THE IRAQI JOURNAL OF
VETERINARY MEDICINE

eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Morphohistology and Biometric Characteristics of Cecal Tonsils of Sonali Chicken at Post-Hatching Ages

Ummay Ayman[1*], Md. A Jahid[2], Md. R Alam[3], Shonkor K Das[1]

[1]Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh, [2]Department of Anatomy and Histology, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Khanpura, Babugonj, Barishal, Bangladesh, [3]Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

***Correspondence:**
ayman@bau.edu.bd

Received: 30 June 2021
Accepted: 10 August 2021
Published: 28 December 2021

DOI:
https://doi.org/10.30539/ijvm.v45i2.1254

Cite:
Ayman U, Jahid MA, Alam MR, Das SK. Morphohistology and biometric characteristics of cecal tonsils of Sonali chicken at post-hatching ages. Iraqi J. Vet. Med. 2021; 45(2): 1-6.

A B S T R A C T

Knowledge of basic structures is prerequisite for acquiring an in-depth idea about the physiology and immunology of the lymphoid system. The study evaluates the age related histomorphometry of cecal tonsil of Sonali chicken at different postnatal stages in Bangladesh as literatures regarding this are very scarce. The investigation was carried out on 25 healthy Sonali chickens representing different stage of postnatal life: days 1, 14, 28, 42, and 56 (n=5). After ethically sacrifice (cervical subluxation method), cecal tonsil was collected and subjected for both gross and histological studies. Haematoxylin and Eosin stain was done for microscopic study. Morphologically, cecal tonsils were located bilaterally at the junction of small and large intestine. It had tubular structure and yellowish white in color. All gross parameters (weight, length, and width) found to be increased significantly ($P<0.05$) throughout the whole study period. Weight was measured 0.022±0.001 g at day 1 and noticed 0.181±0.016 g at the end of study tenure. The microscopic observations revealed that at day 28 encapsulated lymphatic nodules was present along with the diffuse lymphocytes at the lamina propria and submucosa layer, which was absent at the previous study groups. At day 1, only small infiltration of lymphocytes was identified and at day 14, lymphocytes were aggregating to form lymphatic nodules. After that, age related development was noticed in histological features. The findings would be a milestone to give an idea about the gut health and immune status of Sonali chicken and provide a basis for further immunization research.

Keywords: cecal tonsil, histomorphometry, postnatal stages, Sonali chicken

INTRODUCTION

A healthy and functioning defense mechanism allows all species on the planet not only to survive but also flourish in a possibly brutal territory with much less significant endeavor. It also aids in reducing financial damages caused by pharmacological treatment of diseases and birds' loss.

In mammals, a large number of encapsulated lymphatic nodules construct the fundamental details of the lymphoid system; whereas only in few aquatic birds, to be more precise, in swans, ducks and geese; distinctive lymph nodes

exist (1). Fully developed mucosa-associated lymphoid tissue (MALT) is found to be existing in maximum avian families (2). Respiratory system's- Bronchus-Associated Lymphoid Tissue (BALT) and digestive system's-Gut-Associated Lymphoid Tissue (GALT), segregated follicles, Peyer's patches, and tonsila cecalis are parts of MALT. In case of birds, the most common location of MALT is the lamina propria and submucosa of digestive and respiratory pathways (3). In the developmental process of lymphoid tissue, either concentrated or disseminated lymphoid cells are involved and assembled to construct primary and secondary lymphoid follicles separated by inter-follicular accumulated lymphocytes, lymphoblasts or plasma cells (4).

At certain anatomical sites, the lymphoid tissue was noticed to be organized into tonsils (5). The most common entry point for many potentially harmful microorganisms in avian species is the dietary tract. The delicate equilibrium among the components of the chicken gut can adversely affect by a number of causes related to food and pathogenic causal factors which may further influence the performance of production and health status of birds in commercial poultry activities (6).

