Effects of age on gross and microscopic changes of bursa of Fabricius and thymus of commercial broiler chicken

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Abstract
The experiment was carried out in the Laboratory of Anatomy and Histology, Hajee Mohammad Danesh Science and Technology University. Topology and histology were performed of the bursa of Fabricius and thymus of broiler chicken. The mean length, diameter and weight of the bursa and thymus were lower at day-1 (D1) stage and increased gradually at day-35 stage (D35). With increasing age, the bursa became larger with the plicae getting taller and thicker, which contained an enormous number of large polyhedral primary and secondary lymphoid follicles. It was found that the growth rates of bursa of Fabricius from day-1(D1) to day-21(D21) increased gradually but after D21 the growth rate became reduced. Histologically, each lobes of thymus was surrounded by a connective tissue capsule from which septa arises and incompletely divides the glands into lobules. During the day-1(D1) period the lobules were demarcated into cortex and medulla, which becomes larger with age and well demarcated.

Keywords: Broiler chicken, bursa of Fabricius, thymus, gross and microscopic changes

Introduction
Chickens are playing a significant role in national economy and reducing poverty by supplying meat, egg and other by-products in Bangladesh. The main focus of a commercial broiler farm is to increase profits by enhancing maximal yield and maintaining the health condition of the broiler chicken [1]. However, these birds are affected seriously by immunosuppressive and neoplastic diseases such as Gumboro diseases, lymphoid leukemia (avian leuosis), Marek’s diseases. The diseases affecting the lymphoid organs causing disorganization of the organ concerned and leading to increase the morbidity and mortality of the birds and hampering in the development of farming system of birds. Lymphoid tissue can be divided into "central (thymus and bursa of Fabricius)" and "peripheral (spleen and all mucosa associated lymphoid tissues)” tissues [2, 3]. The former are believed to be primary sites of development of lymphocytes. Cell mediated immunity is initiated by a thymus dependent component that is represented by the smaller lymphocytes [4] and humoral immunity is initiated by the bursa dependent component that is represented by larger lymphocytes. The peripheral or secondary lymphoid tissues apparently depend on the central or primary lymphoid tissue for their origin, development and function [5]. In birds they include lymphoid tissue in the spleen and in the alimentary tract including the cecal tonsils [1]. However, both in birds and animals and even in human beings, lymphomas are not treated in the right way due to lack of proper diagnosis and cost management [6]. In Bangladesh different age related observations of lymphoid organs in native chicken [7, 8, 9], similar study only for bursa of Fabricius was done in broiler chicken [10]. Distribution of immunocompetent cells in the different lymphoid tissues in Vencobb chicken [11] and a study was also done on gross and morphometric analysis of lymphoid organs of day old chick in Kadaknath chicken [12], however, the present study reveals the microscopic aspect of the bursa of Fabricius, thymus and in addition attempts were made to bridge the gap between the gross and microscopic anatomy of the lymphoid organs of broiler chickens in Bangladesh. The great importance of these data is bearing on the functional significance of bursa of Fabricius and thymus of the broiler chicken. It is hoped that the present investigation will be a base line for the study of lymphoid organs of broiler chicken and also will provide valuable information to poultry immunologist, pathologist, cell biologist and anatomist.
2. Materials and Methods

The broiler chickens were collected from some selected broiler farm where vaccination and other managemental program were performed properly in Birol and Birgonj upazilla of Dinajpur district, Bangladesh; the samples were collected during the period from March to June’ 2016. The selected birds were no congenital disorders and remarkable disease that may cause any problem in the study of the thymus and bursa of Fabricius. The broiler chickens of Lohman meat breed were divided into six age groups Day-1, Day-7, Day-14, Day-21, Day-28 and Day-35 (D1, D7, D14, D21, D28, and D35 respectively). Each age group was comprised of five chickens. Chickens from each group were killed with cervical subluxation. Food and water was with held two hours before killing, just after killing of the chickens the feathers were removed manually, the bursa of Fabricius was collected by ventral abdominal dissection and thymuses were collected by ventral neck dissection both for gross and histological studies. The gross study included the color, length, weight, diameter and thickness of lymphoid organs. Weights of the lymphoid organs were taken by using digital balance (Table 3) and relative weights of the thymuses were also calculated adopting following formula-

