POSTNATAL GROWTH AND DEVELOPMENT OF LYMPHOID ORGANS AND TISSUES IN DESHI CHICKEN (*GALLUS DOMESTICUS*) OF BANGLADESH

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A thesis submitted in the partial fulfillment of the requirements for the degree of Masters of Science in Anatomy

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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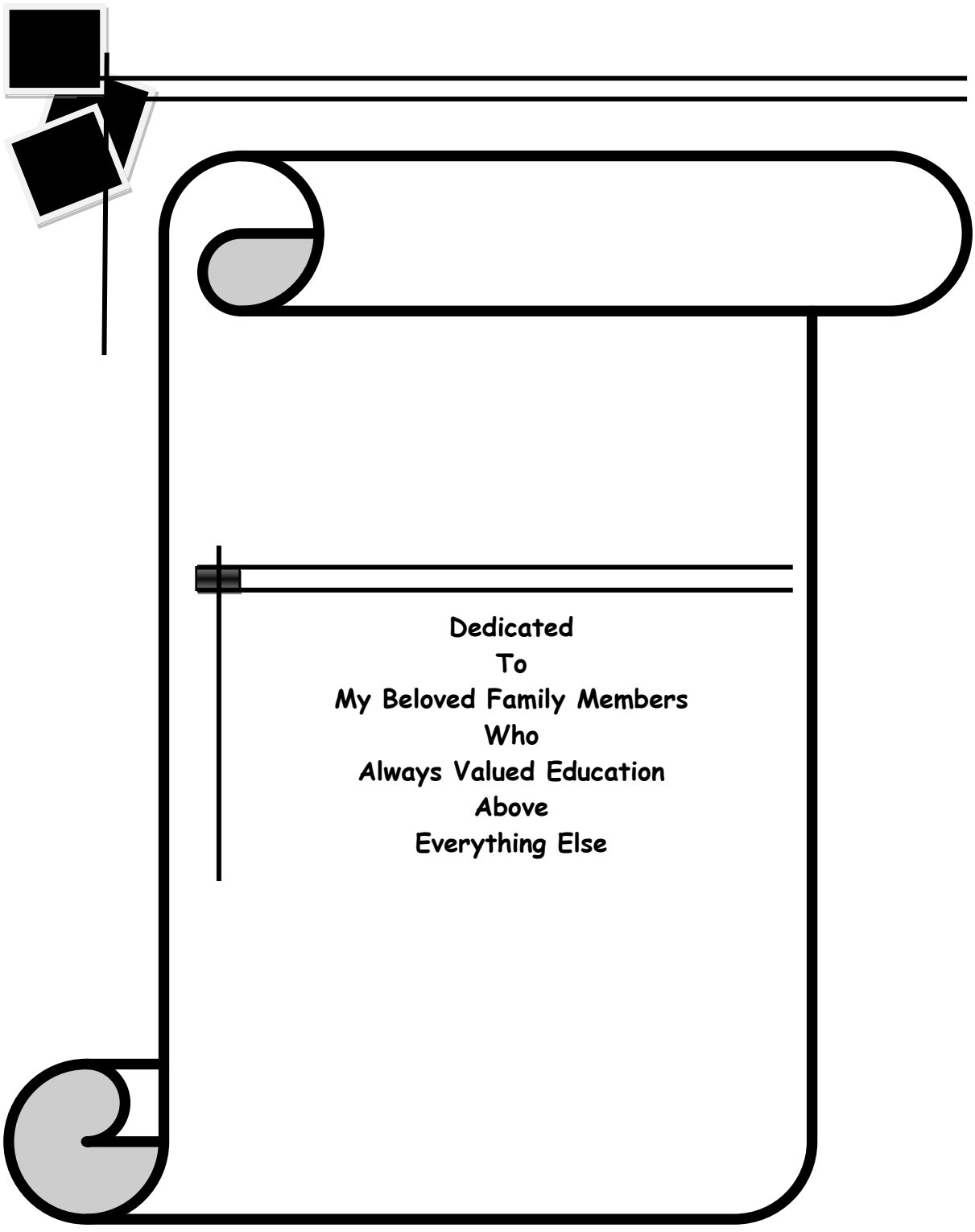
Dedicated
To
My Beloved Family Members
Who
Always Valued Education
Above
Everything Else

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Author
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List of Symbols and Abbreviations

Abbreviations	Elaborations
%	percent
≤	equal or less than
>	more than
°	degree
°C	degree Celsius
Cm	centimeter
Mm	millimeter
µm	micrometer
Gm	gram
Kg	kilogram
Ml	milliliter
Sec	second
Min	minute
N	total number
N	number for each age group
P	level of significance
SD	standard deviation
SDC	scavenging deshi chicken
CDC	captive deshi chicken
MALT	mucosa associated lymphoid tissue
GALT	gut associated lymphoid tissue
BALT	bronchus associated lymphoid tissue
HALT	head associated lymphoid tissue
D	day
ED	embryonic day
H&E	Hematoxylin and Eosin
etc.	et cetera
i.e.	that is

Abstract

The gross and histomorphometrical studies were conducted in scavenging and captive deshi chickens of Bangladesh to know the comparative postnatal growth and development of lymphoid organs and tissues during the period of March to December, 2017. Total 40 non-descriptive deshi chickens were reared in scavenging and intensive system (20 chickens for each) from day 1 to 180 according to standard rearing system. Gross examinations of different lymphoid organs were performed for different ages (day 1, day 30, day 90 and day 180) of both types of rearing chickens. For histomorphometrical study the tissue samples were fixed, processed and stained with H & E stain according to the standard histological procedure and AmScope image measurement software were used for histomorphometry. The gross weight, length and width of thymus, spleen and cecal tonsils were gradually increased significantly ($P \leq 0.05$) with the advancement of ages in both scavenging deshi chickens (SDC) and captive deshi chickens (CDC) but the weight, height and width of bursa were decreased at D_{180} in both types of reared chickens. In both SDC and CDC, the highest weight (1.88 ± 0.13 and 2.04 ± 0.11 gm, respectively), height (1.26 ± 0.11 and 1.48 ± 0.08 cm, respectively) and width (0.76 ± 0.09 and 1.04 ± 0.11 cm, respectively) of bursa was found at D_{90} but the highest number of thymic lobe (6.40 ± 0.54 and 6.40 ± 0.54, respectively), weight (0.98 ± 0.08 and 1.26 ± 0.11 gm, respectively) and length (3.68 ± 0.08 and 3.78 ± 0.08 cm, respectively) of thymus; the highest weight (2.78 ± 0.16 and 3.06 ± 0.18 gm, respectively), length (1.98 ± 0.08 and 2.26 ± 0.16 cm, respectively) and width (1.42 ± 0.08 and 1.62 ± 0.08 cm, respectively) of spleen and the highest weight (2.60 ± 0.10 and 2.72 ± 0.08 gm, respectively), length (1.70 ± 0.10 and 1.82 ± 0.08 cm, respectively) and width (1.18 ± 0.08 and 1.30 ± 0.10 cm, respectively) of cecal tonsil was found at D_{180}. All the gross parameters were found significantly ($P \leq 0.05$) higher in CDC as compared with SDC. Similarly, the histomorphometrical parameters such as length and width of thymic lobules, white pulps of spleen and lymphatic nodules of cecal tonsils were also increased significantly ($P \leq 0.05$) with the advancement of ages in both SDC and CDC but height of epithelium, number and height of mucosal folds, number of follicles per mucosal fold of bursa were decreased at D_{180} in both SDC and CDC. All those histomorphometrical parameters were found significantly ($P \leq 0.05$) higher in CDC as compared with SDC. So, it is concluded that the higher gross and histomorphometrical parameters of lymphoid organs of captive deshi chickens than that of scavenging deshi chickens were due to their different rearing system.

Key words: Bursa, thymus, spleen, cecal tonsil, postnatal, deshi chicken.

Chapter-1: Introduction

The lymphoid system of chicken is divided into primary lymphoid organs and secondary lymphoid organs (Getty, 1975; Day and Schultz, 2014). The primary site for the development of lymphocytes in chicken is the primary lymphoid organ, e.g. Thymus and Bursa of Fabricius. The thymus dependent component is represented by the T-lymphocytes (smaller lymphocytes) which is responsible for cell mediated immunity (CMI) including immune surveillance, whereas, the bursa dependent component is represented by the B-lymphocytes (larger lymphocytes) which transformed into plasma cells in the tissues, synthesizes IgG and plays a vital role in humoral immunity (Dellmann and Eurell, 1998; Bacha Jr and Bacha, 2012). The secondary lymphoid organs apparently depend on the primary lymphoid organs for their origin, development and function (Tizard, 2013). In chicken, the secondary lymphoid organs consists of the spleen and all the mucosa associated lymphoid tissues (MALT). The MALT is consisting of bronchus associated lymphoid tissue (BALT), gut associated lymphoid tissue (GALT) including peyer's patches, isolated follicles and cecal tonsils; and the head associated lymphoid tissue (HALT). The HALT is composed of the harderian gland, lacrimal gland, mucosa of eyelid and nasal cavity (Kajiwara *et al.*, 2003; Khatri and Sharma, 2009; Lee *et al.*, 2010; Uddin *et al.*, 2010).