Nowadays, the avian GALT is in limelight. The need for sound awareness regarding the avian gut health and immunity in producing vaccines is likely to be a key reason behind this growing concern (7). In addition, due to the prohibition on growth-fostering antibiotics; avian GALT based experiment is motivated by the quest for efficient pre and probiotics that enhance the defensive function of avian gut (8-10). These components have both local and systemic effects as through the common mucosal immune system, they can also stimulate natural defenses in other organs (11).

Owing to the presence of huge amount of lymphatic tissue in lamina propria and submucosa that ultimately consists of cecal tonsils, thus cecum provides functions as a protective organ. In fact, the immuno-defence mechanism of the cecal environment continuously controls proliferation of microflora in the cecum as well as avoids the invasion of extra caecal microorganisms (12). Understanding the physiology of the immune system requires comprehensive knowledge of basic structures of the lymphoid tissue and factors that may affect these structures such as age and developmental changes of the animal. Thorough study on morphohistology, immunohistochemistry of the lymphatic tissues has already been performed on different breeds of chickens (deshi and hybrid) of Bangladesh at their different prenatal and postnatal developmental stages (13-15). In case of Sonali chicken, the histomorphology of thymus (16), cloacal bursa (17) and spleen (18) have been investigated.

Therefore, in Sonali chicken, information regarding microstructural features of cecal tonsil and their possible age-related changes is arguably less. Present study aims to evaluate cecal tonsil of Sonali chicken at different after birth representative age groups based on histomorphology and biometric techniques.

MATERIALS AND METHODS

Ethical Approval

The study protocol for the experimental use of animals was approved and performed according to the guidelines established by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh, Bangladesh [AWEEC/BAU/2019(30)].

Animals and Specimen

At Bangladesh Agricultural University (BAU) Poultry Farm, the experimental male Sonali chickens were raised with adequate biosecurity, food and water *ad libitum*. The chicken's management practices, feeding history and vaccination schedule were also considered. Importantly, experimental chickens have not been shown any detectable diseases. Total number of experimental chicken (N=25) was categorized into five groups based on age, starting from hatching day to day 56th at 2 weeks interval (1st, 14th, 28th, 42nd, and 56th, n=5). Cervical subluxation method was performed to ethically sacrifice the chickens. Then, the cecal tonsils were collected for anatomical and biometric study. The entire research was carried out in the Department of Anatomy and Histology, Faculty of Veterinary Science, BAU, Mymensingh, Bangladesh.

Gross-morphometric Study

Immediately after sacrifice, the cecal tonsils were collected and washed in normal saline. After that, samples were weighed (g) and length, width, and thickness were taken into consideration and calculated using slide calipers (mm). By eye estimate, cecal tonsils' color had been recorded. Digital camera was used to take the macroanatomical images directly from the organs.

Histo-morphometeric Study

For histological observation, the cecal tonsils were preserved in Bouin's fluid for 24–36 hours. The samples were then washed in phosphate-buffered saline for three changes 20 minute in each, dehydrated by rising alcohol grade (i.e., 70%, 80%, 90%, 100% (1), 100% (2), and 100% (3) alcohol) for 2 hours in each, cleaned in xylene for 3 changes of 2 hours in each, and finally embedded in paraffin. The paraffin blocks were cut at 6-µm thickness with a sliding microtome (MIC 509, Euromex, Japan). Lukewarm water at 37 °C was used to stretch the sections (KF-WS-100 Tissue Flotation Water Bath). To mount and adhere the sections to the slides, glass slides were painted with adhesive solution (egg albumin) followed by dried on

slide warmer at 37 °C. Harris's Hematoxylin and 1% Eosin Y stain (H & E stain) was aided in the microscopic analysis and finally assembled with Dibutylphthalate Polystyrene Xylene (DPX).

Photomicrographs and Biometric Measurements

The microscopic architectures of cecal tonsils were evaluated through light microscope using both low (10×) and high (40×, 100×) power magnifications. For better demonstration of the study findings, the required photographs were taken from the selected specimens using Carl-Zeiss Photo Microscope (Germany) connected with a digital camera. At the next step, to measure the biometric data of different histological parameters of the tissues, calibrated stage micrometer in µm was used. For biometric assessment, six sections from each age group (total of thirty sections) were taken into consideration. The histological parameters, (thickness of tunica muscularis, length and breadth of lymphatic nodules, and glands of Lieberkühn/mmsq) were measured as part of biometric study.

