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ORIGINAL ARTICLE

Post-natal macro- and microscopic changes of the thymus of Sonali chicken in Bangladesh

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ABSTRACT

Objectives: Avian lymphatic system plays a very significant role in immunity as well as disease control. This research aimed to investigate the histomorphological changes and involution of the thymus of Sonali chicken at different post-natal stages in Bangladesh as the age-related changes of the thymus of Sonali chicken were not described before.
Materials and methods: A gross and microscopic investigation was performed on the thymus of 25 healthy Sonali chickens representing different stages of post-natal life: days 1, 14, 28, 42, and 56. Experimental chickens were sacrificed by cervical subluxation, and the thymus was collected and subjected for both the gross and histological studies. The histological changes were examined with light microscopy after H&E staining.
Results: The thymus was located in close association of the jugular vein, having a long chain of thymic lobes, 5–8 lobes on each side. The statistically significant age-related changes were observed ($p < 0.05$). All gross parameters (weight, length, width, and thickness) found to be increased up to day 42. On day 56, the growth was found to decline from the previous groups. The microscopic observations revealed the same pattern of changes such as gross parameters, i.e., continuously increased till day 42 and then declined ($p < 0.05$). At day 56, the involutory signs such as partial loss of interlobular septa and accumulation of adipose tissue in connective tissue septa were found.
Conclusion: It is well known that in chicken, after a certain period, the thymus got involuted. In Sonali chicken of Bangladesh, the age of involution was notified at day 56/8th week of the post-natal stage, which was correlated both in macro- and microscopic observations.

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Introduction

A sound and intact defense system helps all living beings to endure as well as thrive in a potentially hostile environment without seeming effort. The lymphoid system, a sophistically designed immune system, plays a pivotal role to provide the protection from infections with different etiological agents [1,2]. Advance knowledge of lymphoid system morphology is essential to understand the physiology and immunology of it [3]. Based on anatomy and physiology, the lymphoid system of chicken was divided into two well-defined components consisting of specific organs. The thymus-dependent organs provide an immune response mediated by different cells, whereas the bursa of Fabricius plays a vital role in humoral immunity [4].

The avian lymphatic system acts as a precious model to study the fundamentals of immunology. The embryologists and immunobiologists consider domestic fowl as an excellent research model as it possesses a unique antibody-producing organ, the cloacal bursa [5]. Furthermore, the anatomical separation of lymphoid tissue such as thymic gland and cloacal bursa of domestic fowls, especially chickens, has provided useful experimental models for studying the immune system [6].

The avian lymphoid system represented by a small group of organs and tissues consisting of thymus, spleen,

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bursa, and cecal tonsil. The thymus gland is considered as immunologically the primary lymphoid organ that supports the differentiation of lymphoid progenitor cells along the T-cell pathway. T-cells are the main component of the adaptive immune system which is capable to respond to infectious agents and to increase defensive capabilities with each successive exposure to antigens [2,7–10]. The thymus of chicken is a paired lobulated organ adhering with each other through connective tissue isthmus. Each lobe is encapsulated by thin connective tissue, from which septa arise to divide the lobe into various sized interconnecting lobules [11,12]. Age-dependent structural changes differ the thymus from other lymphoid organs. However, the timetable of the involution process is different among species [2,7]. Understanding normal morphohistological features of the thymus provides a cornerstone to evaluate the lymphoid system function as well as the age-related development of the thymus, which can be used as an indicator of the general health status of the birds [13].

With the increase of age, the morphohistology of thymic gland gradually develops to become mature, and the involution process takes place after a certain period. In the poultry industry, the main reason to do vaccination in the early post-hatch life is the presence of active immune cells during this period which can recognize any antigenic stimulation and do the needful. The comparative study of chicken lymphoid organs in relation to age will unveil their histomorphogenesis as well as provide a clear understanding to study the arrangement of immune cells in lymphoid organs. Although the elaborate work has been done on the lymphoid organs including the thymus of birds, information regarding the histomorphology of the thymus gland in Sonali chicken is arguably less. In the present study, age-related development of the thymus of Sonali chicken was investigated.

Materials and Methods

Ethical approval

The experimental birds were sacrificed humanely following the ethical and welfare guidelines set by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University [approval number: AWEEC/BAU/2019(30)].