Deshi or native chicken is a major local chicken breed in Bangladesh. This breed is being reared in Bangladesh for a long period of time and it has contributed about 19.75% and 25.06% of total meat and egg production, respectively (Dutta *et al.*, 2013). Most of the farmers particularly village women reared this chickens. For this reason, this chickens are usually regarded as a "Walking Bank or Bank Coin" for the rural poor families. These chickens are frequently reared in free range farming system where they scavenge for feeds during the day time and are confined at night. They usually take kitchen waste, seeds, grains, garden left-over, insects, green grasses and all other human refusal that would otherwise go to waste. They attain a weight approximately 1.0 kg at 6 months of age in free range farming system (Rahman *et al.*, 2003; Islam *et al.*, 2012) but their body weight also increases if they are reared in intensive farming system (Ershad, 2005). The lymphoid organs of chicken particularly bursa and thymus, and tissues, e.g. spleen and

cecal tonsils, plays a significant roles in the prevention of disease occurrence (Khalil *et al.*, 2002; Tizard, 2013). Studies on the lymphoid organs of chicken have revealed that the growth and development of lymphoid organs morphology and their functions are related with the growth of chickens (Ciriaco *et al.*, 2003). On the other hand, the growth rates of chickens are related with their rearing systems (Ershad, 2005).

Many scientists have been studied on postnatal growth and development of lymphoid organs in different high yielding birds like Broiler chicken (Nagy and Olah, 2010; Khenenou *et al.*, 2012; Khan *et al.*, 2014), White Leghorn chicken (Betti *et al.*, 1991; Del Moral *et al.*, 1998; Kozuka *et al.*, 2010), Aseel chicken (Haseeb *et al.*, 2014), CARI Shyama and Vanaraja chickens (Jain *et al.*, 2010), Guinea fowl (Onyeanusi *et al.*, 1994) and Geese (Gulmez and Aslan, 1999). A few number of research was conducted on gross and histomorphology of the postnatal lymphoid organs of deshi chickens in Bangladesh (Khalil *et al.*, 2002) where results were compared to broiler chickens as well as other high yielding chickens but there are no single reports are available on postnatal growth and development of lymphoid organs of deshi chickens reared in intensive farming system (called captive deshi chickens). Some authors also reported that due to scavenging nature, the postnatal lymphoid organs of deshi chickens contain more immunocompetent cells than that of high yielding chickens (Khan *et al.*, 2007; Islam *et al.*, 2012). Concerning the above all economic, nutritional and immunological point of view, the comparative postnatal growth and development of lymphoid organs and tissues in deshi chickens reared under scavenging and intensive farming systems are very important. Thus, the present study was undertaken to know the following objectives:-

Objectives:

1. To study the gross and histomorphology of different lymphoid organs and tissues in various postnatal growth and developmental stages of deshi chickens.
2. To know the comparative gross and histomorphology of lymphoid organs and tissues in between scavenging and captive deshi chickens during these stages of growth.

Chapter-2: Review of Literature

For living beings, freedom from disease in environment is dependent on the existence of a complex and highly sophisticated defense system, called lymphoid system (Davison *et al.*, 2011; Song *et al.*, 2012). The lymphoid system of chicken is consisting of unique organs and tissues and divided into two-primary lymphoid organ and secondary lymphoid organs (Getty, 1975; Dellmann and Eurell, 1998). The organs that regulate the production and differentiation of lymphocytes from immature progenitor cells are known as primary lymphoid organs. Primary lymphoid organs of chicken consists of bursa of Fabricius and thymus. Secondary lymphoid organs maintain mature naive lymphocytes and initiate an adaptive immune response. They arise late in fetal life and persist throughout adult life. Secondary lymphoid organs of chicken consists of spleen, bone marrow and mucosa associated lymphoid tissues (MALTs) (Hodges, 1974; Dellmann and Eurell, 1998; Davison *et al.*, 2011). In the present chapter, review of literature of the gross and histological structures of the lymphoid organs and tissues are highlighted as follows:-

2.1 Gross anatomy of bursa of Fabricius

The bursa of Fabricius is a dorsal diverticulum of the cloacal proctodeum and is located at the dorsal surface of proctodeum (Getty, 1975; Dellmann and Eurell, 1998). It is globular or spherical in shape. At four to five months of age it reaches a maximum size of about 3.0 cm long, 2.0 cm wide and 1.0 cm dorsoventrally and weight is about 3.0 gm (Getty, 1975). Khenenou *et al.* (2012) reported that the growth of the bursa of Fabricius of broiler chickens divided into three different phases: the first is characterized by an accelerated growth; this phase begins from the first week of age and continues until the 10^{th} week of age where it reaches its maximum size (6 mm). The second phase begins from the 10^{th} week and finishes on the 20^{th} week of age. From the 20^{th}, fast involution of the Bursa characterized by one reduction in the bursa diameter which passes from 0.50 to 0.62 mm at the 27^{th} week. The bursa of Fabricius reached its peak of development between the 4^{th} and the 12^{th} week of age (Khenenou *et al.*, 2012). The average weight of bursa of Fabricius passes from 0.09 gm to the first postnatal week to reach an average of 1.95 ± 1.217 gm at the 10^{th} week of age and to arrive at 1.725 ± 0.694 gm at the 11^{th} week, at the 23^{rd} week it starts a slow anatomical involution to reach 0.01 gm and a total

regression about the 27th week (Khenenou *et al.*, 2012). The bursa of Fabricius, reached the peak of development enters 10th and 11th week of age. Lillehoj and Trout (1993) confirmed that the bursa of Fabricius of chickens reached its peak of development in between the 4th and 12th week. This can be due to the chickens stock used in work and also to antigenic stimulations (breeding conditions, vaccination, disease etc.).

Sultana *et al.* (2012) reported that the bursa of Fabricius of indigenous duck of Bangladesh was a yellowish white, blind and cylindrical shaped, cecum like elongation, located on the dorsal diverticulum of the proctodeal wall of the cloaca. The weight, length and width of the bursa of Fabricius of duck were 0.22 gm, 1.87 cm and 0.53 cm, respectively.

2.2 Histology of bursa of Fabricius

Histologically, the wall of the bursa of Fabricius of chicken consists of three layers- tunica mucosa, tunica muscularis and tunica serosa (Hodges, 1974; Dellmann and Eurell, 1998; Bacha Jr and Bacha, 2012). Dellmann and Eurell (1998) reported that, the bursa is consisting of long thick mucosal folds (plicae) which are projected into the lumen. The middle region of the plica is thicker than the base and apical part. Numerous follicles filled the lamina propria of each fold. All the follicles have clear margin and they are separated from the adjacent lymphoid tissue by connective tissue fibers, cells and intercellular space. Each bursal follicle is consisting of a peripheral cortex and a central medulla (Hodges, 1974). A layer of undifferentiated epithelial cells occupied the periphery of the medulla, which is separated from the cortex by a capillary layer. The darkly stained cortex is composed of many closely packed small lymphocytes. The paler medulla contains fewer cells of various sizes. The mucosal fold of the bursa is lined by pseudostratified columnar epithelium, except at the apex of each follicle, which is covered by a simple columnar epithelium. The populations of lymphocytes are uniformly distributed and the periphery of the medulla is smooth and regular in appearance in the follicles of chickens (Akter *et al.*, 2006).

Khalil *et al.* (2003) reported that general histological structure of bursa of Fabricius of deshi chicken is similar to many other birds but also some differences. The number of mucosal fold has been stated about 12 in Domestic fowl (Hodges, 1974; King and

McLelland, 1984), about 12-14 in Helmeted Guinea fowl (Onyeanusi *et al.*, 1993), about 11-13 in Geese (Gulmez and Aslan, 1999) and about 11-13 in Hybrid chicken (Betti *et al.*, 1991).

Khenenou *et al.* (2012) stated that in 1st week, the bursa of broiler chicken was characterized by fringes appearances that represent lyphoepithelial follicles and formed later the bursal folds; bordered by an epithelium that separates them from the bursal lumen. Most of the bursal layers were readily observed during the 1st week of age, these layers were serosa; the most external layer; the site of blood and lymphatic vessels route. This layer was followed deeply by the muscularis and mucousa. While the 4th week was marked by the development of bursal follicles; these follicles were well defined and their zones were well separated and the interstitial connective tissue was quite visible. At 10th week, the lymphoid follicles continued their development and increase in size to reach the maximum; the length was 756.55 µm and the width was about 396.25 µm. The cortex and medulla were well demarcated, the lamina propria was well developed and the atrophy of the lymphoid follicles of the bursa started at the 11th week due to connective tissue infiltration of the interfollicular spaces and the follicular lymphoid tissue was gradually replaced. At the 23rd week, a very significant interstitial fibrosis and degeneration of the lymphoid follicles was noticed, the bursal follicles lose their lymphoid characteristics and zone. The follicular structure of the bursa was hardly visible and the interstitial fibrosis was very significant at the 27th week.

Another one study was carried out on broiler chickens by Khan *et al.* (2014) where they observed that each bursal follicle was consist of a peripheral cortex and a central medulla which was found to increase in size at D_{14} and D_{28} ages of broiler chicken. A layer of undifferentiated epithelial cells occupied the periphery of the medulla which was separated from the cortex by a capillary layer. The darkly stained cortex was composed of many closely packed small lymphocytes. The paler medulla contained immature lymphocytes of various sizes. The mucosal fold of the bursa was lined by pseudostratified columnar epithelium, except at the apex of each follicle which was covered by a simple columnar epithelium. The populations of lymphocytes were uniformly distributed and the periphery of the medulla was smooth and regular in appearance in the follicles of Broiler chicken.

Jain *et al.* (2010) studied on CARI Shyama and Vanaraja chickens in India where they noticed that the number of mucosal folds were more in chicks of Vanaraja (10.9) than CARI Shyama (8.5) and in growers of CARI Shyama with an average (16.9) than Vanaraja (13.8). The height of mucosal folds also increased from chicks to grower stage. The surface epithelium of the mucosal folds was simple columnar epithelium but it was pseudostratified columnar in growers. The number of lymphoid follicles per fold was higher in chicks and growers of CARI Shyama (21.55 and 25.14, respectively) than in Vanaraja (14.03 and 18.98, respectively). In chicks, the diameter of follicle was more in Vanaraja (mean value 89.5 µm) than CARI Shyama (mean value 77.5 µm).