Relative weight of the thymus = \frac{\text{Weight of the lymphoid organ}}{\text{Live weight of bird}} \times 100

The length and diameter of thymus on both side of the neck and the bursa of Fabricius were recorded by using digital Vernier calipers (Table 1 & Table 2). Statistical analysis was done by calculating Arithmetic Mean (A.M.), Standard Deviation (S.D.) and Standard Error (S.E.) as per the standard statistical methods.

For histological study the collected samples were preserved for fixation in the “Bouins fluid” [13] for 24 hours and then tissues were dehydrated by using ascending graded of alcohol (70%, 80%, 90%, 95%, 100% and 100%). For each grade of alcohol one hour was provided. After dehydrating the tissue it was transferred to the xylene-1 and xylene-2 each for ninety minutes for clearing purpose. Then the tissues were infiltrated in the liquid paraffin at 60°C temperature for ninety minutes and it was repeated again. Finally the tissues were embedded in paraffin and paraffin blocks were made. The paraffin blocks were cut at 6 μm thickness using microtome machine (Mu 509, Euromex, Japan). After sectioning of paraffin block, the slices were floated on warm water in a water bath at 45°C for stretching. Then the sliced tissue was placed on greeze free clear glass slide using adhesive like Mayer’s egg albumin. Then the glass slides were dried at 37°C temperature for 24 hours in an incubator. After drying the slides the sections were stained with Hematoxylin and Eosin (H & E) stain for general histological study. The histological structures of the thymus and bursa of Fabricius were observed using a light microscope under low (×10) and high (×40) magnification and Photographs were taken from the selected specimens.

3. Results and Discussion

The bursa of Fabricius of chicken is a peculiar organ of the poultry for the production of B- lymphocytes. In the present study it was found that a blind, globular shaped, sac-like, dorsal diverticulum of the proctodeal wall of the cloaca (Fig. 5), the central lumen of the organ was great extent obscured by the presence of about 12 plicae, long folds of the mucous membrane of the bursal wall which resemble villous projections and it was similar with the previous literatures [3, 14]. The color of the bursa of Fabricius of the broiler chicken was whitish at D1 and becomes yellowish white at D35 and agreed with the study [14]. The length of the bursa of Fabricius, its maximum diameter and weight is given at Table -1, 2 & 3. The length of the bursa of broiler chicken at D1 was measured 3.8±0.01 mm and at D35 it was 16.01±0.20 mm (Table 1) and the growth was found to be greater at D35 (Table 1). It was observed that the difference of length of the bursa between each stage from D1 to D35 were statistically significant (P<0.01). The study [15] for deshi chicken more or less similar with the study that the mean length of the bursa of Fabricius of the deshi chicken at ED15 (Embryonic stage Day-15) was 2.80 ± 0.122 mm and at D90 it reached up to 11.00 ± 0.158 mm. The maximum diameter of the bursa of Fabricius of the broiler chicken at D35 it was 11.77±0.21mm and at D1 was found to be 2.21±0.02 mm (Table 2). It was observed that the difference of diameter of the bursa between each stage from D1 to D35 were statistically significant (P<0.01). The result of the present study showed that the diameter gradually increases with the increase of age of the broiler. The present study unanimous with the study [15] where deshi chicken at ED15 was 2.20 ± 0.122 mm and at D90 it reaches up to 8.40 ± 0.187 mm. Similarly, the weight of the bursa was 0.02±0.01gm at D1 and at D35 1.4±0.001gm (Table 3) which was similar to the study [10]. The growth rate of bursa was maximum at D35 (Table 3).

