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Research Article

Effect of selenium-yeast supplementation on gross and histo-architecture of the caecum and its immune response in broiler chickens

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Abstract

Broiler birds (*Gallus gallus domesticus*) are most widely utilized for consumption. With the emergence of the modern era, the broiler is at risk for increased oxidative stress due to its higher rate of muscle growth and metabolism, and by this time, the European Union banned the use of antibiotic growth promoters due to health issues of both livestock and humans. It has been demonstrated that the performance can be improved by a simple replacement of antibiotics with organic selenium. A total of sixty day-old chicks were randomly distributed into the control and experimental groups. Each group had four replicas, with seven birds in each. Selenium yeast was mixed with the basal diet of the treatment group at the dose rate of 0.30 mg/kg diet. The samples were collected on the 1st, 7th, 14th, 21st, 28th, 35th and 42nd days of the experiment. Growth performances of treated birds were better than control birds. Comparatively, the occurrences of goblet cells, distribution of lymphatic nodules, and diffuse lymphatic tissues were more in the treatment group of birds. The caecal tonsil was identified at the neck part of the caeca and increased in size during post hatched period. In conclusion, supplementation of Se-yeast improved caecum in terms of an increased number of goblet cells and lymphocytes, and minimized the IL10 positive reaction, which are responsible for better growth and immunity of the birds.

Keywords: Growth performance, Histomorphology, IL10, Immunohistochemistry, Selenium-yeast

Highlight

- Selenium-yeast supplementations enhance the growth performance of broilers.
- Selenium-yeast supplementations improve the caecal health.
- Selenium-yeast supplementations boost the immune system.

INTRODUCTION

Broiler birds are the most widely utilized poultry varieties for consumption. Modern commercial broilers are reared to reach a standard slaughter weight of about 2 kg in only 5 to 7 weeks (Eriksson *et al.*, 2008). Meat production is related to the growth performance of the birds, depending on the quality of feed supplements and the immunity of the birds. Relative to the whole body length, the gastrointestinal (GI) tract is much shorter in poultry than that of mammalian species, causing the digesta to pass through the entire GI tract faster. However, diet and feeding can have an effect on the passage rate (Hughes, 2008). In order to compromise the rapid transition of nutrient sources, the digestive organs of newly hatched poults undergo both anatomical and physiological changes (Uni *et al*., 1999).

Since on January 1st, 2006, the European Union banned the use of antibiotic growth promoters. As alternatives to antibiotics, several feed additives have been studied, such as probiotics, prebiotics, synbiotics, and some herbal medicines. Among these feed additives, prebiotics have been practised and supplemented extensively into broiler diets in recent years (Castanon, 2007).

Selenium-enriched yeast (Se-yeast) is a common form of Se, which is used as a dietary supplement to intake this important trace mineral. Selenium (Se) is a micro mineral which is an essential element for poultry production, and low or absent of Se in the diet leads to poor growth performance, nutritional muscular dystrophy, exudative diathesis, immune deficiencies, reduced antioxidant status in plasma and various organs

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(Safdari-Rostamabad *et al*., 2017). Nevertheless, with the emergence of the modern era, the broiler is at risk for distress from increased oxidative stress due to its higher rate of muscle growth and metabolism. It has been demonstrated that the performance of the modern broiler can be improved by a simple replacement of sodium selenite with Sel-Plex, an organic form (Edens and Sefton, 2016). Specific commercial yeast cell wall polysaccharides supplied in feed (Bio-Mos®, Alltech Inc.) are able to block fimbriae of pathogenic bacteria, and thus prevent their adhesion to the mucous epithelium. Since adhesion presents the first step in microbial invasion, blocking the receptors may prevent or eliminate the infection. These are also able to adsorb mycotoxins, thus decreasing their toxic effect and mediating their removal from the organism (Kogan and Kocher, 2007).

The present investigation was conducted to explore the effect of supplementation of Se-yeast on growth performance, gross and histomorphological changes of the caecum and immunity of poultry birds.

MATERIALS AND METHODS

Ethical approval

The proposed experiment is duly approved by the Institutional Animal Ethics Committee, Faculty of Veterinary and Animal Sciences, WBUAFS, Kolkata, India. [Ref. No. IAEC/61(V)].

Procurement and distribution of experimental birds

Sixty number of day-old broiler chicks (Ven Cobb 400) were procured from the authorized breeder as per the ethical committee guidelines. The birds were grouped into two viz. control and treatment groups, and each group had four replicates (R-1, R-2, R-3 and R-4). Each replicate had seven birds.

Collection of specimens and preservation

On the very first day, randomly four (4) chicks were sacrificed after a few hours of hatching with euthanasia, and samples were collected. Then at weekly intervals, 4 birds (1 bird/replica) from each group were euthanized by injecting an overdose of Sodium pentobarbital IP at the dose rate of 120 mg/kg (Gourdon, 2016) and samples were harvested carefully after sacrificed. The harvested tissue samples from different parts of the cecum were preserved in 10% neutral buffered formalin (NBF) after necessary cleaning with normal saline and kept for a minimum of 36 to 48 hours at room temperature. Before sacrifice, the live body weight was taken on the day of sample collection.

Methods of gross studies

After the sacrifice of the birds, gross anatomical observations were studied by visual observation.

Methods for histomorphological studies

Tissue processing and slide preparation: Fixed tissues were washed overnight under running tap water, dehydrated by ascending grade of alcohol, cleared with acetone and benzene and impregnated in molted paraffin in a hot air oven in different time arrangements and prepared square blocks. All the hard paraffin blocks were sectioned at 5 µm thickness with the help of semi motorized Leica 2125 DM rotary microtome. The sections were taken on clean grease-free albumenized glass slides, air-dried and preserved. Standard procedure was followed for Haematoxylin and Eosin staining (Luna, 1968), Masson's Trichrome staining (Luna, 1968) and PAS-alcian blue staining (pH 2.5) (Bancroft and Stevens, 1996).

Immunohistochemistry: To assess the in situ activity of IL-10 of the caecum and caecal tonsil, immunohistochemistry (IHC) was conducted on the 7th and 35th days of the control and treatment groups of birds.

The 3-5 µm thick paraffin sections which were obtained from different parts of the caecum, were taken on Poly-L-lysine coated slides. The tissue sections were de-paraffinized and then hydrated with a descending grade of alcohol and double distilled water (ddH_2O). Antigen Retrieval was done in antigen retrieval solution at 95-96^{o}C for 20-25 minutes (Thermo Fisher Scientific). Then, the slides were gently washed with phosphate buffer saline (PBS). The sections were then incubated for 40 minutes in 3% hydrogen peroxide at room temperature. After subsequent washing with PBS, the sections were incubated overnight at 4^{o}C in a humid environment with rabbit polyclonal anti IL10 antibody (Bioss Antibodies) in 1:100 dilution. After washing with PBS, the sections were again incubated with a secondary antibody (Goat anti-Rabbit IgG, Thermo Fisher Scientific) in dilution 1:100 at 37^{o}C. Immuno reactivity was detected after 1 hour of incubation. The sections were treated with freshly prepared DAB solution for 5-7 minutes/until brown colour appeared (Thermo Fisher Scientific). The sections were counter-stained with Mayer's haematoxylin and finally mounted in DPX.