In Geese, the mean, minimum and maximum thickness of bursal medulla is 103.6 µm, 196.0 µm and 316.2 µm, respectively. The minimum, mean and maximum thickness of cortex is 20.72 µm, 89.06 µm and 202.05 µm, respectively (Gulmez and Aslan, 1999).

2.3 Gross anatomy of thymus

The thymus of chicken is a pale red or yellowish color paired organ and each part consists of six to eight separate, different sizes lobes which are connected by a connective tissue isthmus (Getty, 1975; Treesh *et al.*, 2014). One-half of which is lying on the sub-dermal connective tissue of the either side of the neck region (Akter *et al.*, 2006). The structure of thymus of some birds is different from each other by their number of lobes. Pekin duck contain 4-7 lobes on the right side and 4-6 lobes on the left side (Gulmez and Aslan, 1999). Guinea fowl has 7 lobes on the right side and 6 lobes on the left side (Onyeanusi *et al.*, 1994). Water fowl has 5-6 lobes (Getty, 1975; Nickle *et al.*, 1977). In Geese the number of lobes is about 6-9 on the right side and 5-9 on the left side (Gulmez and Aslan, 1999).

Getty (1975) stated that, in White Rock male chicken, the maximum total weight (the left and right together) of thymus is 15.76 gm at 17^{th} weeks of age. Involution is then starts and it is about 2.2 gm at 13 to 19 months of age and in females of similar ages about 0.6 gm but remnants of all the lobes persist up to 16 months of age (Getty, 1975).

Khan *et al.* (2014) reported that, the weight of thymus of broiler chicken at D_1, D_{14} and D_{28} was 0.068 ± 0.0427 gm, 0.153 ± 0.0013 gm and 0.434 ± 0.0231 gm, respectively. The mean weight of the thymus was highest at D_{28} followed by D_{14} and D_1, respectively. The

length of the thymus at D_1, D_{14} and D_{28} was 2.80 ± 0.108 mm, 10.75 ± 0.629 mm and 13.30 ± 0.576 mm, respectively. The mean length of the thymus was highest at D_{28} ages of Broiler and followed by D_{14} and D_1, respectively. The mean width of the thymus was higher at D_{28} than other age groups of chickens (Khan *et al.*, 2014).

Sultana *et al.* (2012) noticed that, in indigenous duck of Bangladesh, each half of thymus was consisted of typically five lobes of various sizes and shape. The color of the thymus was pale white to yellowish white and the shape of the lobes of thymus was elongated and flattened. The length of right and left thymus was 0.83 cm and 0.83 cm, respectively. The breadth of the right and left thymus was 0.30 cm and 0.33 cm, respectively and the weight of the right and left thymus was 0.018 gm and 0.02 gm, respectively.

Song *et al.* (2012) reported that, in Ostrich, the thymus ran along each side of the 5-6 cervical vertebrae and ventrally across the sides of the neck, reaching the costal margin of the costa prima. There have individual variation and lobes differed in size and number across the two sides of the organ. On the left side 2-4 lobes weighing 2.3783 ± 0.0620 gm and 1-5 lobes weighing 2.4133 ± 0.0460 gm are identified on the right. The volume of each lobe decreases from the cranial to caudal. The cranial part of the thymus is connected to the thyroid gland (Song *et al.*, 2012).

2.4 Histology of thymus

Histologically, the thymus is surrounded by a capsule of connective tissue continuous with thin septa that subdivide the lobes into partially separated lobules. The central medulla of each lobule is a branch of tissue that arises from a central stalk in the lobe and is surrounded by a cortex. The thymic cortex consists mainly of an epithelial reticulum and lymphocytes. Lymphoblasts and medium sized lymphocytes predominate in the meshes of the peripheral epithelial reticulum. The thymic cortex stains much darker than the medulla because it contains a greater number of lymphocytes. Medulla contains larger lymphocytes. Some medullary epithelial reticular cells form thymic corpuscles, also called Hassall's corpuscles. Each of this corpuscles consist of one to several calcified or degenerated large central cells which are surrounded by the flat keratinized cells in a concentric arrangement (Dellmann and Eurell, 1998).

A study was carried out on Hubbard chicken by Treesh *et al.* (2014) where they reported that the thymus of one day old chicken was formed by several thymic lobes and lobules. The thymic lobes were surrounded by connective tissue capsules. Connective tissue septa extend from the capsule to divide the thymus into lobules. These capsules and septae contained thymic blood vessels. Each thymic lobule was divided into two parts, outer darkly stained cortex and inner lightly stained medulla. At the age of 7 days, the thymus showed more lobulation with outer dark cortex, filled with numerous lymphocytes (thymocytes) and inner light medulla was formed by large lymphocytes. At the age of 21 days, thymic lobules showed similar structure to that of 7 days old chicken and in addition to appearance of blood capillaries at the corticomedullary junction. At the age of 28 days, the same results were found also in thymic lobules, in addition to few Hassall's corpuscles which started to appear in the medulla at this age. At the age of 42 and 56 days, the thymus showed an increase in the appearance of Hassall's corpuscles in thymic medulla. At the age of 147, 168 and 210 days the thymic lobules showed similar findings to that of 28, 42 and 56 days old, respectively, further increase in Hassall's corpuscles compared to that of the other previously younger ages.

Khan *et al.* (2014) reported that, the thymic lobules are smaller in size at D_1 than D_{28} ages of broiler chickens. The cortex and medulla of the thymic lobules increased in size comparatively at D_{28} ages of chicken (Khan *et al.*, 2014). Sultana *et al.* (2012) observed that histological structure of thymus in indigenous duck was similar to the chicken and the length and breadth of the thymic lobules were 226.68 ± 10.18 µm and 165.78 ± 8.86 µm, respectively.

2.5 Gross anatomy of spleen

In chicken, the spleen is brownish red color, small and rounded, about 1.5 cm in diameter and weighing about 3.0 gm in the female and 4.5 gm in the male. It lies on the right surface of the junction of the proventriculus and gizzard. Accessory spleens sometimes found in the chickens (Getty, 1975). The color, size and shape of the spleen vary with different species of birds (Nickle *et al.*, 1977).

Khan *et al.* (2014) reported that, in broiler chicken, the weight of the spleen at D_1, D_{14} and D_{28} was 0.030 ± 0.0033 gm, 0.517 ± 0.0097 gm and 1.974 ± 0.0497 gm, respectively.

The length of the spleen at D_1, D_{14} and in D_{28} was 3.88 ± 0.315 mm, 13.58 ± 0.217 mm and 18.93 ± 0.394 mm, respectively. The mean width of the spleen was higher at D_{28} than other groups of chicken.

In Duck and Geese, it is more triangular, with a flat dorsal and a curved ventral surface (Getty, 1975). The length and width of spleen in indigenous duck of Bangladesh is 0.94 cm and 0.68 cm, respectively and the weight is 0.18 gm (Sultana *et al.*, 2012).

In Ostrich, the spleen was dark red and elliptical and was wedged against the right kidney. Each spleen was about 4.8 cm in length and 1.5 cm in diameter. There were accessory spleens in 2 of 6 experimental animals (Song *et al.*, 2012).

2.6 Histology of spleen

Histologically, the spleen is surrounded by a thick connective tissue capsule invested by the peritoneum. The capsule consists of two layers- a layer of dense irregular connective tissue and a layer of smooth muscle. The total thickness and relative amount of smooth muscle vary with the species. The capsule, trabeculae and reticular fibers support the splenic parenchyma composed of a red pulp involved in the storage of red blood cells and a white pulp rich in lymphocytes and active in immune responses (Dellmann and Eurell, 1998). The red pulp is less distinct and these are scatteredly distributed among the white pulp, composed mostly of red blood cells. The white pulp is composed of network of reticular cells and reticular fibers within which small, medium and large sized lymphocytes and plasma cells are diffusely distributed. It contains sheathed arteries and lymphatic nodules. The red pulp of the spleen is formed from venous sinuses and anastomosing cord of reticular cells, macrophages, lymphocytes and blood cells. The immunocompetent cells are found both in the red and white pulps. Central arteriole is found in the white pulp. The network of the splenic tissue is consisting of a network of reticular cells and fibers (Hodges, 1974; Dellmann and Eurell, 1998; Sultana *et al.*, 2012).

Histological structure of the spleen in indigenous duck of Bangladesh is more or less similar with the spleen of chicken. The length and breadth of white pulp is 129.05 ± 5.8 µm and 103.43 ± 3.65 µm, respectively (Sultana *et al.*, 2012).

Song *et al.* (2012) stated that, in Ostrich, the tegument of spleen is thin, ranging from 25.0 to 41.5 μm. It extends into the splenic parenchyma. The parenchyma includes both white and red pulp, with no clear demarcation between them. In the white pulp the periarterial lymphoid sheath is thin (12.5 μm). There are numerous rounds or elliptical structures in the region that resembled ellipsoids. The volume of each ellipsoid is small and the diameter ranging from 25.0 to 44.5 μm (Song *et al.*, 2012).

2.7 Gross anatomy of cecal tonsil

Within the large intestinal region, cecal tonsils (CTs) are located in the proximal part of each of the ceca and represent the most studied structures of the avian GALT (Davison *et al.*, 2011). Two ceca open into the digestive tract at the junction of the ileum and rectum. Three regions of this organ present slightly different histological features. The proximal portion contains prominent villi. In the adult bird, large masses of diffuse and nodular lymphatic tissue infiltrate the lamina propria and submucosa of this portion, forming grossly visible cecal tonsils (Getty, 1975; Dellmann and Eurell, 1998). Akter *et al.* (2006) stated that, the cecal tonsils of broiler chickens were in the proximal one third of the paired tubular cecum which lies along each side of the large intestine. They were broad tubular in shape.