Statistical Analysis

Statistical Package for the Social Sciences (IBM SPSS; version 22.0, IBM Corp., Armonk, NY, USA) software was used for analyzing the data. One-way ANOVA test was done to compare all of the biometric data collected in this study. The post hoc Duncan's test was performed following that to separate significant means. In all trials, the findings were represented as mean±standard error of mean (SEM). Statistically, If $P \leq 0.05$, the variations were deemed to be significant (19).

RESULTS

Gross Observations

The cecal tonsils of Sonali chicken were found bilaterally at the junction of small and large intestine laying down at the proximal part of the two ceca. The cecal tonsils had tubular structure, yellowish white in color. They had the peritoneum covering and due to that its surface was smooth (Figure 1).

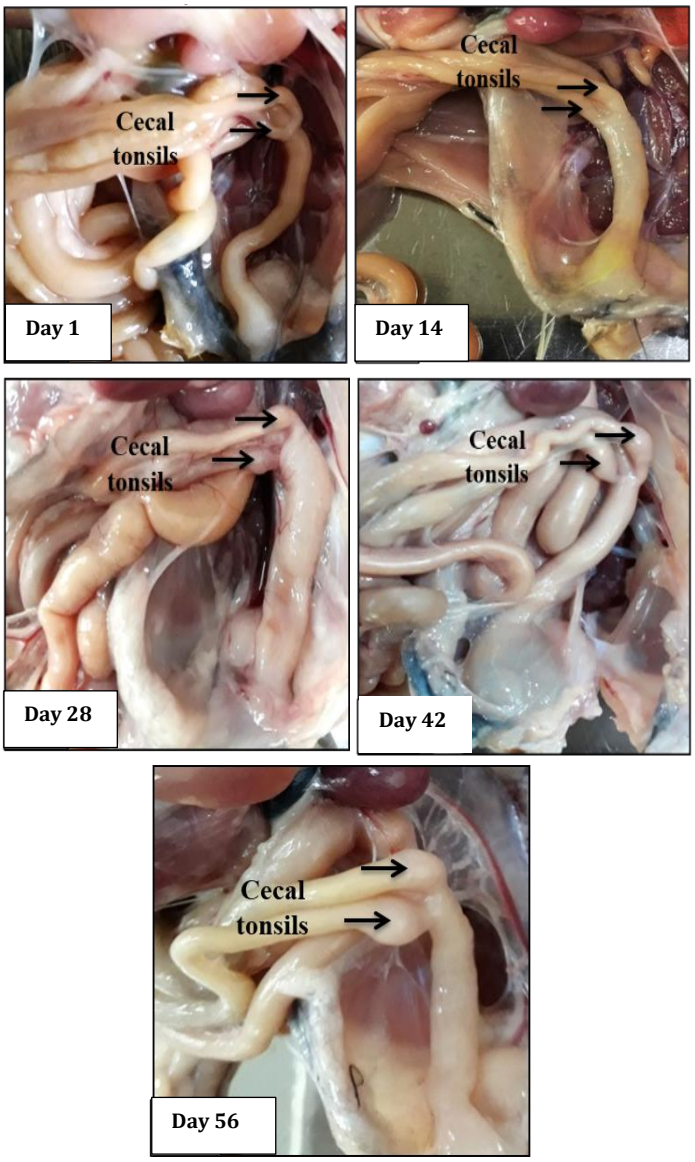


Figure 1. Gross photographs of the cecal tonsil of Sonali chicken. Cecal tonsil located bilaterally at proximal part of paired ceca. This lymphoid organ develops according to age and become more tubular structure

Table 1 shows the changes observed in the average weight, length, and width of cecal tonsils of Sonali chicken over course of the study tenure. The results showed that with increasing age, significant numerical escalations were noticed in all groups.