Statistical analysis

All the data recorded was analysed statistically as per the standard method given by Snedecor and Cochran (1994), with the help of SPSS 17.0 software.

Photomicrography and micrometry

Photomicrography was made by Leica DM2000 microscope. Leica Qwin Images Analyser software was used for micrometry.

RESULTS

Growth performance

The average (Mean±SE) live body weight (ABW) and body weight gain (AWG) of all the birds of different groups were calculated at the age of 7th, 14th, 21st, 28th, 35th, and 42nd day and presented in Table 1 and Table 2, respectively. The data showed that supplementation of Se-Ye at the dose rate of 0.30 mg/kg diet significantly ($p<0.05$) increased the ABW of poultry birds on days 7th and 35th, but on the other days of the experiment, the treatment group did not significantly differ from the control group (Fig. 1).

On the 7th and 35th days, the AWG differed significantly ($p<0.05$) and treated birds showed the best result. On the other days of the experiment, there was no significant ($p<0.05$) difference between the groups. However, between the days, the AWG significantly ($p<0.05$) increased with the advancement of age up to the 35th day and then on the 42nd day, it somehow decreased (Fig. 2). The maximum AWG was found between the 28th and 35th days (5th week) of all the groups of the experiment (Table 2).

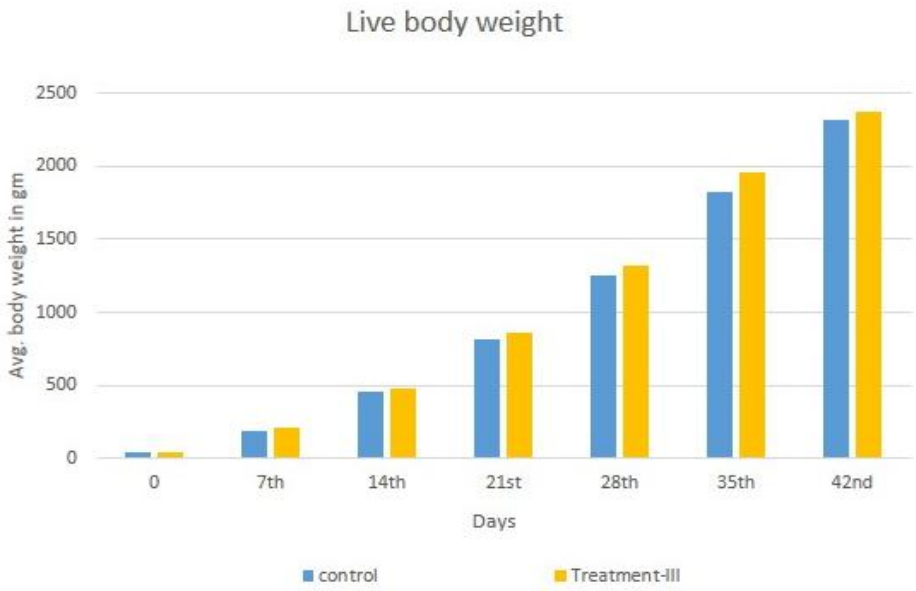

Fig. 1. Graphical presentation and age-wise comparison of the average live body weight of all the groups of poultry birds

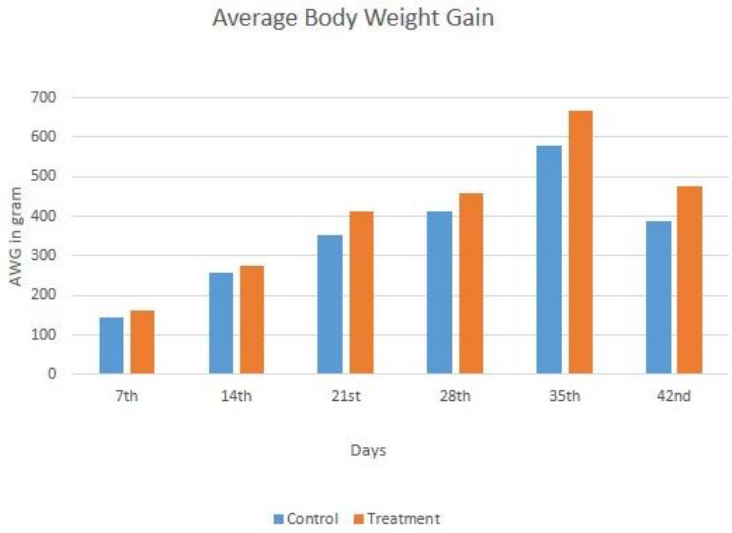

Fig. 2. Graphical presentation and age-wise comparison of the average body weight gain of all the groups of poultry birds

Table 1. Measurement of the live body weight (Mean±SE) in grams of the control group and all the other treatment groups of poultry birds during the period of the experiment

Groups / Days	Control	Treatment-III	p-value
0	46.237^{v}±0.926	46.237^{v}±0.926	
7th	190.823^{bu}±4.193	207.997^{au}±5.783	0.116
14th	458.373^{t}±16.406	473.430^{t}±12.093	0.850
21st	818.913^{s}±19.091	865.357^{s}±4.279	0.249
28th	1249.667^{r}±17.798	1317.973^{r}±1.650	0.387
35th	1826.667^{bq}±23.333	1961.000^{aq}±15.751	0.073
42nd	2315.000^{p}±36.171	2370.000^{p}±22.913	0.298
p-value	0.000	0.000	

Means bearing different superscripts (a,b,c,d…) in the same row differ significantly ($p<0.05$); Means bearing different superscripts (p,q,r,….) in the same column differ significantly ($p<0.05$)

Table 2. Measurement of body weight gain (Mean±SE) in grams of the control group and all the other treatment groups of poultry birds during the period of experiment

Groups / Days	Control	Treatment-III	p-value
0			
7th	144.587^{bt}±3.633	160.470^{at}±6.872	0.186
14th	257.867^{s}±9.085	273.913^{s}±12.714	0.807
21st	352.937^{br}±24.219	411.457^{ar}±13.149	0.168
28th	414.047^{q}±25.623	459.357^{qr}±28.240	0.400
35th	577.917^{bcp}±17.741	665.263^{ap}±21.054	0.065
42nd	389.167^{r}±14.530	475.000^{q}±43.301	0.424
p-value	0.000	0.000	

Means bearing different superscripts (a,b,c,d…) in the same row differ significantly ($p<0.05$); Means bearing different superscripts (p,q,r,….) in the same column differ significantly ($p<0.05$)

Gross morphology

Caecum: The paired caeca were like blind pouches situated on either side of the ileum and connected with each other by the mesentery and the adipose tissues (Fig. 3). These originated from the junction of the ileum and colorectum and extended cranially towards the liver. Their colour was yellowish-grey in chicks and brownish-grey to greenish-brown in adults. In the naked eye, each caecum could be divided into three parts viz. short proximal part- the neck, a long middle thin- walled tube- the body and a short apex.