It was observed that the difference of weight of bursa between each stage from D1 to D35 stages were statistically significant (P< 0.01). The greatest weight of bursa of the hybrid chicken was 4.25 gm at 10 weeks [14] but the study [15] showed that the relative weight of deshi chicken was highest at Embryonic Day-18 (ED18) of prenatal life. In graphical representation it was found that the growth rates from D1 to D21 increased gradually but after D21 the growth rate became reduced (Fig. 1, 2 & 3). So, the period from D21 to D28 is more critical for affecting the bursa related diseases in broiler chicken. Histologically the bursa has developed plicae at the D1 stage. The lumen did not contain mucoid substance. In some bursa the middle region of the plicae was thicker than the base and apical part. Cortex and medulla were not well differentiated in all the lymphatic follicles. The medulla was very small in some follicles and it contains germinal centre which indicates there active stage of lymphopoiesis. Amount of connective tissue was small in amount inside the core of plicae. Lamina propria contains minimum amount of connective tissue agreement with the study of Cornack DH [16]. Under high power objective, it was observed that the lymphatic follicles were largely packed with lymphocytes with prominent nucleous which was similar to the previous study [16]. At the D21 the bursa was increasing in size with well developed plicae which was lined by pseudostratified and columnar epithelium (Fig. 6 & 8). The plicae were tall with uniform thickness. Some of the follicles were quite large with prominent lymphocytes. Primary lymphoid follicles were spherical or ovoid with no clear central region. Secondary nodules had a clear zone with germinal center at this stage of development (Fig. 7 & 9). It was clearly observed under the high power objective that smooth muscle fibers present in the wall and agreed with the report [16]. Primary lymphoid follicles were spherical or ovoid with no clear central region. Secondary nodules had a clear zone with germinal center at this stage of development. The germinal center also called the medulla contains lymphoblast which was similar to the study.
In the present study the thymus composed of two chains of lobes located on both sides of neck, named left and right thymus situated in sub dermal connective tissue (Fig. 4). It was revealed that the lobes of the thymus were ovoid at D1 and becomes slightly elongated at D25 of the broiler chickens. These findings were similar to that of the hybrid chicken [14, 19] and desi chicken [15]. In case of D1, it extends throughout the neck, from base of the skull into the thoracic cavity. But in case of adult chicken upper 1/5th of the neck was devoid of thymus, the findings which agreed to the study made by the previous studies [14, 19] and for desi chicken [15]. The study revealed that the thymus were pale white at D1 period of the broiler chicken but becomes yellowish white at D25. But in case of adult stages the color of the thymus of the hybrid chickens were more yellowish then the study and the desi chicken [14, 19]. The observation of the study was contradicting with the study [12], who reported that pale pinkish black coloured lobes in day old Kadaknath chick. It is also similar with Sultana et al. [20] in ducklings and Lochi et al. [21] in Aseel chicken, who stated that each lobe was pale white to yellowish white. This may be due to breed difference. It was found that the mean weight of the thymus gradually increased with the age of the broiler chicken but in case of relative weight it was highest at D1 of life but then it gradually decreased (Table 3). In this context Getty [3] reported that the greatest weight of the hybrid chicken was 15.76 gm at 17 weeks of age and another study [15] showed that the relative weight was greatest at embryonic stage of prenatal life (ED1) of desi chicken. The result of the study showed that the mean length of the neck occupied by the thymus of the broiler chicken at D1 was 25.63 ± 0.26 mm and at D25 it reached up to 64.84 ± 0.20 mm (Table 1). For desi chicken at D1 was 23.00 ± 0.948 mm and at D25 it reached up to 63.84 ± 0.969 mm [15]. The mean diameter of the thymus of broiler chicken at D1 was 3.61 ± 0.09 mm at D25 it reaches up to 8.12 ± 0.03 mm (Table 2). The result of the present study showed that the diameter gradually increases with the increase of age of the broiler chicken and similar with the study [15] for desi chicken. The numbers of lobes of the thymus on each side of the neck were usually between 4 to 8 in numbers, while in fowl found 7-9 lobes [22], in birds found 4-7 lobes [19], in native geese found 5-9 lobes [23] and found 6-8 lobes in the thymus of turkey [24]. But in case of hybrid chickens they varied 3 to 8 in numbers [19, 14]. So the variance was similar in case of broiler chicken but for desi chicken [15] showed the number from 6 to 7. It was found that the thymus of broiler chicken was surrounded by connective tissue and adipose tissue from D1 to D25 of life of broiler chicken (Fig. 10). In graphical representation the thymus were very small in length, diameter and weight at D1 and with the advanced of age they attained a maximum growth at D35 (n=5) (Fig. 1.2 & 3). The lobes of the thymus were surrounded by very thin connective tissue capsule from which very thin septa arose and divided the lobes into lobules. It was also found that in early life lobules were homogenous, small in size and the cortex and medulla was demarcated. Few Hassall’s corpuscles were present in the medulla of the thymus during early stage of development (D1) (Fig. 10). Capillaries were found in the parenchyma at this stage. Groups of lymphocytes were present with large nucleus. However, reports in these regard agreed with the study for desi chicken [19]. Hassall’s corpuscles became larger and there number increases at the period in the medulla of the thymus (D25) (Fig. 12). The cortex becomes thicker and was packed with the large number of lymphocytes. Very fine thymic capillaries were also present at all the stages of growth of the thymus. The findings of broiler chicken were similar to the adult hybrid chicken [14, 19].