Table 1: Gross-morphometric observations of cecal tonsil of Sonali chicken[1]

Age group	Average weight (g)	Organ index (%)	Length (mm)	Width (mm)
Day 1	0.022±0.001[a]	0.058±0.004[a]	2.930±0.332[a]	1.450±0.153[a]
Day 14	0.029±0.002[a]	0.032±0.003[a]	5.100±0.301[b]	2.730±0.118[b]
Day 28	0.034±0.005[a]	0.217±0.018[a]	6.590±0.260[c]	3.320±0.085[c]
Day 42	0.105±0.008[b]	0.021±0.002[b]	8.150±0.313[d]	4.450±0.035[d]
Day 56	0.181±0.016[c]	0.025±0.002[c]	9.250±0.705[d]	5.230±0.175[e]
P-value	<0.05	<0.001	<0.001	<0.001

[1]Mean±SEM, n=5. [a-e]Means within a column lacking a common superscript differ significantly ($P \leq 0.05$)

Histological Observations

At the day of hatching, cecal tonsil was composed of tunica mucosa, submucosa, muscularis mucosa and serosa (Figure 2 a). The lining epithelium of the tunica mucosa was simple columnar epithelium with goblet cells (Figure 2 c). In day old chick, only small infiltrations of lymphocytes were found in the lamina propria of tunica mucosa and submucosa layers along with the crypts of Lieberkühn (Figure 2 b). The muscular wall was thin and formed by the smooth muscles. The outer serosa was thin (Figure 2 a).