Caecal tonsil: At the neck part of each caecum, a caecal tonsil was found. These were the small masses of lymphoid tissues, comparatively hard to touch and creamy white in colour in all the groups of poultry birds (Fig. 3). Their shape and size gradually enlarged and became bulged with the advancement of age.

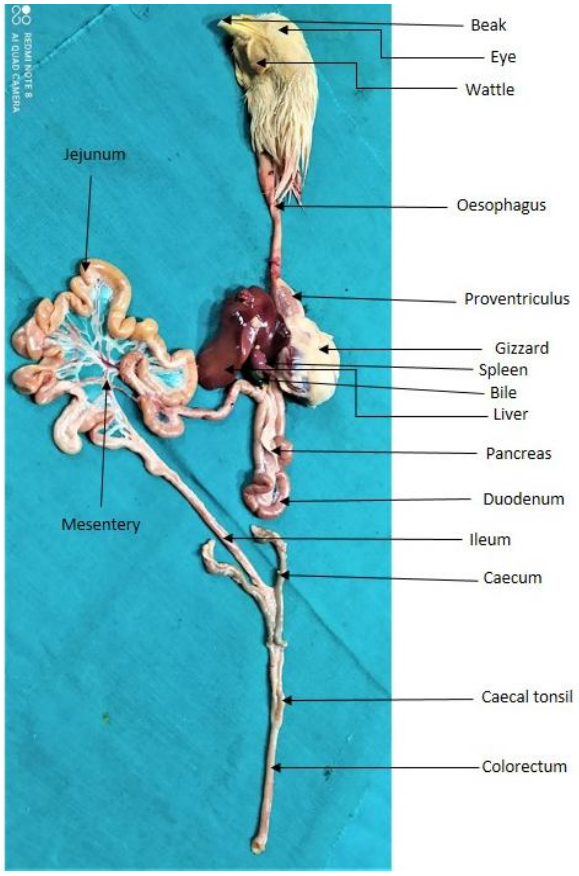

Fig. 3. Photograph showing the different segments of the digestive system of poultry bird

Histo-architecture of caecum

Tunica mucosa: Epithelium- The lining epithelium of the caecum of both the groups was short columnar at the beginning, slightly increased with the advancement of age and granular eosinophilic cytoplasm was observed (Fig. 4). The distribution of goblet cells was increased

with age and their population was maximum in treated birds as compared to control (Fig. 5 & Fig. 6). In the mucosal epithelium as well as the epithelium of the caecal gland, the intra-epithelial lymphocytes were seen infrequently and the clear cell occurrences were few to moderate at the finisher stage of all the groups of birds (Fig. 4 & Fig. 7).

Lamina propria- The lamina propria of the caecum was lightly cellular on day old, 7th and 14th days of age, then became densely cellular on the 21st day of age and after that, the density gradually decreased by the 42nd day of age of both the group of birds (Fig. 8 & Fig. 9). Some portion of lamina propria was occupied by a huge number of intestinal glands (Fig. 9). Lymphatic nodules or solitary lymph nodes were mostly located at the base of the lamina propria and around the plica on the 35th and 42nd days of age (Fig. 9). Compared to the control group, the treatment group showed more amount of lymphoglandular complex (LGC) and diffused lymphatic tissue (DLT) on 28th day and gradually became frequent by the 42nd day of age (Fig. 10 & Fig. 11).

Lamina muscularis mucosae- Lamina mucularis mucosae was thicker in treatment birds as compared to control birds and this was formed with few cell layers. (Fig. 9 & Fig. 11). This layer extended toward the core of plica by 21st day of age, and such type of histological feature was maintained till the day 42nd of the experiment (Fig. 11). Few collagen fibres were fairly distributed (Fig. 12).

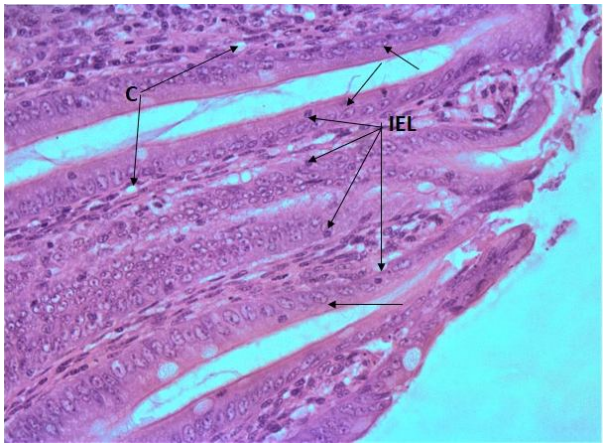

Fig. 4. Photomicrograph showing the cylindrical villi of caecum of the control group of birds at 21st day of age. Long columnar epithelium (Arrows), clear cells (C). H&E 40X

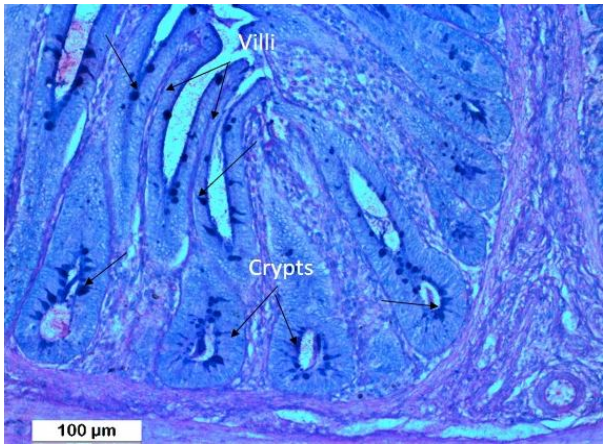

Fig. 5. Photomicrograph showing the histo-architecture of the caecum of control group of birds at 42nd day of age. Goblet cells (Arrows) in villi and crypts. PAS-AB 20X