Table 1: The Mean Length of the thymuses and bursa of Fabricius from day-1 to day-35 at a regular interval (n=5)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mean Length of Thymus</th>
<th>Mean Length of Bursa of Fabricius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day-1</td>
<td>25.63±0.26</td>
<td>3.84±0.01</td>
</tr>
<tr>
<td>Day-7</td>
<td>34.02±0.32</td>
<td>4.84±0.01</td>
</tr>
<tr>
<td>Day-14</td>
<td>43.04±0.55</td>
<td>10.26±0.22</td>
</tr>
<tr>
<td>Day-21</td>
<td>50.82±0.57</td>
<td>15.19±0.19</td>
</tr>
<tr>
<td>Day-28</td>
<td>61.40±0.24</td>
<td>17.01±0.20</td>
</tr>
<tr>
<td>Day-35</td>
<td>64.84±0.20</td>
<td>16.01±0.20</td>
</tr>
</tbody>
</table>

± indicate standard error

Table 2: The Mean diameter of the thymuses and bursa of Fabricius from day-1 to day-35 at a regular interval (n=5)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mean Diameter of Thymus</th>
<th>Mean Diameter of Bursa of Fabricius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day-1</td>
<td>3.61±0.09</td>
<td>2.21±0.02</td>
</tr>
<tr>
<td>Day-7</td>
<td>4.26±0.03</td>
<td>3.14±0.03</td>
</tr>
<tr>
<td>Day-14</td>
<td>5.09±0.02</td>
<td>7.32±0.02</td>
</tr>
<tr>
<td>Day-21</td>
<td>5.61±0.11</td>
<td>9.07±0.01</td>
</tr>
<tr>
<td>Day-28</td>
<td>6.60±0.09</td>
<td>10.08±0.03</td>
</tr>
<tr>
<td>Day-35</td>
<td>8.12±0.03</td>
<td>11.77±0.21</td>
</tr>
</tbody>
</table>

± indicate standard error

Table 3: The Mean weight of the thymuses and bursa of Fabricius from day-1 to day-35 at a regular interval (n=5)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mean weight of Thymus (gm)</th>
<th>Relative Weight of Thymus (gm)</th>
<th>Mean weight of bursa of Fabricius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day-1</td>
<td>0.31±0.01</td>
<td>0.85±0.02</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Day-7</td>
<td>0.42±0.01</td>
<td>0.45±0.01</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>Day-14</td>
<td>0.51±0.01</td>
<td>0.26±0.01</td>
<td>0.21±0.00</td>
</tr>
<tr>
<td>Day-21</td>
<td>1.56±0.01</td>
<td>0.33±0.001</td>
<td>0.04±0.002</td>
</tr>
<tr>
<td>Day-28</td>
<td>4.14±0.01</td>
<td>0.40±0.01</td>
<td>1.4±0.002</td>
</tr>
<tr>
<td>Day-35</td>
<td>6.17±0.03</td>
<td>0.37±0.01</td>
<td>1.4±0.001</td>
</tr>
</tbody>
</table>