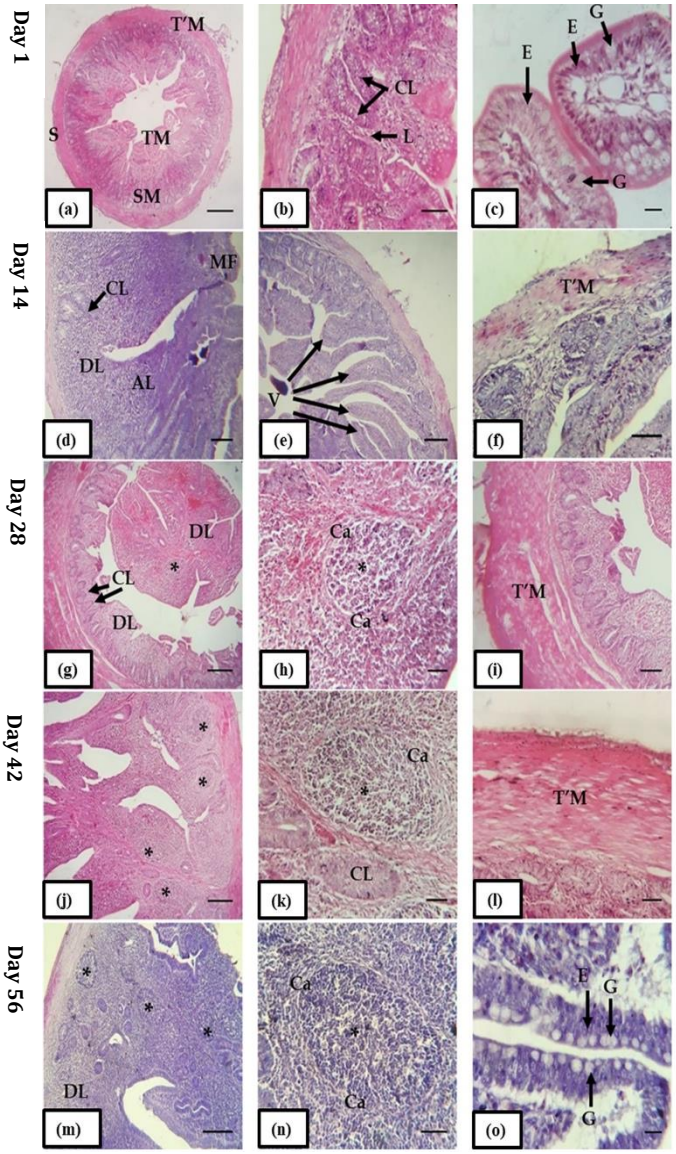


Figure 2. **a**: Four histological layers (10×); **b**: Lymphocytes (L) at lamina propria and submucosa layer (40×); **c**: Simple columnar epithelium (E) with goblet cells (G) (100×); **d**: Mucosal fold (MF) (10×); **e**: The villi (V) (10×); **f**: Tunica muscularis (T'M) (40×); **g**: Distinct lymphatic nodules (*) and diffuse lymphocytes (DL) (10×); **h**: Encapsulated (Ca) lymphatic nodule (*) (40×); **i**: Tunica muscularis (T'M) (40×); **j**: Lymphatic nodules (*) (10×); **k:** Partially encapsulated (Ca) lymphatic nodule (*) (40×); **l:** Tunica muscularis (T'M) (40×); **m:** Diffuse lymphocytes (DL) and lymphatic nodules (*) (10×); **n:** Fully encapsulated oval shaped lymphatic nodules (*) (40×); **o:** Simple columnar epithelium with goblet cell in lamina epithelia layer, H & E stain. TM-tunica mucosa, SM-submucosa, T'M-tunica muscularis, S-serosa, CL-crypts of Lieberkühn , Scale bar: 5 µm (10×), 1 µm (40×), 0.5 µm (100×)

At day 14 of postnatal life, the mucous membrane was much elevated, its bulging surface projecting long tall folds into lumen. Mucosa in cecal tonsil has two forms, one form which contains aggregated and diffuse lymphocytes, may or may not be covered by short villi. The second form or the adjoining part resembles the small intestine that is containing villi (Figures 2 d, e). The whole thickness of lamina propria of tonsillar part was occupied by an accumulation of lymphocytes and was noticed to be aggregating to form lymphatic nodules (Figure 2 d).

The microscopic study revealed that at day 28 of postnatal stage, encapsulated lymphatic nodules was present along with the diffuse lymphocytes at the lamina propria and submucosa layer, which was absent at the previous study groups (Figures 2 g, h). On all other histological features of cecal tonsil, age related development was noticed like the base of the mucosal folds and tunica muscularis layer was thicker (Figure 2 i). At experimental tenure of day 42, the length of the mucosal folds increased. The number of encapsulated lymphatic nodules was also found increased in the lamina propria and submucosa layer (Figures 2 j, k). At this stage, the lymphatic nodules start to appear throughout the whole mucosal fold (Figure 2 j). The thickness of tunica muscularis increased than the previous groups (Figure 2 l).

Day 56 of post hatch stage, within the fold and at the base of the fold, abundant lymphocytes and lymphatic nodules were present (Figure 2 m). Fully encapsulated oval shaped lymphatic nodules were appeared (Figure 2 n). The thickness of tunica muscularis was greatly increased (Figure 2 o).

Biometry of Microscopic Parameters of Cecal Tonsil

Thickness of Tunica Muscularis

The changes of thickness of tunica muscularis of cecal tonsil during the experimental period in different groups revealed that as the age increased, the thickness of tunica muscularis also increased which was statistically significant (P<0.001). The thickness of tunica muscularis was found 81.80±17.151 µm on day 1 and measured 351.94±40.030 µm on day 56 (Figure 3).

Length and Breadth of Lymphatic Nodules

At day 28 of development, lymphatic nodules appeared in the cecal tonsil, which increased in number and size throughout time. Regarding the length of lymphatic nodule, the microscopic results revealed that the lengths of lymphatic nodule were 256.50±22.133 µm, 331.40±28.363 µm, and 509.38±33.015 µm on days 28, 42, and 56, respectively. On days 28, 42, and 56, the breadths of lymphatic nodule of cecal tonsil were 183.42±27.460 µm, 242.44±34.840 µm, and 295.58±46.175 µm, respectively.

These age dependent changes in the length were statistically significant (P<0.05) while breadth of lymphatic nodules was statistically non-significant (P>0.05) (Figure 3).