2.8 Histology of cecal tonsil

Structurally less well defined, isolated lymphoid follicles are present throughout the small and large intestine with greatest density in the apical region of the cecum. These so-called cecal tonsils are macroscopically visible in the Chicken, Duck and Geese as well vascularized ampullar dilatations of each cecal base (Del Cacho *et al.*, 1993; Kitagawa *et al.*, 1998; Casteleyn *et al.*, 2010; Davison *et al.*, 2011). The CTs are large lymphoid aggregates, structurally similar to the peyer's patches that contain multiple follicles and are overlaid by M-cell rich epithelium (Kitagawa *et al.*, 2000; Davison *et al.*, 2011).

The CT is consists of four histological layers i.e. tunica mucosa, submucosa, muscularis and serosa (Hodges, 1974; Dellmann and Eurell, 1998; Bacha Jr and Bacha, 2012). Their lining epithelium is simple columnar epithelium. The bases of the mucosal folds (villi) are thick and the apexes are pointed or rounded in chicken. More diffuse lymphoid tissue and unorganized lymphatic nodules are present both in the mucosa and submucosa of the

CTs of chicken. In Broiler chickens, the length and breadth of lymphatic nodules of the cecal tonsil are 255.20 ± 20.46 μm and 186.08 ± 24.90 μm, respectively (Akter *et al.*, 2006).

Chapter-3: Materials and Methods

The present study was undertaken to know the comparative gross and histomorphology of the postnatal growth and development of lymphoid organs and tissues in deshi chickens of Bangladesh being reared under scavenging and intensive system. The study was conducted during the period of March to December, 2017.

3.1 Study area and study population

Tangail district of Bangladesh was selected as the study area for this study and study population was the non-descriptive deshi chicken (*Gallus domesticus*).

3.2 Collection of day old chicks

A total of 40 (forty) day old non-descriptive deshi chicks were collected and then categorized into two groups (20 chicks in each group) for rearing in both scavenging and intensive systems.

3.3 Rearing of chickens in scavenging system

Twenty chicks were reared in scavenging system (also called traditional or free range system) up to 180 days in Tangail district. They were scavenged for feeds during the day time and confined at night. Also a small amount of feed has been supplied as a supplement **(Figure 3.1)**.

3.4 Rearing of chickens in intensive system

Another twenty chicks were reared in intensive system up to 180 days in the same place where sufficient amounts of feed and water has been supplied as a commercial farming system. The standard farming method was followed for rearing chicken in the litter.

3.5 Laboratory preparation

3.5.1 Required instruments

Scissor, scalpel, scalpel handle, scalpel blade, pencil scale, tray, glass rod, large beaker, Petridis, volumetric flask, electric balance, P^H meter, aluminum foil, small beaker, cylinder, funnel, deep fridge, plastic bag, pencil, pen, knife, calibrated scale, tag paper, sieve, L-shaped angles, gloves, thread, needle etc.

3.5.2 Required media and reagents

Distilled water, Disodium hydrogen phosphate, Sodium dehydrogenate phosphate, Diethyl ether, 10% Formalin, 10% Buffered formalin, Bouin's solution (picric acid, glacial acetic acid, formaldehyde), Alcohol (100%, 95%, 90%, 80%, 70%, 50%), Xylene, Melted paraffin, Hematoxylin, Eosin, Hydrochloric acid etc.

3.5.3 Preparation of 10% neutral buffered formalin solution

About 900 ml of distilled water was measured in a measuring cylinder and then taken into a volumetric flux. Then 100 ml of commercial formaldehyde (37-40%) (Merck, Germany) was mixed with it. Sodium phosphate monobasic 4.0 gm and Sodium phosphate dibasic (anhydrous) 6.5 gm (P^H 7.2 ± 0.5) was added. Then P^H was examined with P^H meter (HI 2212 P^H/ORP Meter, HANA Instruments) and adjusted the P^H at 6.5 by adding diluted hydrochloric acid (HCL) drop by drop with proper precaution.

3.5.4 Preparation of Bouin's solution

Picric acid was added to the 750 ml of distilled water in a volumetric flux and stirred well till sedimentation. Picric acid solution was then filtered well. Thus, prepared 750 ml of the saturated picric acid solution was added to 250 ml of formaldehyde. After adding 50 ml of glacial acetic acid to the solution, it was shaking and mixed well. Thus, prepared 1050 ml of Bouin's solution was kept with proper label, initial and date.

3.5.5 Preparation of Harris's Alum haematoxylin and 1% eosin stock solution

Harris's Alum haematoxylin and stock 1% aqueous eosin solution were prepared according to standard protocol (Gridley, 1957). Working eosin solution was prepared through mixing of 1 part 1% aqueous eosin stock solution and 3 parts of 80% alcohol.

3.6 Laboratory examination

3.6.1 Determination of live weight of chickens

At first live weight of chicken was measured by using sensitive electronic balance (Mettler Toledo B154, ± 0.001 gm, China) prior to sacrifice of the chicken.

3.6.2 Sacrifice of chickens and collection of their lymphoid organs and tissues

The chickens were sacrificed by 'halal' slaughtering method. The carcasses were soaked in water to wet the feathers. The lymphoid organs (bursa of Fabricius, thymus, spleen and cecal tonsil) were carefully dissected out intact from day old chicks (D_1) which was followed by D_{30}, D_{90} and D_{180} **(Figure 3.2)** and washed with normal saline solution. After that, these samples were placed in a tray. The sample was collected in different day, gross anatomical observations and measurements of lymphoid organs were done on those following day and samples for histological study was also collected on the respective day with 10% neutral buffered formalin solution and transferred to the Department of Anatomy and Histology, CVASU.

3.6.3 Measurement of the weight of lymphoid organs and tissues

A visual observation, such as color and shape was performed after collection of lymphoid organs. Then weights of individual lymphoid organs (bursa of Fabricius, thymus, spleen and cecal tonsil) were taken in grams (gm) using a sensitive electronic balance (Mettler Toledo B154, ± 0.001 gm, China).

3.6.4 Determination of the height and length of lymphoid organs and tissues

The height of bursa of Fabricius and the length of thymus, spleen and cecal tonsils were measured by using slide calipers (0-150 digital caliper, Shinko Denshi Co. Ltd, Japan) as well as calibrated scales and were expressed in centimeter (cm).

3.6.5 Determination of the width of lymphoid organs and tissues

The width of bursa of Fabricius, spleen and cecal tonsils were measured by using calibrated scales and was expressed in centimeter (cm).

3.7 Tagging of specimen

Individual lymphoid organs (bursa of Fabricius, thymus, spleen and cecal tonsil) were tagged with specific tag paper using thread and needles.

3.8 Fixation of lymphoid organs and tissues

Then individual lymphoid organs (bursa of Fabricius, thymus, spleen and cecal tonsil) immersed in 10% neutral buffered formalin solution for a period of 72 hours for complete fixation **(Figure 3.4)**.

3.9 Tissue processing for microscopic examination

3.9.1 Washing of fixed tissue

After 72 hours of fixation the desired tissue specimens were washed in running tap water for 4-5 hours **(Figure 3.5)**. It was done to prevent any unexpected interference with subsequent processes as it helps to remove excess fixative from tissue samples.

3.9.2 Dehydration

To remove water content from tissue samples dehydration was performed through the passing of tissue sample into successive ascending concentration of alcohol **(Figure 3.6)**. It was done in following sequences:

50% Alcohol for 2 hours
60% Alcohol for 2 hours
70% Alcohol for 2 hours
80% Alcohol for 2 hours
90% Alcohol for 2 hours
95% Alcohol for 2 hours
100% Alcohol for 2 hours
100% Alcohol for 2 hours

3.9.3 Cleaning

After completion of dehydration, the tissue specimens were passed through successive changes of xylene until the alcohol from the tissue was replaced. This processing was performed in following sequences:

Alcohol (50%) + Xylene (50%) for 12 hours or overnight
Xylene (100%) for 2 hours
Xylene (100%) for 2 hours

3.9.4 Infiltration

When the tissue samples became clearly transparent, i.e., cleaning was completed by xylene; the specimens were placed into melted paraffin in the oven at 58-60°C to evaporate the xylene and the tissue spaces were infiltrated with melted paraffin. Different graded of paraffin was used in following sequences:

↓
Paraffin (50%) + Xylene (50%) for 2 hours
Paraffin (100%) at 60°C for 3 hours
Paraffin (100%) at 60°C for 3 hours

3.9.5 Embedding

After completion of infiltration, the tissue samples were placed in between two L-shaped angles and it was filled with melted paraffin for making paraffin block **(Figure 3.7)**.

3.9.6 Sectioning

Then microscopic section was cut at 6 μm thickness using a sliding microtome machine (Leica SM2010R V1.2 English-09/2008 Sliding Microtome Machine, Germany) **(Figure 3.8)** with the help of disposable carbon blade. Water bath was set at 60°C temperature. The slide was labeled by diamond pencil as labeled by tag. Gelatin was used as an adhesive to attach the section on glass slides. Then the slide was dried in air **(Figure 3.9)** for a period of 12 hours before staining.

3.9.7 Staining

The tissues were stained with hematoxylin and eosin **(Figure 3.10)** for visualization under the light microscope. Following protocol was followed for staining (Gridley, 1957).

↓
100% Xylene-I for 2 min
100% Xylene-II for 2 min
100% Alcohol-I for 2 min
100% Alcohol-II for 2 min
95% Alcohol for 2 min
70% Alcohol for 2 min

Running tape water for 2 min
Hematoxylin for 10-15 min
Running tape water for 2 min
1% Acid alcohol for 1-2 short dips
Running tape water for 5 min
Lithium carbonate for 2-4 dips
Running tape water for 5-10 min
1% Eosin for 2-4 min
70% Alcohol for 30 sec
95% Alcohol for 2 min
100% Alcohol-I for 2 min
100% Alcohol-II for 2 min
50% Alcohol + 50% Xylene for 2 min
100% Xylene-I for 2 min
100% Xylene-II for 2 min

3.9.8 Mounting

Mounting of these stained slides were completed with cover slides by using "Canada Balsam" and then those mounted slides were allowed to harden in air.