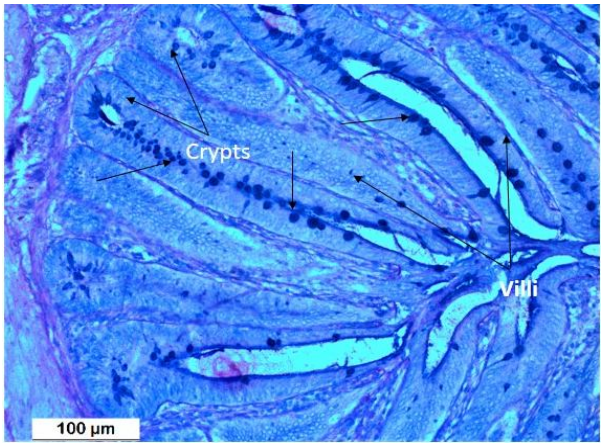

Fig. 6. Photomicrograph showing the histo-architecture of the caecum of treatment group of birds at 42nd day of age. Goblet cells (Arrows) in villi and crypts. PAS-AB 20X

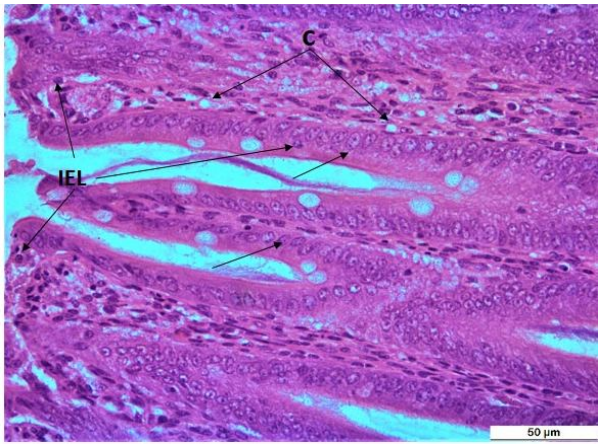

Fig. 7. Photomicrograph showing the villi of caecum of the treatment group of birds at 21st day of age. Tall columnar epithelium (Arrows), clear cells (C). H&E 40X

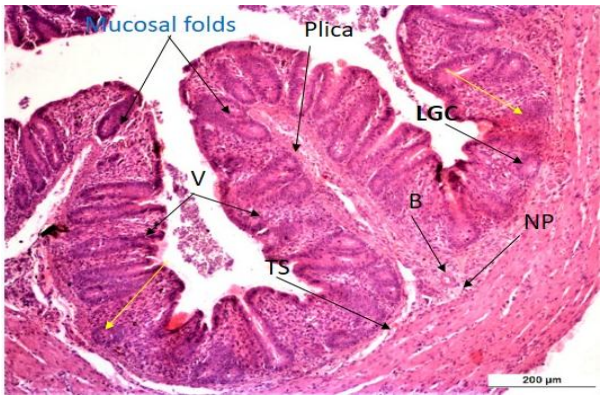

Fig. 8. Photomicrograph showing the histo-architecture of the caecum of the control group of birds at 21st day of age. Note- densely cellular L. propria, blood vessels (B), villi on plica (V), lymph nodules (Arrows), tunica submucosa (TS), nerve plexus (NP). H&E 10X

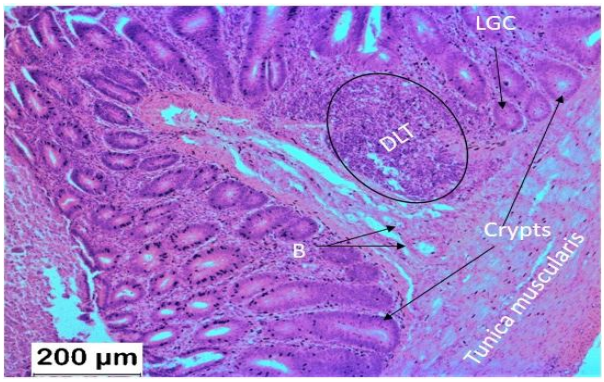

Fig. 10. Photomicrograph showing the histo-architecture of the caecum of treatment group of birds at 42nd day of age. Note- less density of L. propria, blood vessels (B), tunica serosa (Arrow). H&E 10X

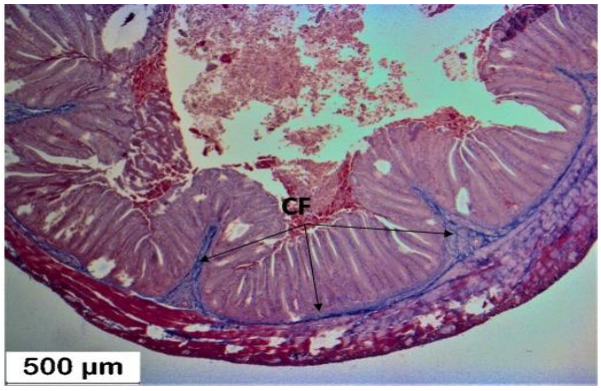

Fig. 12. Photomicrograph showing the histo-architecture of the caecum of treatment group of birds at 42nd day of age. Collagen fibres (CF) in plica, lamina propria and T. submucosa. MT 4X

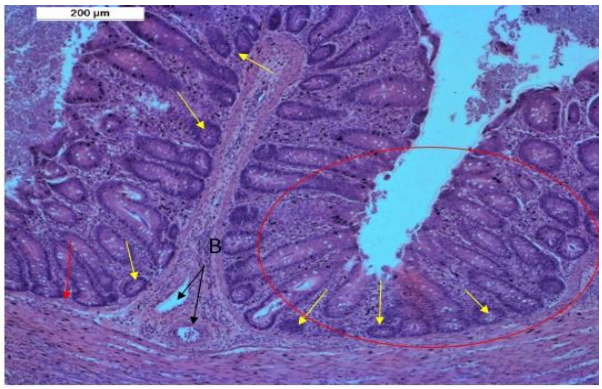

Fig. 9. Photomicrograph showing the histo-architecture of the caecum of control group at 42nd day of age. Note- decreased density of lamina propria, intestinal glands (Circle), lymphatic nodules (Yellow arrows), lamina muscularis (Red arrow), blood capillaries (B). H&E 10X

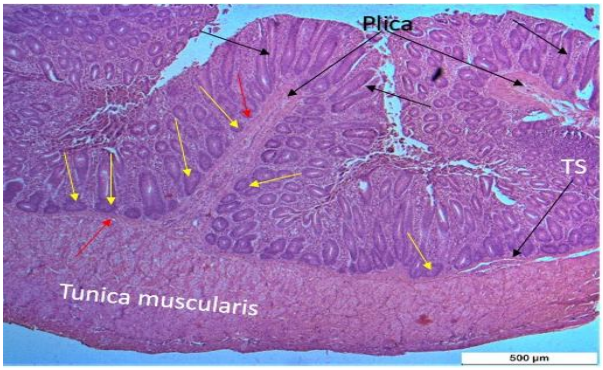

Fig. 11. Photomicrograph showing the histo-architecture of caecum of treatment group of birds at 42nd day of age, lymphatic nodules (Yellow arrows), L. muscularis (Red arrows), tubular glands (Black arrows), T. submucosa (TS). H&E 4X