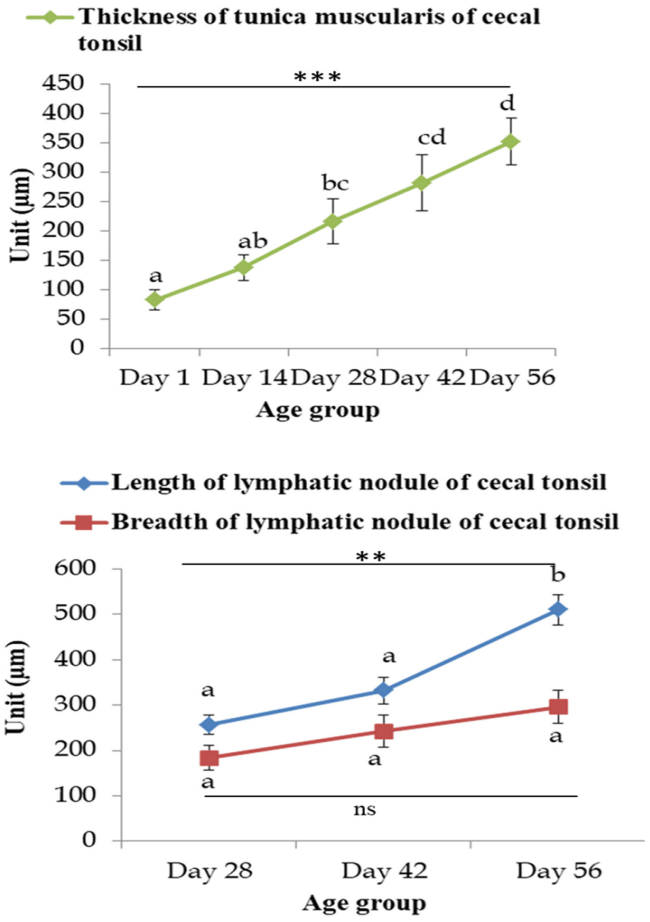
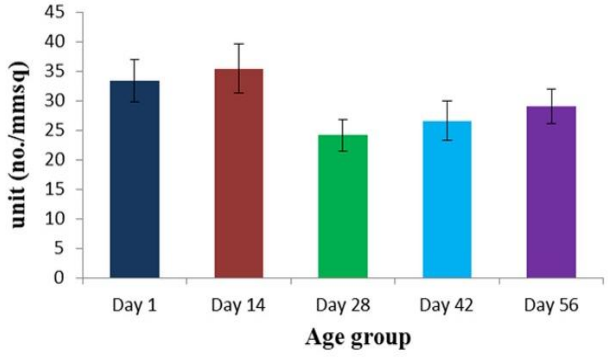


Figure 3. Analysis of thickness of tunica muscularis and length and breadth of lymphatic nodules (mean±standard error mean). [a-d]Values with different letters within the same line differ significantly (P≤0.05)



Figurer 4. Analysis of glands of Lieberkuhn/mmsq (mean±standard error mean). P>0.05.

Glands of Lieberkuhn/mmsq

Here, some fluctuation was observed. When focused on the intestinal glands, the number climbed up from day 1 to day 14; however, with the appearance of lymphatic nodules the number went down and again found to be increasing as developmental step up to day 56 (Figure 4).

DISCUSSION

Gross Observations

The cecal tonsils of Sonali chicken were tubular structure and laid down at the proximal part of large intestines. In this experiment, the shape of the cecal tonsil was similar to that documented in the hybrid chicken (20) and in broiler chicken (21, 22). The color of the cecal tonsil of Sonali chicken during postnatal stages found to be yellowish white. In deshi chicken, the color of cecal tonsil was greenish yellow at postnatal stage (23). Breed variation may be the reason behind this color variation. The result helps to understand that as age increases, the cecal tonsil increases in weight and become more prominent. These findings were similar to the study in deshi chicken (23). In this study, it was observed that the length of cecal tonsil of Sonali chicken was 2.930±0.332 mm at day 1 and it reached up to 9.250±0.705 mm at day 56; that means the length was gradually increased. Similarly, the width of cecal tonsils were found 1.450±0.153 mm at day 1 and reached up to 5.230±0.175 mm at day 56. Similar result was observed before (23) in deshi chicken. The results could not be compared with hybrid chicken due to unavailability of literature in this regard.