± indicate standard error
Fig 1: Length of lymphoid organs (thymus and bursa) of broiler chicken at different stages of growth and development (n=5)

Fig 2: Diameter of lymphoid organs (thymus and bursa) of broiler chicken at different stages of growth and development (n=5)

Fig 3: Weight of lymphoid organs (thymus and bursa) of broiler chicken at different stages of growth and development (n=5)

Fig 4: Gross photograph of thymus (T) of broiler chicken.

Fig 5: Gross photographic of bursa of Fabricius (B) of broiler chicken

Fig 6: The bursa of broiler chicken at day-21 (D21) of growth and development showing developed plicae (DP) which is lined by pseudostratified and columnar epithelium (E). The plicae are tall with uniform thickness. All the lymphatic follicles (F) are not the same size and shape. H & E stain. X 10.

Fig 7: The bursa of broiler chicken at day-28 (D28) of growth and development showing developed plicae (DP) which is lined by pseudostratified and columnar epithelium (E). The plicae are tall with uniform thickness. All the lymphatic follicles are not the same size and shape. H & E stain. X 10.
Fig 8: The bursa of broiler chicken at day-21 (D21) of growth and development showing lymphoid follicles (F) contain lymphoblast and lymphocytes. H & E stain. X 40.

Fig 9: The bursa of broiler chicken at day-28 (D28) of growth and development showing lymphoid follicles (F) contain lymphoblast and lymphocytes. H & E stain. X 40.

Fig 10: The thymus of broiler chicken at day-1 (D1) of growth and development showing Hassal’s corpuscles (H). H & E stain. X 40.

Fig 11: The thymus of broiler chicken at day-28 (D28) of growth and development showing Hassal’s corpuscles (H). H & E stain. X 40.

Fig 12: The thymus of broiler chicken at day-35 (D35) of growth and development showing Hassal’s corpuscles (H). H & E stain. X 40

4. Conclusion
The mean length, diameter and weight of the bursa of Fabricius of broiler chicken at D1 stage were 3.8±0.01 mm, 2.21±0.02 mm and 0.02±0.01 gm respectively, which were increased gradually and at D35 were 16.01±0.20 mm, 11.77±0.21 mm and 1.4±0.001 gm. Histologically, the bursa of D1 stage was filled up by plicae and devoid of mucoid substances. But with increasing age the bursa became larger with the plicae getting taller and thicker, which contained an enormous number of large polyhedral primary and secondary lymphoid follicles. Thin wall of the bursa of D1 stage became thicker with increasing age. The day from 21st to 28th days is more important for broiler rearing for bursa related diseases. The mean weight, diameter and the extent of the neck occupied by the thymus of broiler chicken at D1 were 0.31±0.01 gm, 3.61±0.09 mm and 25.63±0.26 mm, that were increased gradually at D35 were 6.17±0.03 gm, 8.12±0.03 mm, 64.84±0.20 mm respectively. Hassall's corpuscles were found during D1 stage which increased in diameter and number at D35 stage of broiler chicken. The capsule and trabeculae becomes thicker with the growth and development of the thymus. Further study will provide for the focusing of the lymphatic organs related diseases of human and similarities or dissimilarities with chicken.

5. Acknowledgement
I express my profound gratitude to Almighty Allah for giving me the opportunity to carry on this research. With great pleasure I would like to express my heartfelt gratitude and indebtedness to the Dean of the faculty of veterinary and animal science, my colleagues and lab assistant for providing the necessary facilities during the research work.

6. References