Villi: In the present study, the villi of the caecum were short and conical on the day old to 7th days, short and cylindrical with a blunt apex on the 14th to 21st days of investigation of control birds (Fig. 4). From the 28th to 42nd days of age, villi and plica were together observed and these villi were spatula-shaped and short in height (Fig. 13). In the treatment group of birds, the caecum had a moderate number of villi which were short and broad in shape and few were short and cylindrical on the 14th day of age. On the 21st and 28th days of the experiment, they appeared club-shaped, but most of them were short and cylindrical in shape, and this architecture continued up to the end of the experimental day (Fig. 14). The plica contained a bunch of short villi which had blunt apex. In the control birds, the incidence of the villi was low from 0 to the 14th day, fair on the 21st and 28th days and moderate on the 35th and 42nd day of age (Fig. 8). On the other hand, the villi's incidence was fair

in treated birds in the early days of rearing and became plenty from the 28th day of age and onwards (Fig. 14). The base of the villi and mucosal folds were thick and infiltrated profusely with lymphoid cells on the 28th day and onwards (Fig. 14). These folds contained many short and cylindrical villi and tubular glands which were directly opened into the lumen (Fig. 11) and also some DLT and lymph nodules were distributed alongside the plica and on the apex of the folds (Fig. 8 & Fig. 9). The lumen appeared to be symmetrical since all of the villi on the mucosal folds were of the same height (Fig. 15). The capillary bed was observed to moderate in quantity in the core of the villi by the 42nd day of age (Fig. 9).

Crypts of Leiberkuhn: It is a short and narrow tubular gland. On the first days of the experiment, the crypt epithelium was cuboidal, then became short columnar on the 28th day, and finally became tall columnar on the 42nd day. With the advancement of age, the number of crypts gradually increased, and their shape and size also changed. In comparison, the number of crypts was more frequent in the treatment group than in control birds. In the control group, it was tubular, and in the treatment group, it was oval elongated (Fig. 10 & Fig. 13). On the 14th day of age, there were relatively few goblet cells in the glandular epithelium, but by the 28th day of age onward, they were frequent (Fig. 5). However, goblet cell occurrences were comparatively more in treated birds (Fig. 6). The lymphoglandular complex was distinct in the treatment group on the 42nd day of age (Fig. 10). Moreover, in the glandular epithelium, trans-epithelial lymphocytes (TEL) were well visible (Fig. 13).

Tunica submucosa: The submucosa was a thin continuous layer of connective tissue that was identified on the 14th day of age in the caecum of the control group of birds. Although, in the first week, this layer was not clearly demarcated, but from the 21st to 42nd days of age, this layer became moderately thick (Fig. 8). By the 21st day of age, they revealed a fair quantity of blood vessels and nerve plexus and from the 28th day to the 42nd day of age, these were frequent (Fig. 8 & Fig. 9). Collagen fibres were fairly distributed.

Whereas, in the treatment group of birds, the submucosa was distinctly thick and continuous layer on the 14th day of age. The thickness of this layer increased by the 28th to 42nd days of age and at certain locations along with lamina muscularis, extended towards the core of the mucosal folds (Fig. 11). Numerous blood capillaries and nerve plexus were found on the 28th day to the 42nd day of age (Fig. 14). The collagen fibres were found in moderate form (Fig. 12).

Tunica muscularis: The tunica muscularis layer was thin in the first two weeks and gradually became thicker with the advancement of age (Fig. 8). However, the maximum thickness was observed on 28th day of age in both groups but thickness was higher in treated birds as compared to control (Fig. 14). Apart from this, amoderate amount of collagen fibres were interspersed in this layer at all the days of investigations in both groups of birds (Fig. 16).

Tunica serosa: The tunica serosa layer of the caecum of both groups was very thin at the primary stage of life and was not wavy and corrugated. With the advancement of age, this layer was gradually becoming wavy and corrugated, while on the 21st day of age, it was strongly wavy in appearance (Fig. 15). The collagen fibres were scanty up to the age of the 21st day and then gradually became numerous afterwards in the treatment group of birds (Fig. 16).

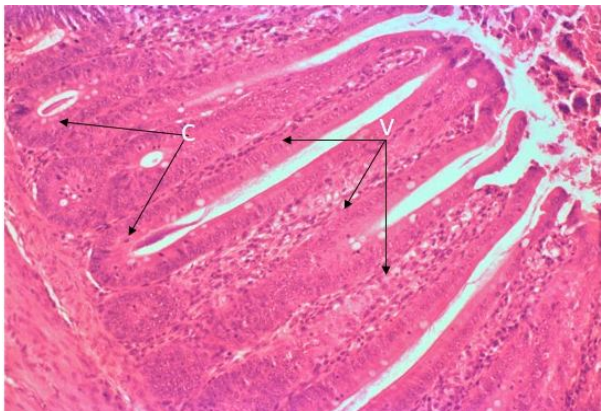

Fig. 13. Photomicrograph showing the histo-architecture of the caecum of the control group of birds at 42nd day of age. Spatula shaped villi (V), crypts with wide lumen (C). H&E 20X

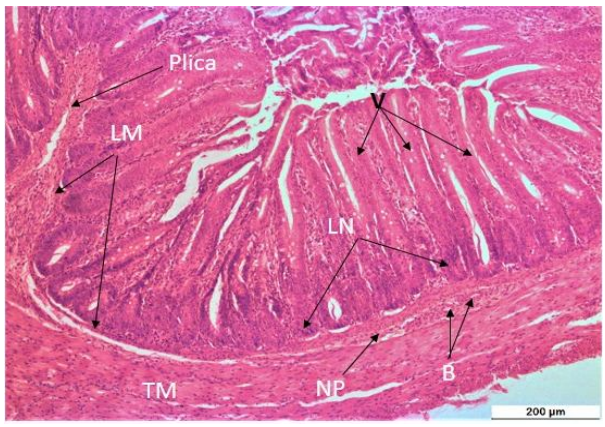

Fig. 14. Photomicrograph showing the histo-architecture of the caecum of the treatment group of birds at 28th day of age. Lamina muscularis (LM) plenty of club shaped villi (V), lymphatic nodules (LN), blood capillaries (B), nerve plexus (NP), tunica muscularis (TM). H&E 10X

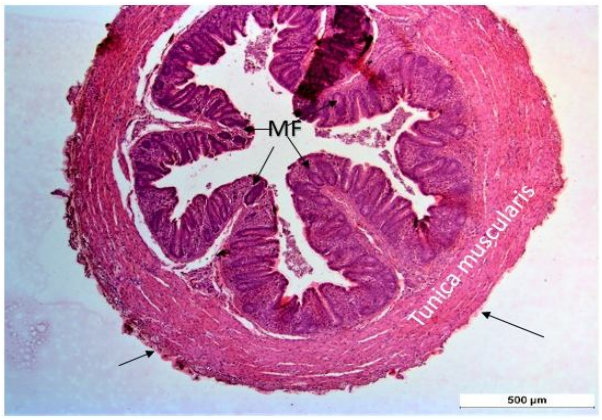

Fig. 15. Photomicrograph showing the histo-architecture of the caecum of the control group of birds at 21st day of age. Mucosal folds (MF), tunica serosa (Arrows). H&E 4X