Histological Observations

The histological layers of cecal tonsils composed of tunica mucosa, submucosa, tunica muscularis and serosa. The lining epithelium of the tunica mucosa was simple columnar epithelium with goblet cells. The muscular wall was thick and formed by the smooth muscles. The outer serosa was thin. These findings were found in agreement with the reports in White Leghorn chickens (20), in native chicken (15) and in broiler chicken (21, 25). But some histological features in Sonali chicken did not agree with the others. In newly hatched Sonali chicken, only small infiltrations of lymphocytes were found in the lamina propria of tunica mucosa and submucosa layers. The lymphocytes were found aggregating at day 14 and distinct lymphatic nodules appeared in the mucosal folds at day 28. These nodules become more distinct as age increases. The presence of lymphatic nodules at day 1 was recorded in deshi chicken (23). In turkey, at 1 week of age, the lymphoid aggregation in the lamina propria-submucosa of cecal tonsil have been recorded (24). It was reported that an increase of lymphocytes proceeded by 10th day and tonsilla cecalis completed its fundamental architecture by the beginning of third week (26). This variation in result may be due to breed variation or exposure to the antigens which triggers the formation of lymphatic nodules. The histological measurements like thickness of tunica muscularis and length and breadth of lymphatic nodules

tends to increase during the whole period of observations up to day 56. No literatures were available to compare these results of Sonali chicken.

In conclusion, this work constitutes the first histo-morphometric study of cecal tonsil at postnatal stages of Sonali chickens of Bangladesh. It is expected that our results will contribute to guide further studies on the postnatal stages of cecal tonsil of Sonali chicken at molecular level as well as their specific role against exposure of antigen or external stimuli.

ACKNOWLEDGEMENTS

We sincerely thank "The Ministry of Science and Technology, Bangladesh" for providing NST fellowship to the first author to successfully accomplish the research. Grant number [1005/499].

AUTHOR CONTRIBUTIONS

SKD, UA and MRA designed the study. UA conducted the experiment and data collection. UA contributed to the conception and design of the data analysis and interpretation. UA and MAJ prepared the preliminary manuscript. All authors critically revised the manuscript and finalized the manuscript.