Sonali chicken

The experimental Sonali chickens (male chickens) were reared in Bangladesh Agricultural University (BAU) Poultry Farm, Mymensingh-2202, Bangladesh, with proper hygienic conditions, food and water *ad libitum* with strict biosecurity. Feeding history, vaccination schedule, and management practices of the chicken were taken into consideration. The collected chickens were free from any developmental disorder or detectable diseases. A total number of 25 (N = 25) chickens were used for the experiment and were divided into five age groups: day old, 14th day, 28th day, 42nd day, and 56th day; each group contained five chickens (n = 5).

Chickens from each group were sacrificed by the cervical subluxation method. Then, the thymus glands were collected for gross morphometrical and histological evaluation. The investigation was performed in the Department of Anatomy and Histology, Faculty of Veterinary Science, BAU, Mymensingh-2202, Bangladesh.

Morphometric study

After collection, the samples were weighed (gm) and measured for length, width, and thickness of the thymus by slide calipers. The color of the samples was recorded by eye estimation. The unit of length, width, and thickness measurement was millimeter (mm). The gross anatomical images were captured directly from the organs by using a digital camera.

Histological study

For histological observation, the thymus was preserved in Bouin's fluid for 24–36 h. Then, the samples were washed in phosphate-buffered saline for three changes 20 min in each, dehydrated through ascending grade of alcohol (i.e., 70%, 80%, 90%, 100% (1), 100% (2), and 100% (3) alcohol) for 2 h in each, cleaned in xylene for 3 changes of 2 h in each, and finally embedded in paraffin. The paraffin blocks were cut at 6-μm thickness with a rotatory microtome. The microscopic study was facilitated by Harris's Hematoxylin and 1% Eosin Y stain and finally mounted with Dibutylphthalate Polystyrene Xylene (DPX). Images of the microscopic structure of the thymus were taken by using a Carl-Zeiss Photo Microscope (Germany) connected with a digital camera. The histological parameters of thymic lobule (length, breadth, and thickness of the cortex and breadth of the medulla) were measured using a precalibrated ocular micrometer.

Statistical analysis

The analysis of the collected data was performed using the Statistical Package for the Social Sciences (SPSS; version 22.0) software. The *post hoc* Duncan's test was performed following one-way analysis of variance to analyze gross and histological parameters of thymus. The results were displayed in the form of mean ± mean of the standard error. In case of p values ranging below 0.05, the differences were considered to be statistically significant [14,15].

Results

Gross observations

In the gross observation, the thymus of the Sonali chicken was located in close association of the jugular vein, having a long chain of thymic lobes on both sides of the neck (Fig. 1).

The caudal end of each lobular string extended up to the thoracic inlet, whereas the cranial end originated from the level of the third cervical vertebra. On each side, there were 5–8 lobes of various size and shape which lying on the connective tissue beneath the skin of the neck. Up to day 14, the lobes of the thymus were found more elongated and flattened (Fig. 1). From day 28, the lobes had become flattened ovoid, elongated, or irregular shaped (Fig. 1). The color of the gland up to day 28 was found brownish (Fig. 1), but day 42 onward, the color transformed to yellowish-white from pale white (Fig. 1). The thymic lobes were surrounded by a considerable amount of loose connective tissue and adipose tissue (Fig. 1).

Measurements of each thymic lobe were taken using slide calipers, with the following dimensions being determined: length, taking the craniocaudal axis measurement; thickness, using the measurement of the dorsoventral axis; and width, evaluating the measurement of the laterolateral axis.

A significant change in the average weight of thymus among different age groups was observed ($p < 0.05$) (Fig. 2). The average weight of the thymus of Sonali chicken at day 1 was 0.023 ± 0.006 gm which increased to 0.347 ± 0.026 gm at day 42 but at day 56, it was found to be 0.279 ± 0.050 gm, indicating the average weight of thymus gradually increased up to day 42. However, on day 56, the weight begins to decrease from the previous groups.

The different morphological parameters of the thymus (length, width, and thickness) followed a similar pattern of changes such as average weight during post-hatch stages of development. This change was statistically significant ($p < 0.001$) (Fig. 3).

