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Impact of IBDV Vaccination on Bursa of Fabricius in Rainbow Rooster Chickens: A Gross, Serological and Ultrastructural Analysis

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Abstract

This study aimed to evaluate the effects of IBDV vaccination on the gross morphology, serology, and ultrastructural integrity of the Bursa of Fabricius in Rainbow Rooster chickens from birth to 28 weeks of age. A total of 57 birds of both sexes were used. Blood samples (1 ml) were collected from the wing vein at 0, 4, 8, 12, 16, 20, 24, and 28 weeks for serological analysis. Ultrastructural studies were conducted on birds aged 8th and 12th weeks. Morphological observations revealed a progressive increase in body weight and bursal size, peaking at 12th weeks in both vaccinated and control groups before declining. The bursa appeared creamy white, soft, and oval to spherical. Length, width, and thickness significantly increased upto the 12th week. The maximum number of plicae was noted at the 4th weeks. Serologically, antibody titers peaked in vaccinated birds between weeks 12th and 16th, whereas titers in the control group were undetectable from week 8th onward. Ultrastructural analysis showed a well-defined cortex and medulla in bursal follicles, which are composed of reticular cells, lymphocytes, and macrophages.

Keywords: Bursa of Fabricius, Rainbow Rooster, IBDV Vaccination, Gross, Serology, Ultrastructure.

INTRODUCTION

Rainbow Rooster is a multicolored, low maintenance, dual-purpose chicken suitable for backyard and free-range farming. It matures between 4–6 months and lays 160– 180 eggs over ten months, starting around 160 days of age (Value Magazine team, 2019). Poultry meat is a rich source of iron, zinc, selenium, omega-3 fatty acids, protein, and vitamins (Singh *et al*., 2021). Immunity and digestion are critical for poultry health and growth. In chickens, the Bursa of Fabricius plays a key role in B lymphocyte development and immune response (Glick *et al*., 1956; Cooper *et al*., 1965). It appears on embryonic day 5 and matures by 20 weeks (Fellah *et al*., 2014), later undergoing involution. The Bursa also supports lymphocyte maturation and transport (Nagy *et al*., 2020). Infectious Bursal Disease (IBD) or Gumboro disease affects chicks aged 3–6 weeks, targeting immature B cells and causing immunosuppression (Caston *et al*., 2008; Williams and Davison, 2005). This study compares the Bursa of Fabricius in vaccinated and non-vaccinated birds based on anatomical and serological parameters to better understand its role in avian immunity.

Materials and Methods Location of the experiment

The present research was conducted in the College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, in the Department of Anatomy and Histology for gross morphology, Department of Veterinary Microbiology for serological tests, and ultrastructural morphology was evaluated at AIIMS, Delhi.

Experimental design

The study involved 57 Rainbow Rooster chickens of both sexes from the Livestock Product Management (LPM) farm at the College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (CAU), Aizawl, Mizoram. Chickens were divided into two groups: 30 in the control group and 27 in the treatment group. The treatment group received vaccinations

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at 14 and 21 days old and was raised separately. The Bursa of Fabricius samples were collected by sacrificing three birds from each group every four weeks from 0 to 28 weeks, except for the treatment group at day 0. The birds were sacrificed using the cervical dislocation (Jaksch, 1981) method. The Institutional Animal Ethics Committee (IAEC) granted ethical approval for using the birds in this experiment.

Collection and preparation of samples

The cervical dislocation technique was used to sacrifice chickens from each group. Two hours before slaughter, food and water were withheld. The Bursa of Fabricius was obtained through ventral abdominal dissection. The live body weights of the birds were recorded using an electronic weighing balance before the Bursa was removed. Samples were taken for gross examination immediately after the birds were sacrificed. Each sample was then individually weighed (in grams). The samples were washed with normal saline and blotted dry using blotting paper. The Bursa of Fabricius was visually inspected for its colour, consistency and shape. The dimensions of the Bursa, including length, width and thickness, were measured using vernier callipers. Data obtained during the study were analyzed using a standard procedure with the help of SPSS 20.0.