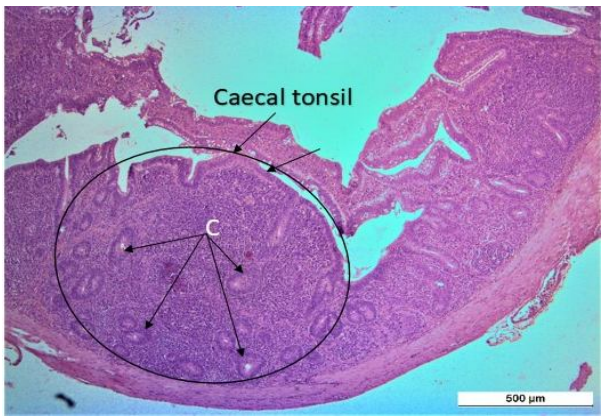

Fig. 17. Photomicrograph showing the histo-architecture of the caecum of the treatment group of birds at 14th day of age. Simple columnar epithelium (Arrow). H&E 4X

Caecal tonsil: Under histological observation, the caecal tonsil was located in the mucosal fold of tunica mucosa at the proximal part of the caecum and occupied the whole lamina propria of the fold. It appeared as thick nodular elevations and became massive due to increase in density of the lymphoid tissue accumulation with the advancement of age (Fig. 17). The villi around the tonsil were short and thin with a tip of finger-like apex (Fig. 18).

The caecal tonsil of the control group of birds was occupied by many lymph nodules, numerous diffuse lymphoid tissues and there was huge lymphocyte infiltration (Fig. 18). The lymph nodules were spherical in shape and were surrounded by a delicate smooth muscle tissue layer which was the continuation of muscularis mucosae and fine branches of inner circular muscle of the caecum (Fig. 19). They were present mainly at the base of the lamina propria, few of them seated on the submucosa of the caecum, and few of them

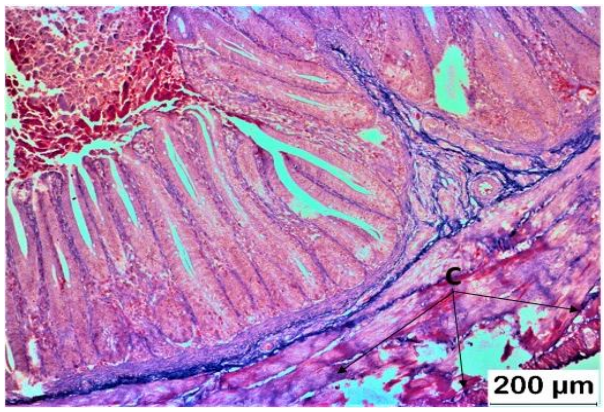

Fig. 16. Photomicrograph showing the histo-architecture of the caecum of treatment group of birds at 35th day of age. Collagen fibres (C) in tunica muscularis and tunica serosa. MT 10X

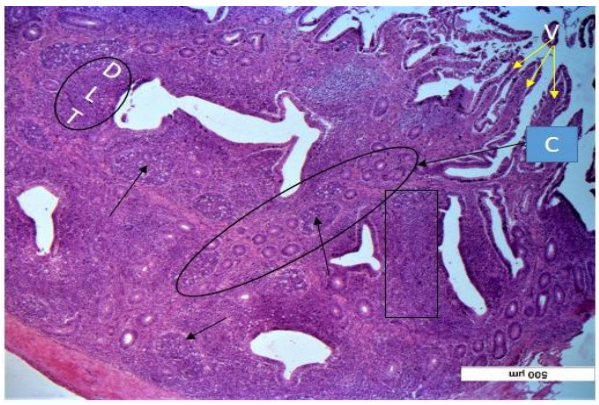

Fig. 18. Photomicrograph showing the histo-architecture of the caecal tonsil of the control group of birds at 42nd day of age. Villi (V), lymph nodules (Arrows), lymphocyte infiltration (Rectangle), leiberkuhn glands (C). H&E 4X

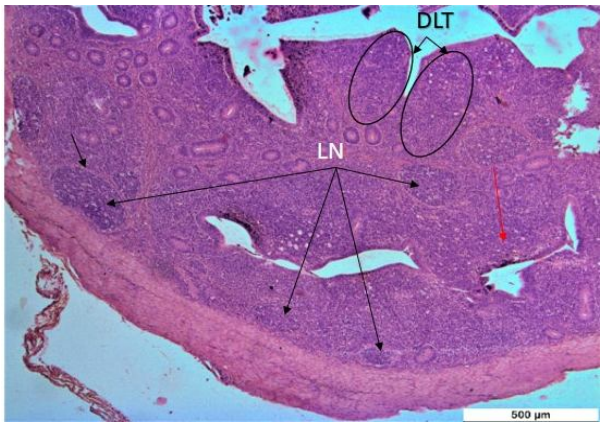

Fig. 19. Photomicrograph showing the histo-architecture of the caecum of the treatment group of birds at 42nd day of age. Lymphatic nodules (LN), delicate muscle tissue (Arrow), lymphocytic infiltration (Red arrow). H&E 4X

were interspersed here and there in the tonsil (Fig. 19). They were frequently populated with lymphocytes and among them, plasma cells and macrophages were interspersed. At the base, it had a big germinal centre (Fig. 20). The diffuse lymphoid tissue and the lymphocyte infiltration were distributed throughout the whole tonsil (Fig. 21).

In case of treatment groups, the occurrence of lymph nodules, diffused lymphatic tissues, and lymphocyte infiltration were more compared to the control birds (Fig. 22). Some intervening connective tissue compartmentalized the tonsil and became thicker gradually. A few Leiberkuhn glands in both groups were visible in dispersed condition and were oval to spherical in shape. With the advancement of age, their number was increased and arranged in lines from the base to luminal epithelium in all the cases (Fig. 17 & Fig. 18).

The lymphoglandular complexes (LGC) were also noticed in all the groups which were elongated oval structure laid on the base of the tonsil and more frequent in treatment groups (Fig. 21). A typical feature was observed that the surface epithelium had made a deep crypt into the nodular unit. These crypts were known as fossula and it was continued by the Lieberkuhn gland (Fig. 22).