Histological observations

The thymus of Sonali chicken at day 1 was covered by a very fine connective tissue capsule which was nearly visible (Fig. 4A). From the capsule, fine connective tissue septum was arisen and extended into the parenchyma of the gland, which divided the gland into lobules (Fig. 4A and B). The lobules were heterogeneous; they had a different size and shape like pyramidal or polygonal. In some lobules, the

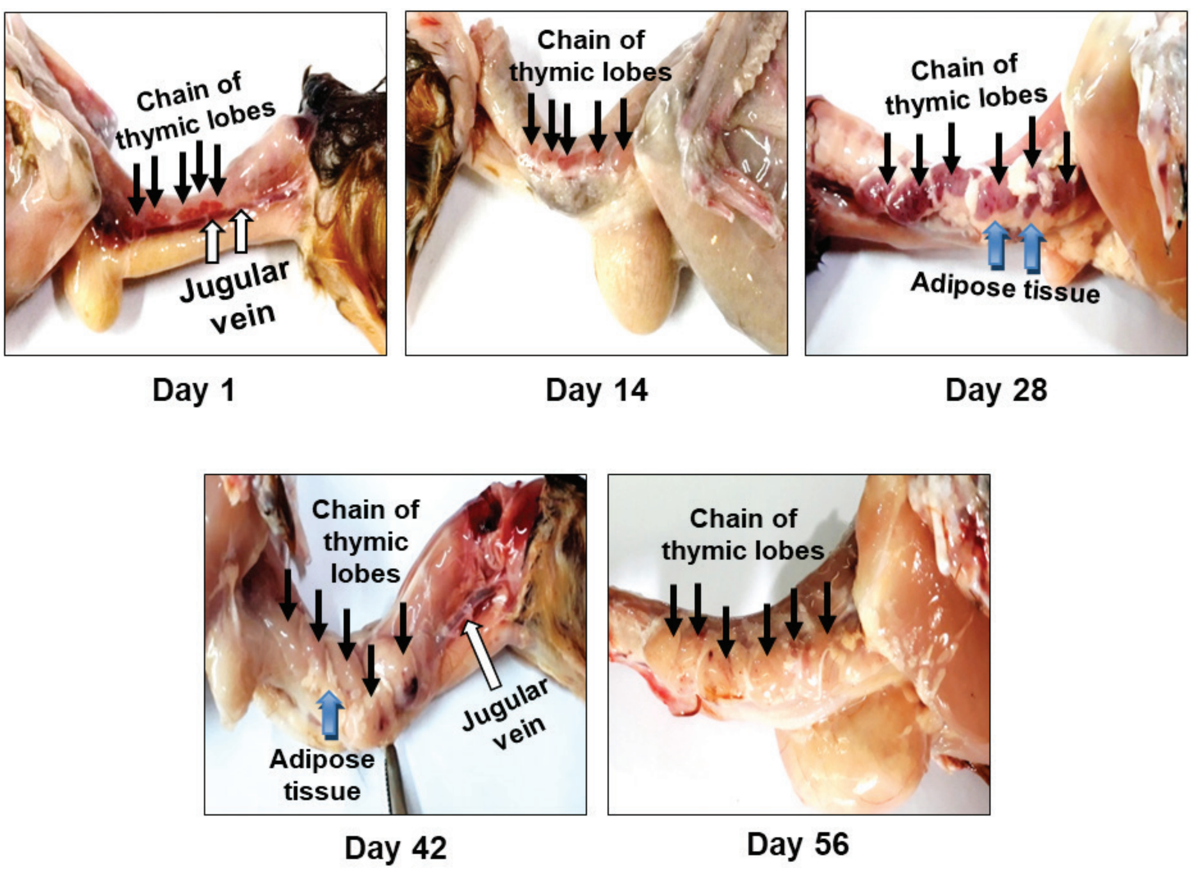

Figure 1. Gross photograph of the thymus of Sonali chicken at different post-natal stages; chain of thymic lobes (black arrow); jugular vein (white arrow); adipose tissue (blue arrow).