3.10 Histomorphometry

For obtaining histomorphmetric data of lymphoid organs and tissues in different postnatal stages, 5 photomicrographs were taken from each age group of tissues of lymphoid organs and tissues (thymus, bursa of Fabricius, spleen and cecal tonsil) in both scavenging and captive deshi chicken using a photomicroscope (AmScope Trinocular Compound Microscope, Model T490 B-MT) and AmScope image measuring software (x86, 3.7.3036 version). At first stage micrometer was calibrated in micrometer (each division 10 µm) and placed under the 10x and 20x objective lens and then images were taken. Simultaneously, images from tissue sections were taken under same 10x and 20x objective lens of the microscope. Then those images were placed in AmScope image measuring software and then the actual length, height and width of respected lymphoid

organs and tissues were determined automatically and thus the obtained result was measured in micrometer (µm) **(Figure 3.11 and Figure 3.12)**.

3.11 Data analysis

At first all data obtained from this study were entered and stored into an excel sheet (Microsoft Excel-2007). Then the data were transferred to statistical software, STATA-13 (STATA Corp., Texas, USA) to perform statistical data analysis. Unpaired sample t-test was done to compare means of different variables between two chickens groups (scavenging and captive chickens). A p-value of equal or less than 0.05 ($P \leq 0.05$) was considered as significant for this test. Results were expressed as arithmetic mean ± standard deviation (Mean ± SD).

Pictorial presentation of materials and methods

Figure 3.1: Deshi chickens

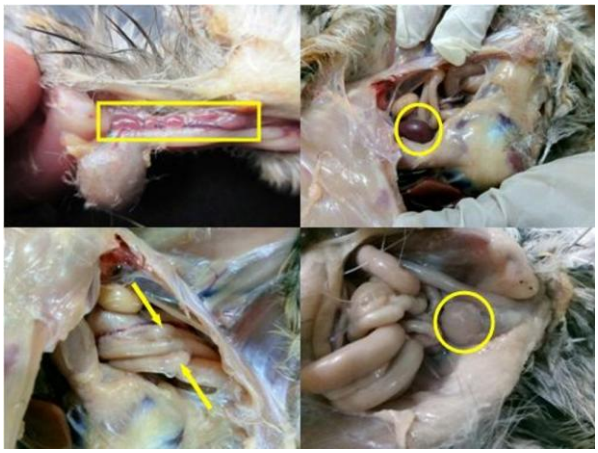

Figure 3.2: Collection of sample

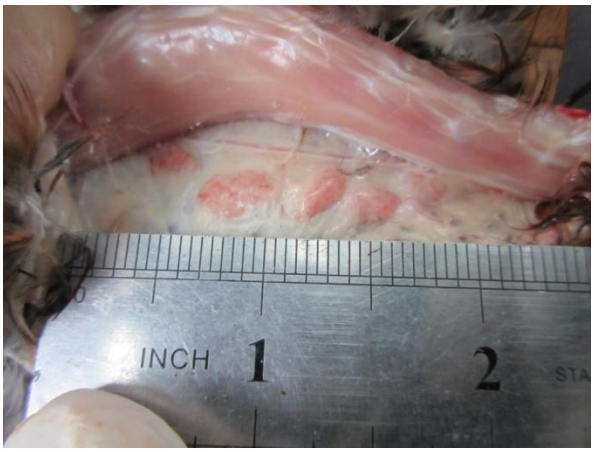

Figure 3.3: Gross measurement technique of samples

Figure 3.4: Fixation of sample

Figure 3.5: Washing of tissues in running tap water

Figure 3.6: Dehydration of tissues by using ascending graded alcohol

Figure 3.7: Paraffin block preparation

Figure 3.8: Sectioning of tissue by microtome machine

Figure 3.9: Keeping of slides in a rack

Figure 3.10: H & E staining process

Figure 3.11: Microscopic examination

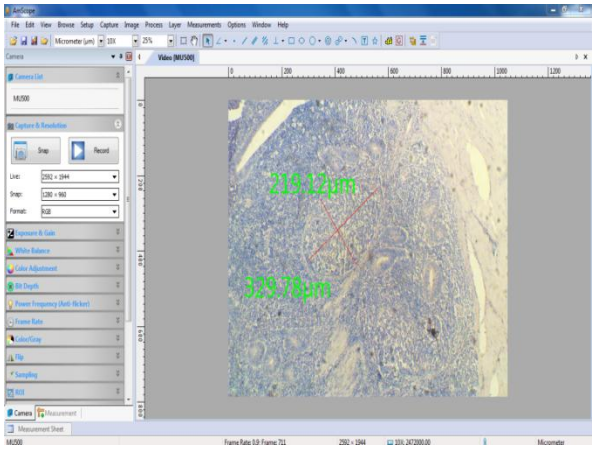

Figure 3.12: Measurement of histological parameter by using AmScope software

Chapter-4: Results

The gross and histological studies of lymphoid organs and tissues were carried out in both scavenging deshi chicken (SDC) and captive deshi chicken (CDC) during their postnatal growth and developmental stages and the results are presented under following captions:-

4.1 Gross morphology of lymphoid organs and tissues in SDC and CDC

4.1.1 Bursa of Fabricius

The bursa of all age groups in both scavenging and captive deshi chickens were globular in shape with slightly dorsoventrally compressed and it was located on the dorsal surface of proctodeum. The color of bursa was whitish cream in all age groups of chickens **(Figure 4.1)**.

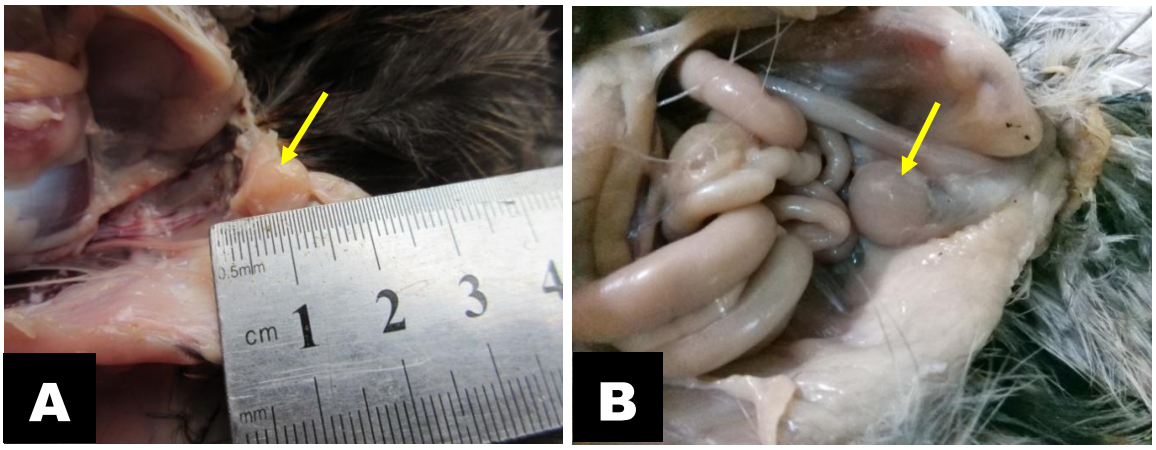

Figure 4.1: Arrow headed area showing the 90 days old bursa of Fabricius in scavenging deshi chicken (A) and captive deshi chicken (B).

The gross morphometrical measurements of bursa of both SDC and CDC are presented in **figure 4.2**. It observed that the mean weight, height and width of bursa in both SDC and CDC were increases from D_1 to D_{90}. At D_{180} in both SDC and CDC, involution of bursa was indicated by decreasing weight, height and width. The mean weight, height and width of bursa were significantly ($P \leq 0.05$) higher in CDC than that of SDC in all age

groups except day old (D_1) chicks. In both SDC and CDC, the highest mean values of weight (1.88 ± 0.13 gm, 2.04 ± 0.11 gm, respectively), height (1.26 ± 0.11 cm, 1.48 ± 0.08 cm, respectively) and width (0.76 ± 0.11 cm, 1.04 ± 0.11 cm, respectively) of bursa were found at D_{90}.