For serological observation, on day 14, coinciding with the day of vaccination for both groups of birds, 1 milliliter of blood samples was collected from the wing vein for serological testing. Similar samples were taken at 0, 4, 8, 12, 16, 20, 24, and 28 weeks of age. The separated sera were stored at -20! for serological assays. An indirect ELISA was performed following the method by Islam *et al*. (2003) to detect serum antibodies, with slight modifications. Results interpretation was also based on the guidelines by Islam *et al*. (2003). The average serum antibody titers were compared throughout the experiment at different intervals and correlated with the gross and histomorphological parameters. For ultrastructural observation, the Bursa of Fabricius from birds aged 8^{th} and 12^{th} weeks was utilized for ultrastructural studies in this research. Fresh tissue samples from both groups were cut into 1-2 mm pieces after gross morphological examination. These samples were then fixed in Carnovsky's fixative (2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2) for 4 hours at 4°C. After washing in 0.1 M buffer (three changes of 15 minutes each), the samples were preserved in 0.2 M sodium cacodylate buffer until further use. The tissue samples were then sent to AIIMS, New Delhi, for Transmission electron microscopy observation.

RESULTS AND DISCUSSION

Gross morphology

In this study, the body weight of Rainbow Rooster chickens increased significantly from 0 days to 28 weeks in both control and treatment groups, with vaccinated birds consistently weighing more (Fig. 1). These findings differ from those of Hashimoto and Sugimura (1976), who observed peak body weight in White Pekin ducks at 11 weeks, likely due to breed and dietary differences.

The weight of the Bursa of Fabricius in both control and treatment groups of Rainbow Rooster chickens showed a fluctuating pattern with age. The Bursa was present from birth to the 24^{th} week, with weight increasing until the 12^{th} week (Fig. 2) before gradually declining. A significant difference ($p<0.05$) between the control and treatment groups was noted at 16^{th} and 24^{th} weeks of age. (Fig. 3), likely due to increased immune cell activity during the growth phase between the 8^{th} and 16^{th} weeks. Involution began at the 20^{th} week, with plical fusion noted by the 24^{th} week (Fig. 4) and completion by the 28^{th} week. These findings differ from Deka *et al*. (2020), who observed the Bursa in Pati ducks only up to the 16^{th} week. Other studies also reported earlier peak Bursal weights, He *et al*. (2015) and Lavanya *et al*. (2019) in quails at the 5^{th} week, Ward and Middleton (1971) in Mallards at the 8^{th} week, Gultiken *et al*. (2010) in turkeys at the 9^{th} week, Khenenou *et al*. (2012) in broilers at the 10^{th} week, and Bickford *et al*. (1985) in White Leghorns at the 14^{th} and 16^{th} weeks.

The colour of the Bursa of Fabricius in all experimental birds appeared creamy white in both

control and treatment groups, consistent with findings by Sultana *et al*. (2011) in ducklings and Deka *et al*. (2020) in Pati ducks. In contrast, Onyeanusi *et al*. (1993) reported a pale pink bursa in Helmeted guinea fowl, Jain *et al*. (2010) observed a gray bursa in CARI Shyama, He *et al*. (2015) found it brown in Yellow quail, and Mahanta *et al*. (2018) noted a whitish bursa with a blackish tinge in Kadaknath chickens.

The shape of the Bursa of Fabricius in all experimental birds was oval to round in shape. These findings are consistent with those of Glick (1979) in chickens, Ebru *et al*. (2015) in Long-legged buzzards, He *et al*. (2015) in Yellow quail and Wu *et al*. (2021) in young Leiothrix lutea. However, in contrast to these results, Hashimoto and Sugimura (1976), Nagy *et al*. (2004), Lavanya *et al*. (2019) and Deka *et al*. (2020) described the Bursa of Fabricius as long and cylindrical in White Pekin ducks, Quail, Japanese quail, and Pati ducks, respectively. These differences may be attributed to breed variations.

The consistency of the Bursa of Fabricius in all experimental birds from both groups was soft. These findings are similar to those reported by Sultana *et al*. (2011) in ducklings and Deka *et al*.(2020) in Pati ducks. The width and length of the Bursa of Fabricius increased from birth to 12th weeks of age, then began to decline.

The maximum width and length were observed at 12th Weeks in both groups (Table 1). However, He *et al*. (2015) and Lavanya *et al*. (2019) reported the maximum width and length at the 5th week of age in Yellow quail and Japanese quail, respectively. At 20th weeks of age, the length of the treatment group showed significantly higher values ($p<0.05$) than the control group, suggesting a greater number of cells in the treatment group. These results suggest that IBDV vaccination caused a significant increase in the length and width of the Bursa in the treatment group.