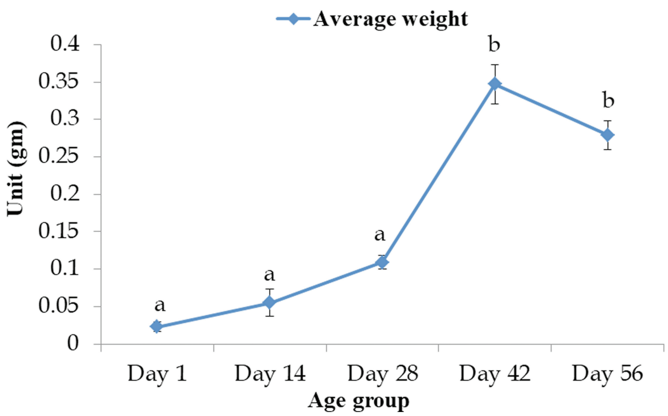

Figure 2. Analysis of average weight (mean ± mean of the standard error). **Values with different letters (a, b) within the same line differ significantly ($p < 0.05$).

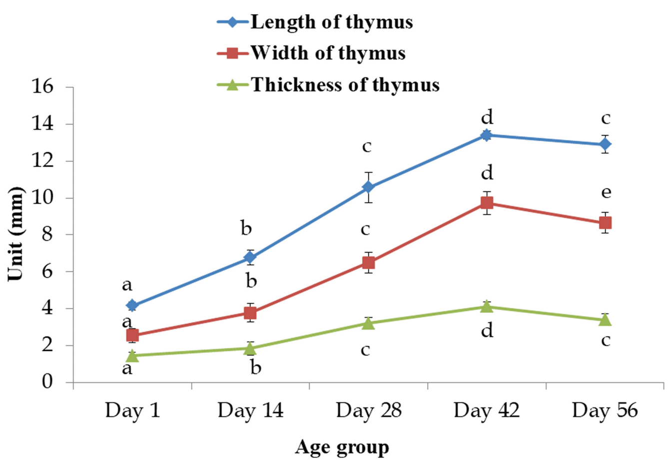

Figure 3. Analysis of length, width, and thickness (mean ± mean of the standard error). **Values with different letters (a, b, c, d, and e) within the same line differ significantly ($p < 0.001$).

outer dark staining cortex and inner light staining medulla were well demarcated while within some lobules, there was no clear definition among the cortex and medulla filled with cortical lymphocytes (Fig. 4A). With high power magnification, it was found that the medulla had developing Hassall's corpuscle with no uniform size and shape (Fig. 4C).

At day 14 of post-hatch life, the number of lobules and thickness of septa were increased than that of day 1 (Fig. 4D and F). Capsule covering the thymus was visible (Fig. 4E). Demarcation among the cortex and medulla becomes distinct at this stage (Fig. 4D). The number of Hassall's corpuscle was increased within the medulla.

On day 28 of post-natal development, all the components of the thymus were well developed than the previous groups. Connective tissue septa in between lobules became thicker (Fig. 4I). Inside the medulla, light staining Hassall's corpuscle was found (Fig. 4G). Endothelial capillary appeared at the corticomedullary junction at this stage of development, which was absent before (Fig. 4H).

At day 42, all the components were well developed and more distinct than those of the previous groups. The cortex was packed with a large number of lymphocytes that were deeply stained with basophilic dye. Medulla was palely stained due to fewer lymphocytes than the cortex (Fig. 4J). The cords of lymphocyte were prominent in the medullary area (Fig. 4K). The septa were thicker than the previous groups (Fig. 4L).

At day 56 of age, the signs of involutory changes occurred. The involutory changes of the thymus noticed were that the clear distinction among cortex and medulla was lost (Fig. 4M), partial loss of interlobular septa, the interlobular septum was present at the cortical region, whereas at the medullary area, the septum was absent in some lobules as a result of that three or more lobule had a common medulla (Fig. 4N) and accumulation of adipose tissue in connective tissue septa (Fig. 4O). An increased number of Hassall's corpuscles were found in the medulla (Fig. 4M).

The length of the thymic lobule was 197.80 ± 8.182; 563.00 ± 13.015; 1,042.72 ± 19.293; 1,455.00 ± 22.186 and 1,215.20 ± 28.408 µm, respectively, at different post-natal stages of development. The length of the lobule was the highest at day 42, whereas reduced at day 56. Similarly, the breadth of thymic lobule was increased up to day 42, and at day 56, the breadth of the lobule was decreased. The breadth of thymic lobule was 99.70 ± 2.743 µm at day 1; 242.98 ± 6.755 µm at day 14; 617.22 ± 9.682 µm at day 28; 986.72 ± 26.202 µm at day 42 and 552.50 ± 17.172 µm at day 56 ($p < 0.001$) (Fig. 5).