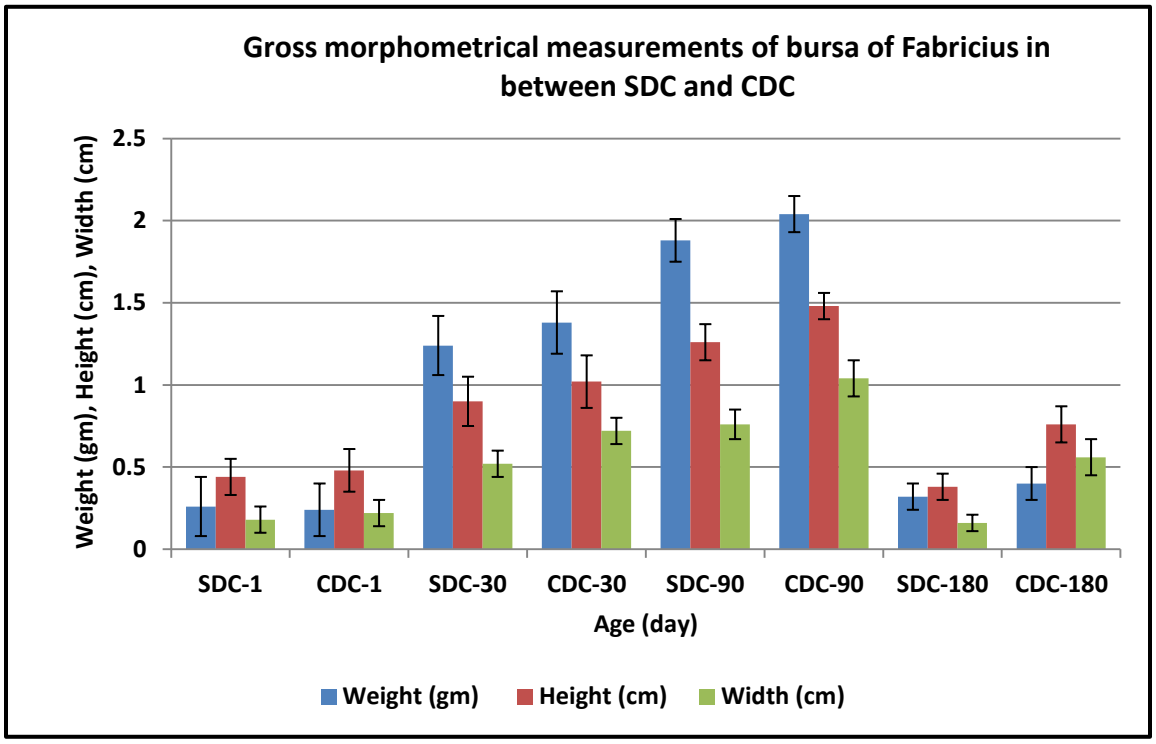

Figure 4.2: The present graph is showing the weight, height and width of bursa of Fabricius in different ages of scavenging deshi chicken (SDC) and captive deshi chicken (CDC). The growth of the bursa is found higher at D_{90} in both SDC and CDC; N=40 (n=5) and each bar represent Mean ± SD.

4.1.2 Thymus

The thymus of all age groups in both SDC and CDC were a paired organ, located on parallel to the vagus nerve and internal jugular veins, extended from the anterior cervical region to the thoracic region on either side of the neck. Each lobe was well separated from the other and they were connected as a chain and lying in the sub-dermal connective tissue of the neck region. The color of the thymus of deshi chicken was pale red to yellowish red **(Figure 4.4)**. The mean numbers of thymic lobe, weight and length of thymus in both SDC and CDC were increases from D_1 to D_{180}. In both SDC and CDC, the highest mean value of weight (0.98 ± 0.08 gm, 1.26 ± 0.11 gm, respectively) and

length (3.68 ± 0.08 cm, 3.78 ± 0.08 cm, respectively) of thymus were found at D_{180}. The mean weight and length of thymus were higher in CDC as compared to SDC in all age group except day old (D_1) chicks. Among these all gross parameters of thymus only weight and length were significantly ($P \leq 0.05$) higher in CDC. The mean numbers of thymic lobe was equal in the same age group in both SDC and CDC **(Figure 4.3)**.

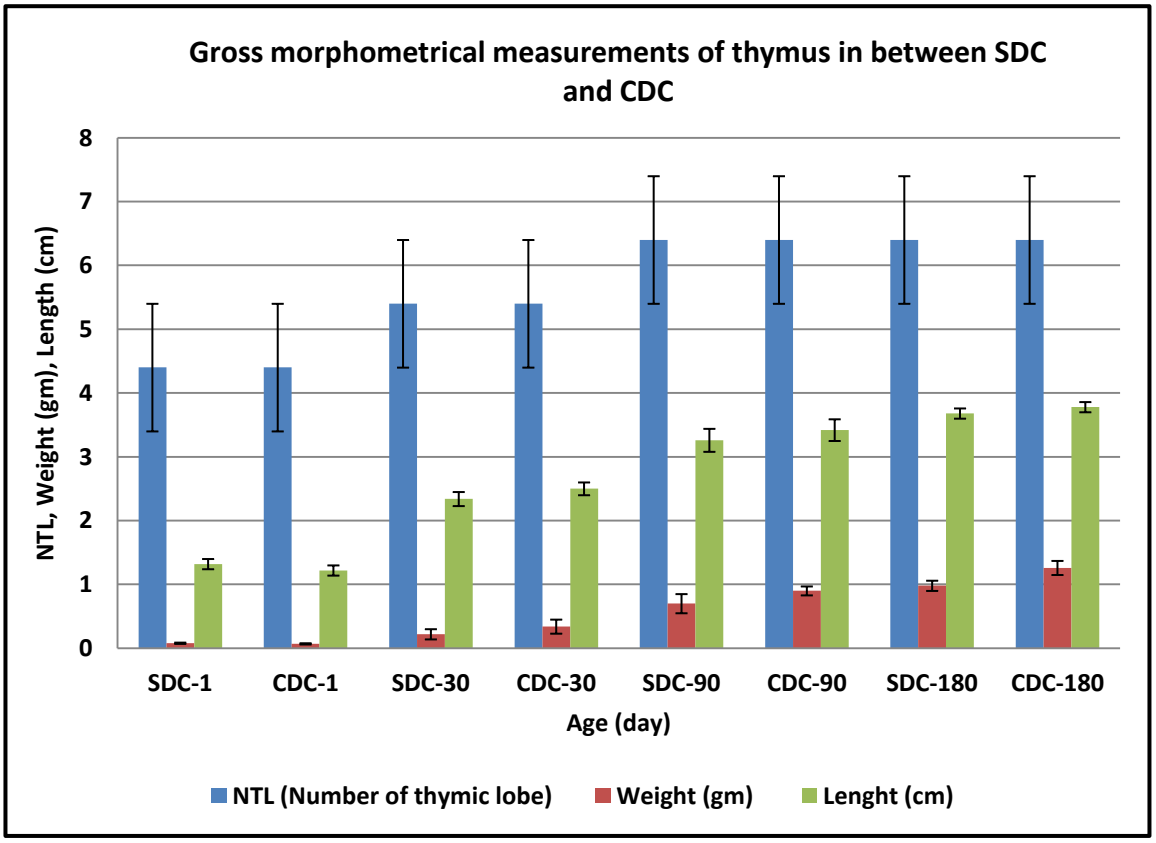

Figure 4.3: The present graph is showing the number of thymic lobe, weight and length of thymus in different ages of scavenging deshi chicken (SDC) and captive deshi chicken (CDC). The growth of the thymus is found higher at D_{180} in both SDC and CDC; N=40 (n=5) and each bar represent Mean ± SD.

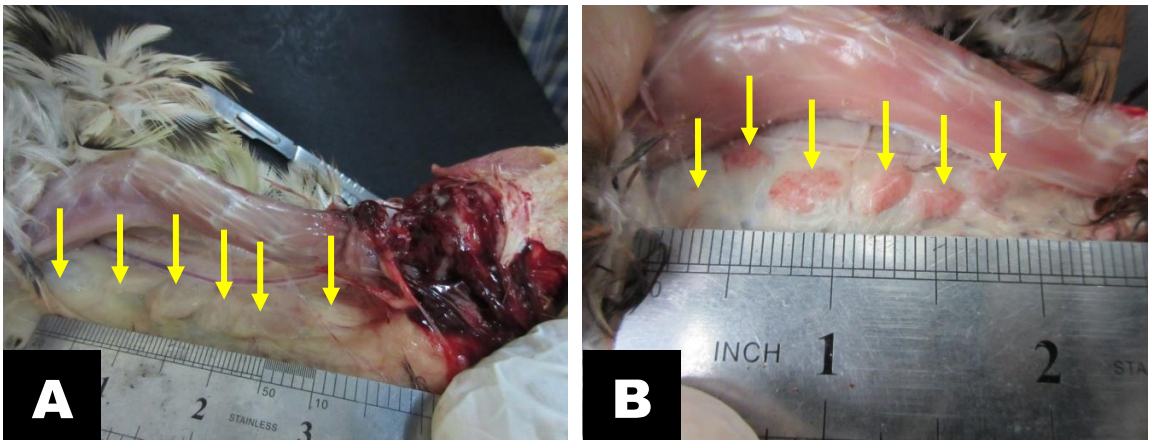

Figure 4.4: Arrow headed area showing the 90 days old thymus in scavenging deshi chicken (A) and captive deshi chicken (B).

4.1.3 Spleen

The present study revealed that the spleen of all age group in both SDC and CDC were somewhat rounded shape and brownish red in color **(Figure 4.5)**. It was found that the mean weight, length and width of spleen in both SDC and CDC were gradually increased from D_1 to D_{180}. In both SDC and CDC, the highest mean value of weight (2.78 ± 0.16 gm, 3.06 ± 0.18 gm, respectively), length (1.98 ± 0.08 cm, 2.26 ± 0.16 cm, respectively) and width (1.42 ± 0.08 cm, 1.62 ± 0.08 cm, respectively) of spleen were found at D_{180}. The mean weight, length and width of spleen were higher in CDC than that of SDC. The weight, length and width of spleen were significantly ($P \leq 0.05$) more in CDC of all age group except D_1 chicks group **(Figure 4.6)**.

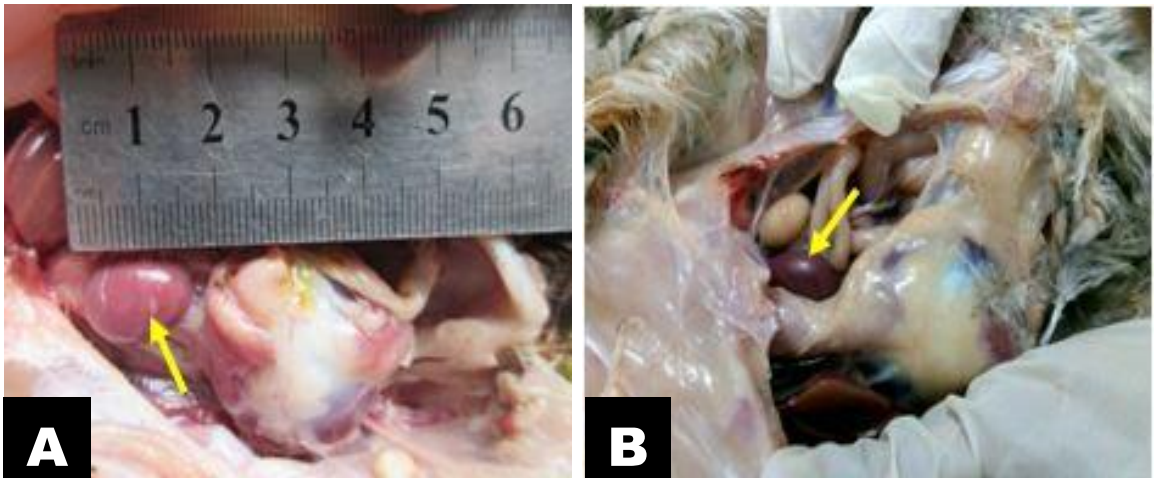

Figure 4.5: Arrow headed area showing the 90 days old spleen of scavenging deshi chicken (A) and captive deshi chicken (B).