The thickness of the Bursa of Fabricius increased from birth to 12th weeks, reaching its maximum in both groups (Table 2).These findings contrast with Lavanya *et al*. (2019), who reported the maximum thickness in Japanese quail at the 5th week of age. In this study, the Bursal thickness was significantly higher ($p<0.05$) in the treatment group compared to the control group from 8th to 16th weeks of age (Table 2). The greater thickness in the treatment group may indicate a higher number of immune cells present in the follicles.

The number of plicae peaked between the 4th and 8th weeks in both control and treatment groups before gradually decreasing. The control group showed more plicae than the treatment group, likely due to biological variation. Plicae fusion began by the 24th week (Fig. 4), and irregularity in plicae indicated the onset of involution, which was slower in the treatment group. These findings are consistent with Bickford *et al*. (1985) in White Leghorns and Dahariya *et al*. (2020) in Hansli and Vencobb broilers. In contrast, Hashimoto and Sugimura (1976) reported Bursal regression after the 13th week in White Pekin ducks, Khenenou *et al*. (2012) at the 20th week in broilers, Deka *et al*. (2020) at the 24th week in Pati ducks, and Yadav *et al*. (2020) at the 28th week in Kadaknath chickens. These variations are likely due to breed differences.

Serological and ultrastructural morphology

This study measured serum antibody titres in control and treatment groups using indirect ELISA at multiple intervals from day 0 to the 28th week. Both groups showed initial titres due to maternal antibodies. The treatment group received a primary vaccination at the 2nd week and a booster at the 3rd week. A notable rise in antibody titre was observed in the treatment group from the 12th to 16th weeks, followed by a gradual decline up to the 28th week (Table 3). In contrast, the antibody levels of the control group dropped to undetectable levels after the 8th week. These findings agree with Jakka *et al*. (2014) in White Leghorn chickens.

The ultrastructure of the Bursa of Fabricius was examined in 8th and 12th week-old birds. It is shown that a distinct basement membrane separates the cortex and medulla of the lymphoid follicles

in both control and treatment groups. Reticular epithelial cells were primarily found in the medulla, with characteristic pale, oval nuclei and cytoplasm containing ribosomes, mitochondria, and Strands of rough endoplasmic reticulum (Figs.5 & 6). These findings are consistent with Frazier (1974) and Yadav *et al*. (2020). Lymphocytes were present in both cortical and medullary regions,with smaller lymphocytes localized to the cortex and larger ones in the medulla Larger lymphocytes had more cytoplasmic organelles, aligning with observations by Frazier (1974), Yadav *et al*.(2020), and Udoumoh *et al*. (2022). Macrophages, primarily located in the medulla, contained phagocytosed material in cytoplasmic vacuoles, matching descriptions by Frazier (1974), Yadav *et al*. (2020), and Olah *et al*. (2022). No ultrastructural differences were observed between control and treatment groups, although direct comparisons were limited due to a lack of relevant literature.

CONCLUSION

The study demonstrates that IBDV vaccination results in delayed involution of the Bursa of Fabricius, indicating sustained lymphoid activity in the vaccinated Rainbow Rooster chickens. A significantly stronger antibody response was observed in the vaccinated group compared to the unvaccinated controls, highlighting the vaccine's efficacy in stimulating humoral immunity. Furthermore, IBDV vaccination was associated with enhanced lymphoid proliferation and contributed positively to the overall growth and immune system development. These findings underscore the critical role of timely vaccination in promoting immune competence and health in poultry.

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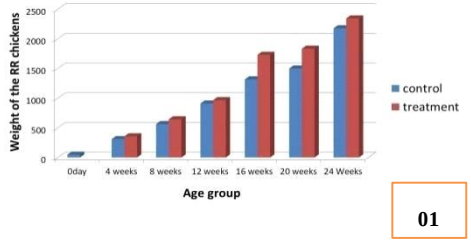

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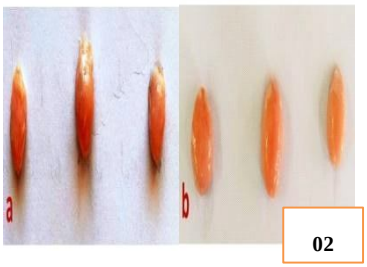

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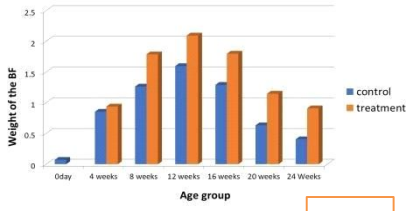

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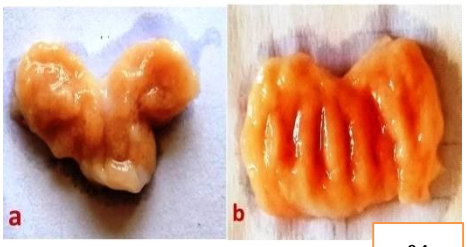
Fig 1: Weight of the Rainbow Rooster chicken (gm), **Fig 2:** Bursa of Fabricius of 12th weeks old birds: a) control group and b) treatment group, **Fig 3:** Weight of the Bursa of Fabricius (gm).