The thickness of the cortex could not be measured at day 56 due to the involutory changes. Up to day 42, the thickness of the cortex was found increasing. At day 1 the thickness was 42.32 ± 3.435 µm, 82.42 ± 4.796 µm at day 14; 257.36 ± 17.407 µm at day 28 and 347.32 ± 13.608 µm at day 42. The breadth of the medulla of thymic lobule was 66.10 ± 5.862, 182.86 ± 16.100, 300.98 ± 22.680, and 410.24 ± 81.861 µm at day 1, 14, 28, and 42, respectively. Similar to the thickness of the cortex, the breadth of the medulla of thymic lobule also could not be measured at day 56 due to involutory changes (Fig. 6).

Discussion

Gross observations

The gross observations revealed that the chain of thymic lobes of Sonali chicken was symmetrically present on lateral aspects of the neck with the close proximity of jugular vein. The shape and color of the gland varied according to the age. These data were similar to the previously reported studies [5,10,13,16].

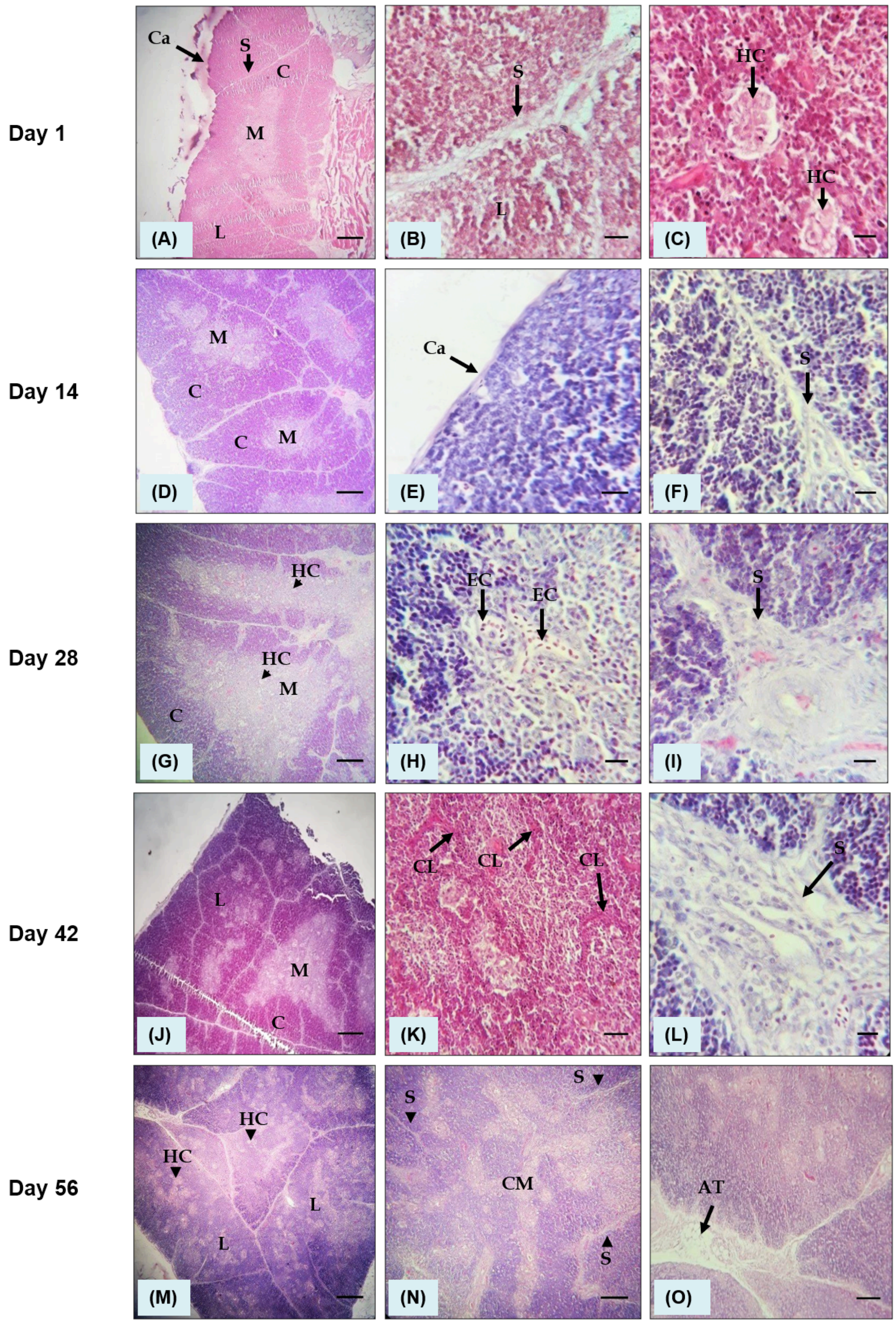

Figure 4. Microscopic images of the thymus of Sonali chicken at different post-natal stages; (**A**) Connective tissue septa (S), thin capsule (Ca), cortex (C), medulla (M), lobule (L) (5 µm); (**B**) Thin connective tissue septa (S) between lobules (L) (0.5 µm); (**C**) Developing Hassall's corpuscles (HC) (0.5 µm); (**D**) Distinct cortex (C) and medulla (M) (5 µm); (**E**) Visible capsule (Ca) (1 µm); (**F**) Connective tissue septa (S) (0.5 µm); (**G**) Cortex (C) and medulla (M); Hassall's corpuscle (HC) (5 µm); (**H**) Endothelial capillary (EC) (0.5 µm); (**I**) Connective tissue septa (S) (0.5 µm); (**J**) Cortex (C) and medulla (M) (5 µm); (**K**) Cords of lymphocytes (CL) (1 µm); (**L**) Connective tissue septa (S) (0.5 µm); (**M**) Lobule (L), Hassall's corpuscle (HC) (5 µm); (**N**) Three or more lobules shearing a common medulla (CM), septa (S) (5 µm); (**O**) Adipose tissue (AT) (1 µm). **Scale bar:** 5 µm (×10), 1 µm (×40), and 0.5 µm (×100).

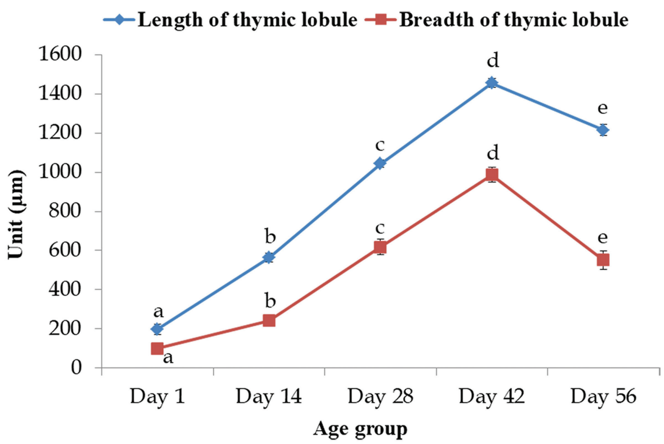

Figure 5. Analysis of length and breadth (mean ± mean of the standard error) of thymic lobules. **Values with different letters (a, b, c, d, and e) within the same line differ significantly ($p < 0.001$).

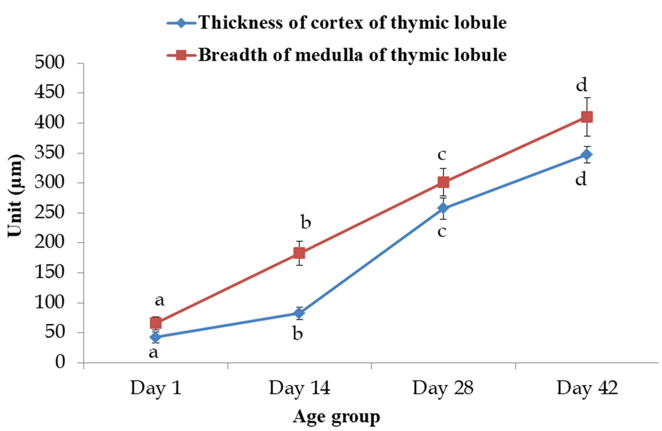

Figure 6. Analysis of thickness of cortex and breadth of the medulla (mean ± mean of the standard error) of thymic lobules. **Values with different letter (a, b, c, and d) within the same line differ significantly ($p < 0.001$).