CONFLICT OF INTEREST

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

REFERENCES

1. Casteleyn C, Doom M, Lambrechts E, Van den Broeck W, Simoens P, Cornillie P. Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. Avian Pathol. 2010; 39(3): 143-150.
2. Matsumoto R, Hashimoto Y. Distribution and developmental change of lymphoid tissues in the chicken proventriculus. J Vet Med Sci. 2000; 62(2):161-167.
3. Khatri M, Sharma JM. Response of embryonic chicken lymphoid cells to infectious bursal disease virus. Vet. Immunol. Immunopathol. 2009; 127(3-4): 316-324.
4. Ogra PL. Mucosal immune response in the ear, nose and throat. Pediatr. Infect. Dis. J. 2000; 19(5 Suppl): S4-8.
5. Ola ´h I, Nagy N, Magyar A, Palya V. Esophageal tonsil: a novel gut-associated lymphoid organ. Poult Sci. 2003; 82(5): 767-770.
6. Yegani M, Korver DR. Factors affecting intestinal health in poultry. Poult Sci. 2008; 87(10): 2052-2063.
7. Nagy N, Igyártó B, Magyar A, Gazdag E, Palya V, Oláh I. Oesophageal tonsil of the chicken. Acta Vet Hung. 2005; 53(2): 173-188.
8. Haghighi HR, Abdul-Careem MF, Dara RA, Chambers JR, Sharif S. Cytokine gene expression in chicken cecal tonsils following treatment with probiotics and *Salmonella* infection. Vet Microbiol. 2008; 126(1-3): 225-233.
9. Khan MZI, Akter SH, Islam MN, Karim MR, Islam MR, Kon Y. The effects of selenium and vitamin E on the lymphocytes and immunoglobulin-containing plasma cells in the lymphoid organ and mucosa-associated lymphatic tissues of broiler chickens. Anat Histol Embryol. 2008; 37(1): 52-59.
10. Janardhana V, Broadway MM, Bruce MP, Lowenthal JW, Geier MS, Hughes RJ, Bean AG. Prebiotics modulate immune responses in the gut-associated lymphoid tissue of chickens. J Nutr. 2009; 139(7): 1404-1409.
11. Cesta MF. Normal structure, function, and histology of mucosa-associated lymphoid tissue. Toxicol Pathol. 2006; 34(5): 599-608.
12. Kitagawa H, Hiratsuka Y, Imagawa T, Uehara M. The apical caecal diverticulum of the chicken identified as a lymphoid organ. J Anat. 1996; 189: 667-672.
13. Karim MR, Khan MZI, Haque Z. The dynamics of immunocompetent cells in the major lymphoid organs and mucosa-associated lymphoid tissues of the chemotherapy treated chickens. J. Bangladesh Soc. Agric. Sci. Technol. 2005; 2(1): 5-8.
14. Nasrin M, Khan MZI, Siddiqi MNH, Masum MA. Mobilization of immunoglobulin (Ig)- containing plasma cells in Harderian gland, cecal tonsil and trachea of broilers vaccinated with Newcastle Disease Vaccine. Tissue Cell. 2013; 45(3): 191-197.
15. Rahman ML, Islam MR, Asaduzzaman M, Khan MZI. Lymphoid tissues in the digestive tract of Deshi chicken (*Gallus domesticus*) in Bangladesh. Pak. J. Biol. Sci. 2003; 6(13): 1145-1150.
16. Ayman U, Alam MR, Das SK. Post-natal macro- and microscopic changes of the thymus of Sonali chicken in Bangladesh. J Adv Vet Anim Res. 2020a. 7(2): 324-330.
17. Ayman U, Alam MR, Das SK. Age-related development and histomorphological observations of bursa of Fabricius in sonali chicken. J Adv Biotechnol Exp Ther. 2020b. 3(1): 20-28.
18. Ayman U, Alam MR, Das, SK. The spleen of Sonali chicken: Morphohistology and biometric analysis at post hatching ages. Asian J Med Biol Res. 2021; 7(1): 69-75.
19. Igbokwe CO, Ezenwaka K. Age-related morphological changes in the thymus of indigenous large white pig cross during foetal and postnatal development. Anatomy. 2017; 11(1): 12-20.
20. Hodges RD. The Histology of the Fowl. London: Academic Press. (pp. 84, 180-187), 1974.
21. Akter SH, Khan MZI, Jahan MR, Karim MR, Islam MR. Histomorphological study of the lymphoid tissues of broiler chickens. Bangl. J. Vet. Med. 2006; 4(2): 87-92.
22. Majeed MF, Al- Asadi FS, Al Nassir AN, Rahi EH. The morphological and histological study of the caecum in broiler chicken. Bas. J. Vet. Res. 2009; 8(1):19-25.
23. Khalil M. Age related histomorphological changes of lymphoid organs of deshi chicken of Bangladesh [Master]. Mymensingh, Bangladesh, Department of Anatomy and Histology: Bangladesh Agricultural University; 2001.
24. Hussein AA, Reshag AF. Immunehistochemical and histomorphological study of postnatal development of the cecal tonsils in Turkey (*Meleagris Gallopavo*). Iraqi J. Vet. Med. 2019; 43(1): 85-92.
25. Paul SR, Das P, Ghosh RK. Comparative histological studies on the caeca of goat, pig, dog and broiler chicken. Indian J. Anim. Hlth. 2017; 56(1): 85-90.
26. Hoshi H, Mori T. Identification of the bursa-dependent and thymus-dependent areas in the Tonsilla caecalis of chickens. Tohoku J. Exp. Med. 1973; 111(4): 309-322.