th weeks in Kadaknath chickens, while Hashimoto and Sugimura (1976) recorded a peak at 13th weeks in White Pekin ducks. In contrast, Ayman *et al.* (2020) documented continuous follicular growth in Sonali chickens. Notably, a significant increase in follicle count at the 8th and 12th weeks in the treatment group may be attributed to an elevated immune cell response. Plicae length (Fig 10) and breadth

(Fig 11) increased significantly up to the 12th week, followed by a gradual decline. Peak length and breadth occurred at 12 to 16th weeks in the control group and 8 to 12th weeks in the treatment group. Ayman *et al.* (2020) reported continuous growth in Sonali chickens up to 56 days. A highly significant difference of plical length between the control and treatment groups was observed from 4th to 12th weeks possibly due to increased immune cells. The maximum height of the lining epithelium (Fig 12) in the control group, was observed between the 8th and 12th weeks, while in the treatment group, it peaked between the 4th and 12th weeks. A significant difference was noted at 4th weeks of age in the treatment group compared to the control. The thickness of the cortex (Fig 13) increased until the 12th week, then gradually declined in both groups, showing significant differences. A notable difference between the groups was observed in the treatment group at the 8th and 12th weeks likely due to bursal maturation and increased immune cells. The control group showed maximum medullary breadth (Fig 14) at 12th weeks, while the treatment group peaked between 8th to 12th weeks. The breadth increased until the 12th week, then gradually declined, with a significant difference between the two groups at the age of 16th and 20th weeks. Ayman *et al.* (2020) observed continuous growth of lining epithelium, cortex thickness and medullary growth in Sonali chickens from 0-56 days.

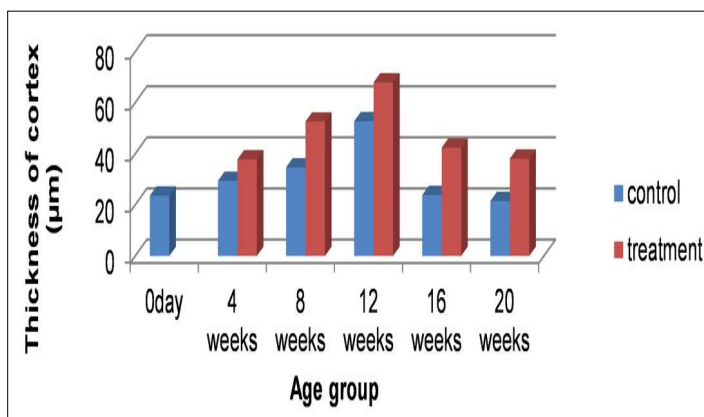


Fig 13: Cortical thickness of bursal follicles in Rainbow Rooster chickens from 0-day to 20th weeks of age (µm).

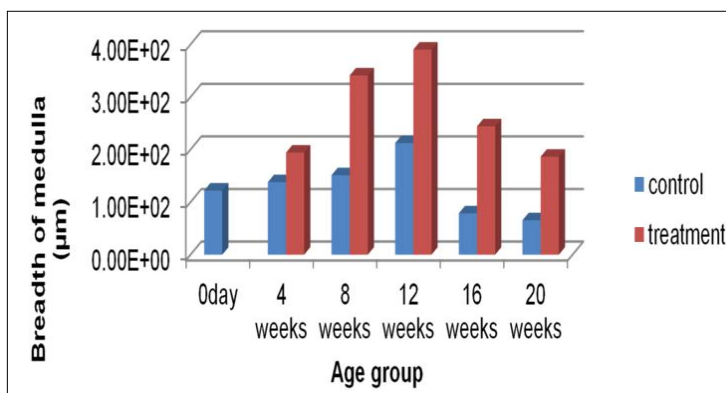


Fig 14: Medullary breadth of bursal follicles in Rainbow Rooster chickens from 0-day to 20th weeks of age (µm).

CONCLUSION

From the study, we can conclude that, the involution of the bursa was prolonged in the treatment group. There was early development of secondary follicles in the bursa of Fabricius in the treatment group. A significant increase in lymphocyte infiltration was observed in the bursal follicles of the treatment group. The treatment group showed a higher antibody response compared to the non-vaccinated control group.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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