In the current investigation, the lobes of thymus on each side of the neck were among 5–8 in numbers in Sonali chicken, whereas in deshi chicken, the numbers were 6–7 [5] and in hybrid chicken, the number varied from 2 to 9 [13,16,17]. The current research showed that average weight, length, width, and thickness were increased with the increase of age up to day 42. However, on day 56, all these parameters were suddenly decreased from the previous groups. In deshi chicken, the average weight of thymus was increased up to 3 months [5,10]. Hence, the variation of the weight of thymus might be due to breed variation and environmental or managemental effects. We are aware that there was a scarce literature related to length, width, and thickness of thymus of deshi or hybrid chicken including Sonali chicken.

Histological observations

The thymus of Sonali chicken was encapsulated with a thin connective tissue that was nearly visible on day 1 but became more distinct as age increased. From the capsule, thin connective tissue septa arose and entered into the parenchyma of the gland, dividing the gland into lobules. The number of lobulation increased with the increase of age. Each thymic lobule consisted of the inner light-stained medulla containing Hassall's corpuscles and outer dark-stained cortex. The number and size of Hassall's corpuscle found to be increasing as age increases. The microscopic architecture of thymus was found developing with the increase of age from day 1 to day 42. Up to this stage of development, the cortex, medulla, Hassall's corpuscles, and septa, all these histological components were becoming more developed. These findings up to day 42 found in agreement with the previous results [10,11,16,18–20].

However, on day 56, the signs of involutory changes found in the present study. The involutory signs that appeared in this study were found to be similar to the findings of the previous studies [9,21]. The age, at which the involutory changes occur, found different in Sonali chicken. Khenenou et al. [22] reported, in broiler chicken, that the morphometric and histological evolution of thymus occurred during 28 weeks of post-natal life which was complementary to the sexual maturity. In the case of Sonali chicken, the involutory signs occurred earlier (at 56th days or 8th weeks) which may be due to breed difference or environmental or managemental effects.

The report had shown for the first time that the length and width of thymic lobules increased up to day 42, and at day 56, they decreased abruptly in Sonali chicken. The increase in these two parameters was reported up to day 28 in broiler chicken [15].

Conclusion

Finally, evidence showed that morphometric changes are well correlated with the histological modifications. During post-natal growth and development of the thymus of Sonali chicken, some structural differences have been identified. Moreover, the age of involution is found at day 56 or 8th week of post-hatch age. The involutory signs include the loss of a clear distinction between cortex and medulla, partial loss of interlobular septa, accumulation of adipose tissue in connective tissue septa, and the number of Hassall's corpuscles largely increased in the medulla. Moreover, the further study is required for molecular detection and immunological activities of the thymus in Sonali chicken.

Acknowledgment

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Conflict of interests

There is no competing interest to declare.

Authors' contribution

Shonkor Kumar Das designed the experiment. Ummay Ayman conducted the investigation, data collection, data analysis and prepared the preliminary manuscript. Shonkor Kumar Das and Md. Rafiqul Alam critically revised the manuscript and finalized the manuscript.

References

[1] Cortan RS, Kumar V, Robbin SL. Robbin's pathogenic basis of disease. W.B. Saunders, Philadelphia, PA, pp 163–4, 1998; https://doi.org/10.1002/path.1711600125

[2] Kannan TA, Ramesh G, Ushakumary S. Dhinakarraj G, Vairamuthu S. Thymic Hassall's corpuscles in Nandanam chicken-Light and electronmicroscopic perspective (*Gallus domesticus*). J Anim Sci Technol 2015; 57:30; https://doi.org/10.1186/s40781-015-0064-2

[3] Davison F, Kaspers B, Schat KA. Avian immunology. Academic Press is an imprint of Elsevier, Cambridge, MA, 2008. Available via https://trove.nla.gov.au/work/34195682 (Accessed 15 February 2020).

[4] Janeyway CA, Jones B, Hayday A. Specificity and function of T-cell bearing γδ receptors. Immunol Today 1988; 9:73–6; https://doi.org/10.1016/0167-5699(88)91267-4

[5] Khalil M. Age related histomorphological changes of lymphoid organs of deshi chicken of Bangladesh. MS thesis. Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh, Bangladesh, p 2202, 2001.