Immunohistochemical study: Immunohistochemically, it was revealed that the expression of antigen-antibody reaction of IL-10 was diffused in the lamina propria. (Fig. 23). The occurrences of IL-10 positive cells were maximum in the lamina propria of the caecum and moderate in the caecal tonsil (Fig. 23 & Fig. 24). The IL-10 expression by IHC in the caecum was minimum in treated birds on both days of the experiment compared

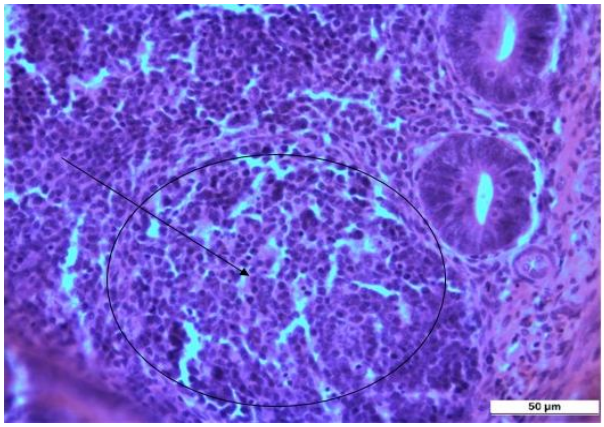

Fig. 20. Photomicrograph showing the lymphatic nodule (Circle) of the caecal tonsil of the control group of birds at 21st day of age. Germinal center (Arrow). H&E 40X

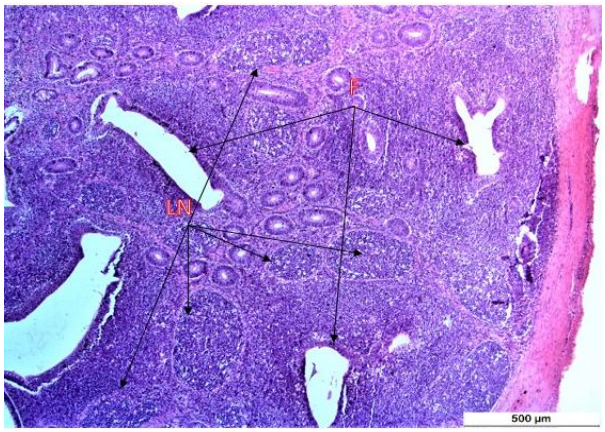

Fig. 22. Photomicrograph showing the histo-architecture of the caecal tonsil of the treatment group of birds at 42nd day of age. Lymphatic nodules (LN), Fossula (F). H&E 4X

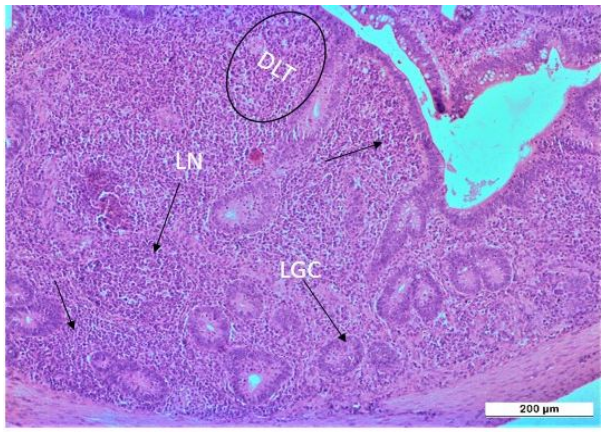

Fig. 21. Photomicrograph showing the histo-architecture of the caecal tonsil of the treatment group of birds at 14th day of age. Lymph nodule (LN), lymphocytic infiltration (Arrows) H&E 10X

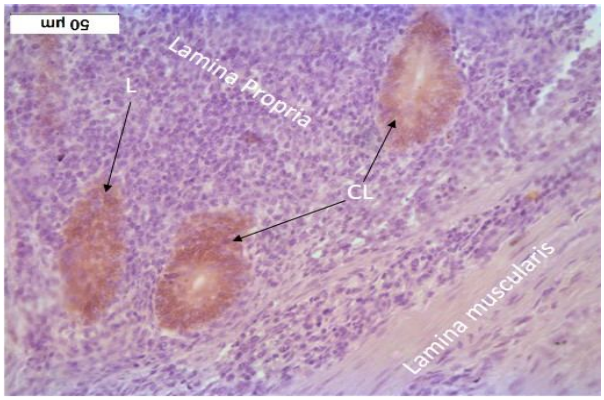

Fig. 23. Photomicrograph showing the lamina propria of the caecum of the control group of birds at 35th day of age. IL-10 positive reaction of crypts of Leiberkuhn (CL) and lymphatic nodule (L). IHC 40X

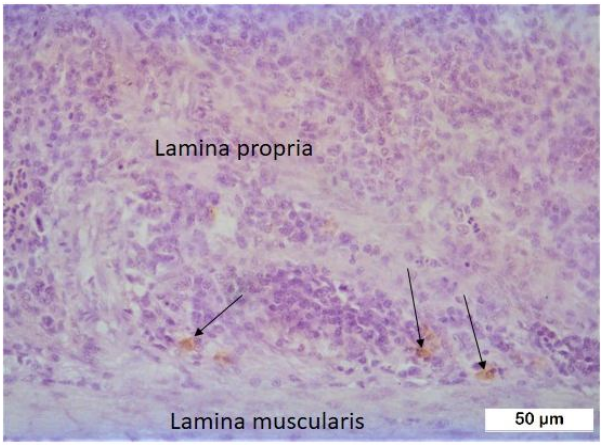

Fig. 24. Photomicrograph showing the crypts of the caecal tonsil of the control group of birds at 35th day of age. IL-10 positive cells (Circle). IHC 40X

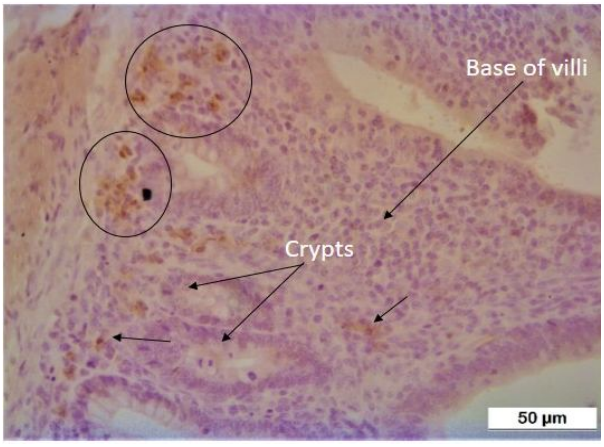

Fig. 25. Photomicrograph showing the lamina propria of the caecum of the treatment group of birds at 7th day of age. Cluster of IL-10 positive cells (Circles), scattered positive cells (Arrows). IHC 40X