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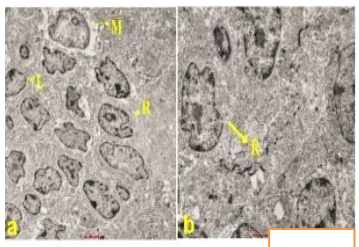

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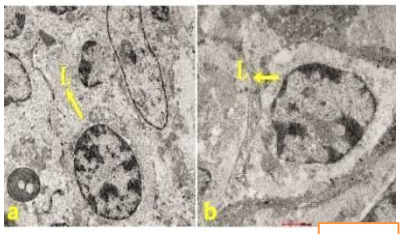

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Fig 4: Plical fusion at 24th weeks of age in a) control group and b) treatment group, **Fig. 5:** Transmission Electron Micrographs of Bursa of Fabricius in Rainbow Rooster chickens at 8th weeks of age (a) control group, (b) treatment group; M: Macrophages, L: Lymphocytes, R: Reticular cells (1000x), **Fig. 6:** Transmission Electron Micrographs of Bursa of Fabricius in Rainbow Rooster chicken at 12th weeks of age (a) control group, (b) treatment group; L: Lymphocytes (10000x).

Table 1: Width and length of Bursa of Fabricius (mm), *: Significant, NS: Non-significant

Age of the birds/groups	Width of the Bursa of Fabricius (mm)		T-value (Width)	Length of the Bursa of Fabricius (mm)		T-value (Length)
	Control	Treatment		Control	Treatment	
0-day	4.94 ± 0.30	-	-	6.07 ± 0.40	-	-
4 weeks	9.12 ± 0.82	12.72 ± 1.15	0.06^{ns}	11.74 ± 1.65	14.08 ± 1.34	0.33^{NS}
8 weeks	10.25 ± 0.46	14.67 ± 2.03	0.10^{ns}	13.65 ± 0.24	15.44 ± 2.55	0.52^{NS}
12 weeks	13.23 ± 0.89	15.68 ± 0.87	0.12^{ns}	14. 25 ± 0.65	17.40 ± 1.17	0.08^{NS}
16 weeks	12.89 ± 1.46	12.91 ± 1.69	0.99^{ns}	11.70 ± 0.61	16.07 ± 2.05	0.23^{NS}
20 weeks	11.45 ± 0.79	12.74 ± 1.86	0.56^{ns}	11.39 ± 0.62	14.32 ± 0.74	0.04*
24 weeks	8.69 ± 3.99	9.32 ± 4.61	0.92^{ns}	10.94 ± 0.2	12.56 ± 0.76	0.11^{NS}
P-value	0.05*	0.55^{ns}		0.00**	0.54^{NS}	

Table 2: Thickness of bursa of Fabricius (mm) *: Significant, NS: Non-significant

Age of the birds/groups	Thickness of the Bursa of Fabricius (mm)		T-value
	Control	Treatment	
0-day	1.21 ± 0.07	-	-
4 weeks	1.640 ± 0.11	1.80 ± 0.12	0.38^{ns}
8 weeks	1.74 ± 0.10	2.37 ± 0.95	0.01*
12 weeks	1.99 ± 0.13	2.84 ± 0.27	0.05*
16 weeks	1.30 ± 0.21	2.32 ± 0.07	0.01*
20 weeks	1.07 ± 0.80	1.22 ± 0.35	0.70^{ns}
24 weeks	0.89 ± 0.13	1.19 ± 0.07	0.11^{ns}
P-value	0.00**	0.00**	

Table 3: Antibody titre (log value) against IBDV vaccination in different intervals of time

Age/ Groups	0 day	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	28 weeks
Treatment	1.8060	0.903 ±0	1.806 ±0	1.806 ±0	1.906 ±0.33	1.906 ±0.33	1.705 ±0.13	1.304 ±0.27	1.105 ±0.27
Control	1.8060	0.903 ±0	0.501 ±0.10	0	0	0	0	0	0

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