[6] Kon-Ogura T. Distribution of T-cell subsets in chicken lymphoid tissues. J Vet Med Sci 1993; 55(1):59–66; https://doi.org/10.1292/jvms.55.59

[7] Ciriaco E, Pinera PP, Esnal BD, Laura R. Age-related changes in the avian primary lymphoid organs (Thymus and Bursa of Fabricius). Microsc Res Techniq 2003; 62:482–7; https://doi.org/10.1002/jemt.10416

[8] Lucena CB. Embryonic thymic epithelium differentiation in chicken: study of molecular signals involved in lymphoid progenitor cells colonization. Mestrado Em Biologia Molecular E Genética, Universidade De Lisboa Faculdade De Ciências, Departamento De Biologia Vegetal, 2012.

[9] Leena C, Prasad RV, Kakade K, Jamuna KV. Histology and age related involutory changes of the thymus of Giriraja birds (*Gallus domesticus*), J Vet Anim Sci 2008; 39:40–3. Available via http://www.cvaslibrary.com/journals/1_39_12.pdf

[10] Steppan LB, Nakaue HS, Matsumoto M, Gainer JH, Kerkvliet NI. The broiler chicken as model for immunotoxicity assessment: standardization of in vitro immnulogical assay. Fund Appl Toxicol 1989; 12:773–86; https://doi.org/10.1016/0272-0590(89)90009-2

[11] Treesh SA, Buker AO, Khair NS. Histological, histochemical and immunohistochemical studies on thymus of chicken. Int J Histology Cytol 2014; 1(11):103–11.

[12] Terszowski G, Müller SM, Bleul CC, Blum C, Schirmbeck R, Reimann J, et al. Evidence for a functional second thymus in mice. Science 2006; 312(5771):284–7; https://doi.org/10.1126/science.1123497

[13] Pereira CCH, Freitas AG, Silva MC, Treichel TLE, Calábria KC, Moraes M. Morphology and biometry of the thymus in *Gallus gallus domesticus* (Linnaeus, 1758) from the isa brow lineage. Acta Vet Bras 2017; 11(4):235–40; https://doi.org/10.21708/avb.2017.11.4.7299

[14] Casmir Onwuaso Igbokwe, Kelechi Ezenwaka. Age-related morphological changes in the thymus of indigenous Large White pig cross during foetal and postnatal development. Anat 2017; 11(1):12–20; https://doi.org/10.2399/ana.16.050

[15] Khan MZI, Masum Md, Khan ZI, Aziz ARB, Nasrin M, Siddique NH, et al. Histopmorphology of the lymphoid tissues of broiler chickens in Kelantan, Malaysia. Sains Malays 2014; 43(8):1175–9.

[16] Haseeb A, Shah MG, Gandahi JA, Lochi GM, Khan MS, Faisal M, et al. Histo-morphological study on thymus of Aseel chicken. J Agr Food Technol 2014; 4(2):1–5.

[17] Santana MIS, Silva FOC, Severino RS, Santos ALQ, Drummond SS, Bombonato PP. Vascularização arterial do timo em aves (*Gallus gallus domesticus*) da linhagem Avian Farms. Biosci J 2000; 16(2):3–15; https://repositorio.usp.br/item/001204292

[18] 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; https://doi.org/10.3329/bjvm.v4i2.1289

[19] Kannan TA, Ramesh G, Ushakumary S, Dhinakarraj G Vairamuthu S. Light microscopic studies on thymus of chicken (*Gallus domesticus*). Haryana Veterinarian 2012; 51:116–8.

[20] Senapati MR, Behera PC, Maity A, Mandal AK. Comparative histomorphological study on the thymus with reference to its immunological importance in quail, chicken and duck. Exploratory Anim Med Res 2015; 5(1):73–7.

[21] Bhattacharya, Binay K. Some histomorphological and cytochemical changes in chick thymus during spontaneous age involution. PAUO 1983; 21:71–85.

[22] Tarek K, Mohamed M, Omar B, Hassina B. Morpho-histological study of the thymus of broiler chickens during post-haching age. Int J Poultry Sci 2012; 11(1):78–80; https://doi.org/10.3923/ijps.2012.78.80