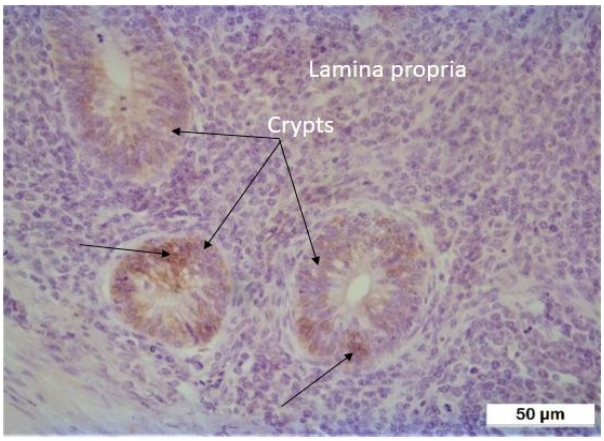

Fig. 26. Photomicrograph showing the lamina propria of the caecum of the treatment group of birds at 35th day of age. IL-10 positive reaction (Arrows). IHC 40X

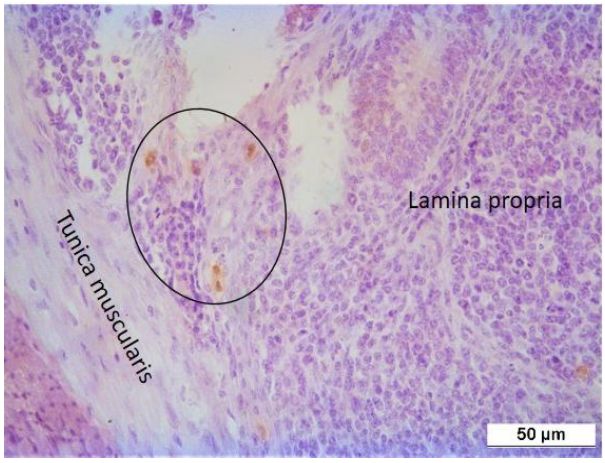

Fig. 27. Photomicrograph showing the immune reaction of caecal tonsil of the treatment group of birds at 7th day of age. Immune positive cells at the base of L. propria (Arrow). IHC 40X

to control birds (Fig. 23 & Fig. 26). When compared between the days, the reaction intensity of both caecum and caecal tonsil was more at the 7th day as compared to the 35th day (Fig. 24-27).

DISCUSSION

Growth performances: The average live body weight and weekly weight gain increased more significantly ($p<0.05$) in the dietary Se-Ye supplemented groups. The dietary selenium supplementation in different doses significantly ($p<0.05$) increased average daily weight gain or at different weekly intervals (Wang and Xu, 2008; Cai *et al.*, 2012; Dalia *et al.*, 2018; Shabani *et al.*, 2019; Pardechi *et al.*, 2020) which is corroborative with the present findings. In the previous studies, the live weight gain was higher in the live yeast and organic selenium-fed groups than in the control group (Rutz *et al.*, 2003; Upton *et al.*, 2008; Ghosh *et al.*, 2012; Saad *et al.*, 2013; Pardechi *et al.*, 2020; He *et al.*, 2021).

Gross: The right and left caeca of broilers arose from the lateral walls of the rectum, close to the junction with the ileum and the two caeca were blind pouches and extended along the line of the small intestine towards the liver and were closely attached along their length by the mesentery. These present findings were previously stated by Nasrin *et al.* (2012) in broilers and Zaher *et al.* (2012) in quail. The caecal tonsil was identified at the neck part of the caeca and increased in post hatched period with age. Similar observation was cited by Nnadozie *et al.* (2019).

Histomorphology: From the present histological observations of large intestine, it was revealed that there was a better histomorphological development in the treatment groups as compared to the control group. It seems from all parameters that a remarkable development of goblet cell population, size of intestinal glands and distribution of LGC and DLT were more in frequency and developed on an average one week earlier than the control group. Similar observations were cited by Zaher *et al.* (2012) in case of quail and also documented by Majeed *et al.* (2009) and İlgün *et al.* (2018) in broiler birds and Pandit *et al.* (2018) in Uttara fowl. The lymphatic tissues in the form of diffused and encapsulated lymphatic nodules of the caecal tonsil were present in the mucosa and submucosa of the caeca. With the advancement of age, the frequency and size of lymphatic nodules increased, and that is why the mucosal villi height decreased. This similar observation was reported by Akter *et al*. (2006) in broilers, Verma *et al*. (2019) in Kadaknath fowl and Ayman *et al*. (2021) in Sonali chickens. Post-hatch lymphocytic infiltration gradually increased in the lamina propria of the caecal tonsil, and tunica submucosa of the caecum showed dense lymphocytic infiltration. A similar observation was cited by Udoumoh *et al.* (2021), who described that at 7, 11, 14, and 21 post-hatch days, dense infiltration of a mixed population of lymphocytes was evident in both the lamina propria and submucosa. This is in agreement with the present observation. Rezaian and Hamedi (2007) examined that the nodular units were tonsil-like structures formed by a mucosal-submucosal prominence. Diffuse and nodular lymphatic tissue filled the lamina propria which was full of lymphocytes, macrophages, and mast cells. Lymph nodules at the base of lamina propria contain big germinal centres. These observations are in accordance with our present findings, and similar results were also documented by Yildiz *et al.* (2019) in quail.

Immunohistochemistry: IL-10 is recognized as a multifunctional cytokine produced by many immune cell types including macrophages, monocytes, dendritic cells and regulatory T-cell subsets and B-cells and a feedback regulator of diverse immune responses to infections (Couper *et al*., 2008). The role of IL-10 in birds is less evident. The present results in both groups suggested that in better gut health conditions IL-10 positive cells decreased. Lee *et al*. (2018) also documented that the *Clostridium perfringens* induced IL-10 production in chicken intestinal epithelial cells. Likewise, Hong *et al*. (2006), Rochell *et al.* (2016) and Arendt *et al*. (2019) hypothesized that IL-10 was increased during *Eimeria* infection in birds. Therefore, the antimicrobial property of Se-Ye reduced the pathogenic bacterial load for those treated birds showed less IL-10 positive reaction. These results are in accordance with the present findings. The caecum of the Se-Ye treated birds revealed the occasional presence of IL-10-positive cells on both days of experiments. It is therefore suggested that the treatment group is the best group in terms of the reduction of gut pathogens. The host immune system coordinates the balance of effector and regulatory immune cells as well as anti and pro-inflammatory cytokines in the physical condition through interaction with microbiota. It is evident that the host immune system senses the gut bacteria not only through recognition of pathogen-associated molecular patterns but in addition by sensing microbial metabolites which influence the host immune response of the gut and beyond (Belkaid and Hand, 2014).

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Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: MMI, PD, BR and IS: Planned, designed and conducted the research; PKD, PD, MMI: Analyzed the data; MMI, PD, IS: Drafted the manuscript and prepared the figures; JM, SI: Critically reviewed the article.

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