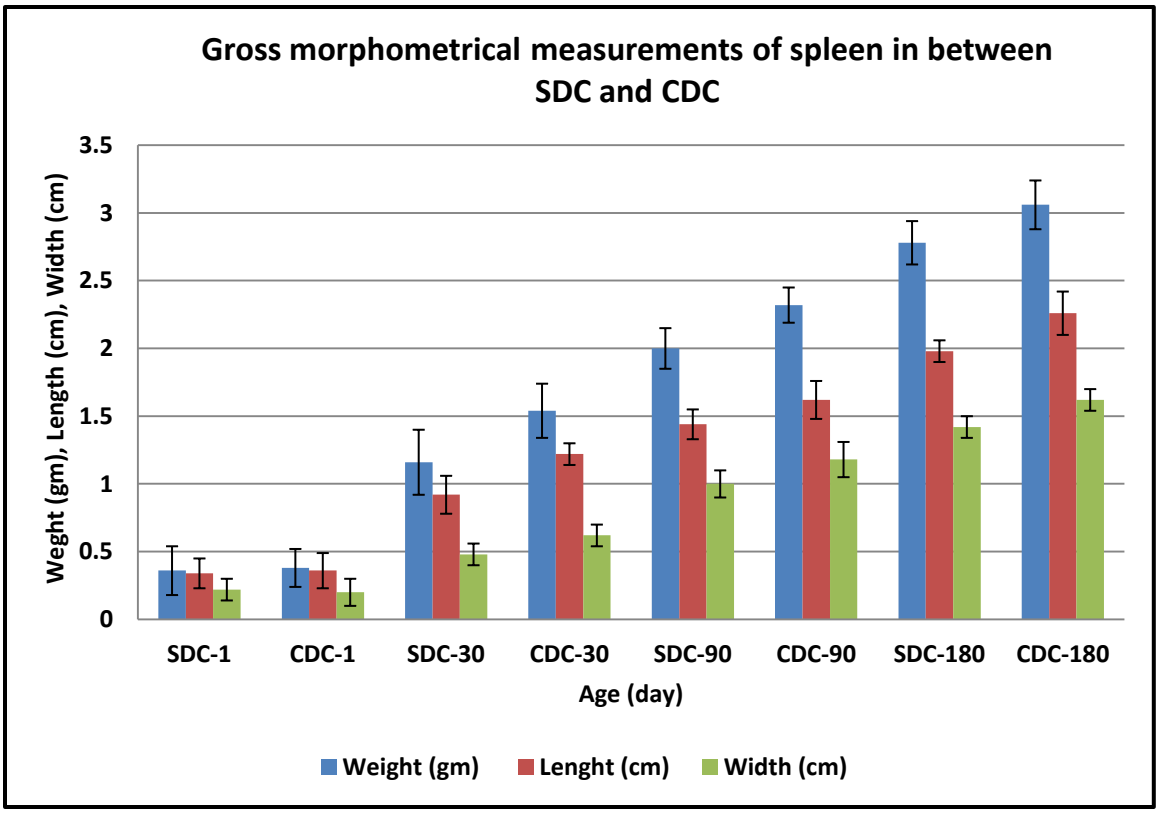

Figure 4.6: The present graph is showing the weight, length and width of spleen in different ages of scavenging deshi chicken (SDC) and captive deshi chicken (CDC). The growth of the spleen is found higher at D_{180} in both SDC and CDC; N=40 (n=5) and each bar represent Mean ± SD.

4.1.4 Cecal tonsil

In the present study the cecal tonsils of all age groups in both SDC and CDC were in the proximal one third of the paired tubular cecum which lies along each side of the large intestine. They were broad tubular in shape **(Figure 4.7)**. The mean weight, length and width of cecal tonsil of both SDC and CDC were gradually increased from D_1 to D_{180}. In both SDC and CDC, the highest mean value of weight (2.60 ± 0.10 gm, 2.72 ± 0.08 gm, respectively), length (1.70 ± 0.10 cm, 1.82 ± 0.08 cm, respectively) and width (1.18 ± 0.08 cm, 1.30 ± 0.10 cm, respectively) of cecal tonsils were found at D_{180}. The mean weight, length and width of cecal tonsils were higher in CDC than that of SDC in all age groups. The weight, length and width of cecal tonsils of all age group except D_1 chicks group were significantly ($P \leq 0.05$) more in CDC than SDC **(Figure 4.8)**.

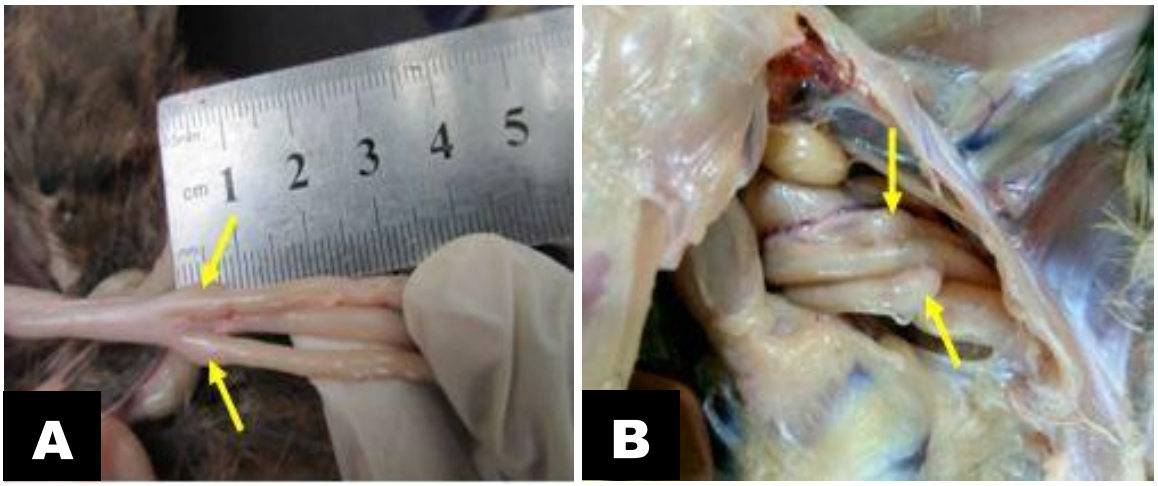

Figure 4.7: Arrow headed area showing the 180 days old cecal tonsil of scavenging deshi chicken (A) and captive deshi chicken (B).

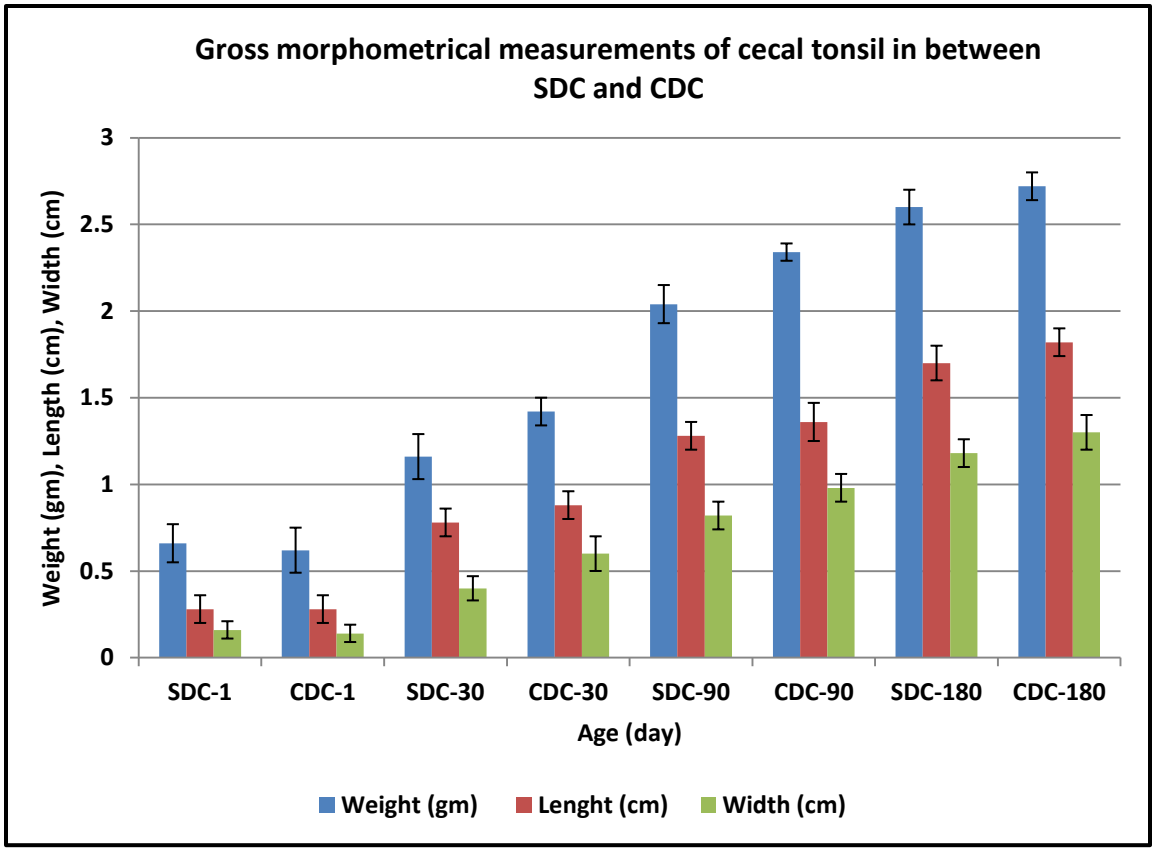

Figure 4.8: The present graph is showing the weight, length and width of cecal tonsil in different ages of scavenging deshi chicken (SDC) and captive deshi chicken (CDC). The growth of the cecal tonsil is found higher at D_{180} in both SDC and CDC; N=40 (n=5) and each bar represent Mean ± SD.

4.2 Histomorphology of lymphoid organs and tissues in SDC and CDC

4.2.1 Bursa of Fabricius

In both SDC and CDC, the bursa of Fabricius was consisted of tunica mucosa, submucosa, tunica muscularis and tunica serosa. Each bursal follicle was consisted of a peripheral cortex and a central medulla. The darkly stained cortex was composed of many closely packed small lymphocytes. The paler medulla contained fewer cells of various sizes. The mucosal folds of the bursa were lined by pseudostratified columnar epithelium, except at the apex which was covered by a simple columnar epithelium. The cores of the folds were filled with lymphoid follicles which were separated by interfollicullar septae **(Figure 4.9)**.

The histomorphometrical observations of bursa of Fabricius are shown in **table 4.1** and observed that the height of epithelium, number of mucosal folds, number of follicles per mucosal folds, height of mucosal folds were increased from D_1 to D_{90} of both SDC and CDC but all those parameters were decreased at D_{180} of both SDC and CDC. In contrast, all those parameters (height of epithelium, number of mucosal folds, number of follicles per mucosal folds, height of mucosal folds) were higher in CDC than SDC at their different ages. A different shaped mucosal fold includes elongated, leaf, club, sessile and pyramidal with secondary branches was also noticed. The mean number of mucosal folds was varied from 6.80 ± 0.84 to 12.00 ± 1.58 in both SDC and CDC. The number of mucosal fold was significantly ($P \leq 0.05$) higher at 30 days (8.80 ± 1.30), 90 days (12.00 ± 1.58) and 180 days (8.20 ± 1.30) old CDC as compared to 30 days (7.80 ± 1.30), 90 days (10.80 ± 1.30) and 180 days (7.20 ± 1.30) old SDC, respectively. The number of follicles per mucosal folds was significantly ($P \leq 0.05$) higher at 30 days (30.20 ± 6.14 µm), 90 days (63.40 ± 7.70 µm) and 180 days (26.80 ± 3.19 µm) old CDC as compared to 30 days (26.80 ± 7.01 µm), 90 days (59.80 ± 7.56 µm) and 180 days (24.00 ± 3.16 µm) old SDC, respectively. Height of epithelium was significantly ($P \leq 0.05$) higher at 30 days (51.50 ± 3.71 µm), 90 days (68.60 ± 4.93 µm) and 180 days (47.00 ± 5.43 µm) old CDC as compared to 30 days (43.40 ± 5.94 µm), 90 days (57.4 ± 5.59 µm) and 180 days (40.80 ± 3.19 µm) old SDC, respectively. Height of mucosal folds was significantly ($P \leq 0.05$) higher at 30 days (879.80 ± 152.04 µm), 90 days (1076.40 ± 154.74 µm) and 180 days

(608.40 ± 103.97 µm) old CDC as compared to 30 days (481.00 ± 144.59 µm), 90 days (1017.00 ± 134.82 µm) and 180 days (560.80 ± 102.53 µm) old SDC, respectively **(Table 4.1)**.

Table 4.1: Histomorphometrical observations of bursa of Fabricius

Age group (day)	Parameter	Chickens group (Mean ± SD)		P-value
		SDC	CDC	
D_1	HE (µm)	29.00 ± 5.47	30.00 ± 3.54	0.74
	NMF (µm)	7.00 ± 0.71	6.80 ± 0.84	0.69
	NFPMF (µm)	14.00 ± 3.16	14.20 ± 2.86	0.91
	HMF (µm)	468.00 ± 105.21	471.60 ± 103.49	0.95
D_{30}	HE (µm)	43.40 ± 5.94	51.50 ± 3.71	0.03*
	NMF (µm)	7.80 ± 1.30	8.80 ± 1.30	0.02*
	NFPMF (µm)	26.80 ± 7.01	30.20 ± 6.14	0.03*
	HMF (µm)	481.00 ± 144.59	879.80 ± 152.04	0.01**
D_{90}	HE (µm)	57.4 ± 5.59	68.60 ± 4.93	0.01**
	NMF (µm)	10.80 ± 1.30	12.00 ± 1.58	0.002**
	NFPMF (µm)	59.80 ± 7.56	63.40 ± 7.70	0.05*
	HMF (µm)	1017.00 ± 134.82	1076.40 ± 154.74	0.03*
D_{180}	HE (µm)	40.80 ± 3.19	47.00 ± 5.43	0.05*
	NMF (µm)	7.20 ± 1.30	8.20 ± 1.30	0.009**
	NFPMF (µm)	24.00 ± 3.16	26.80 ± 3.19	0.04*
	HMF (µm)	560.80 ± 102.53	608.40 ± 103.97	0.02*

Note: N = 40 (n = 5 for each age group); HE = Height of epithelium; NMF = Number of mucosal fold; NFPMF = Number of follicles per mucosal fold; HMF = Height of mucosal fold; µm = micrometer; SD = Standard deviation; *Significant ($P \leq 0.05$); **Highly significant ($P \leq 0.01$).

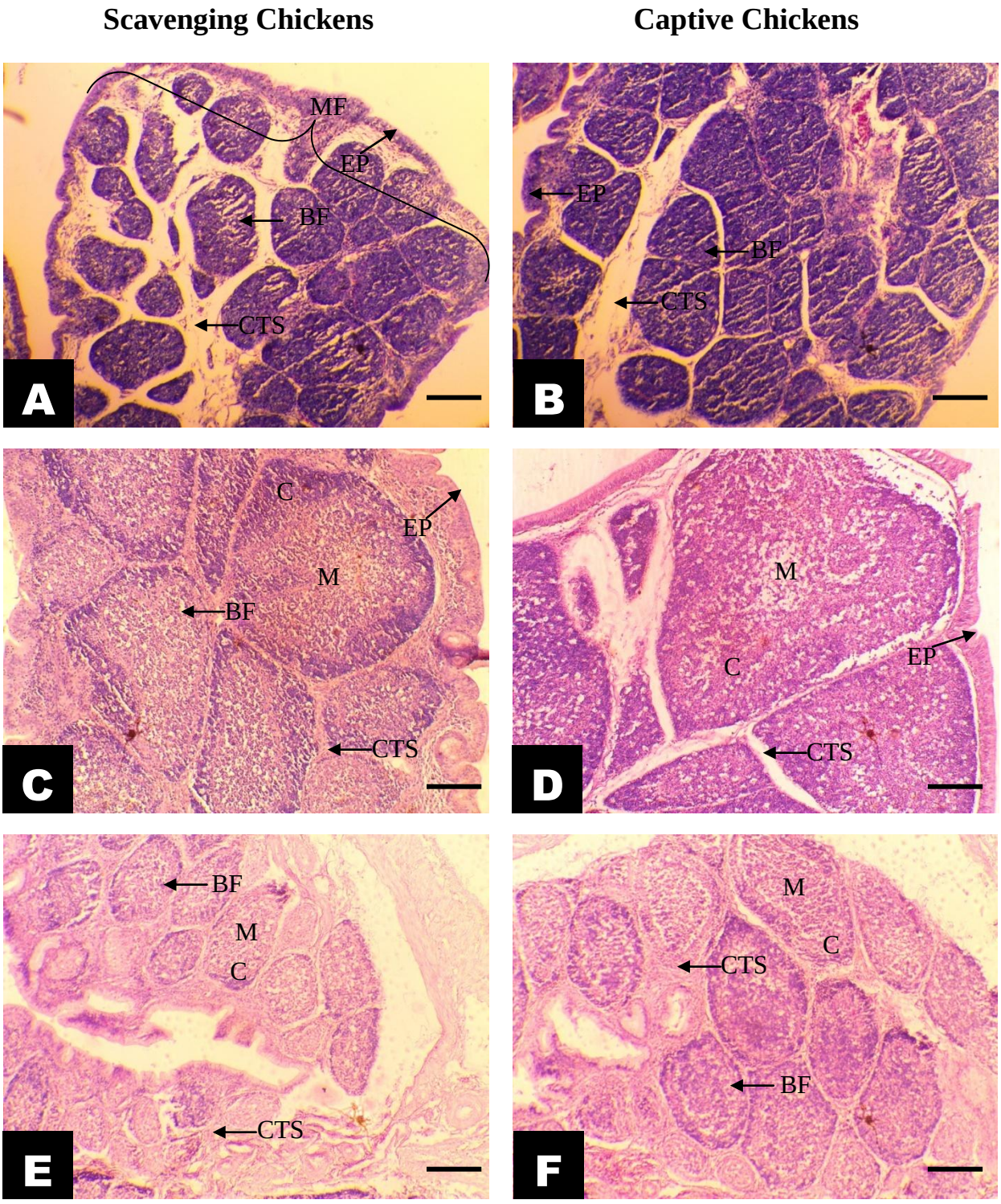

Figure 4.9: Histological structure of 30 days (A, B), 90 days (C, D) and 180 days (E, F) old bursa of Fabricius in both SDC (left column) and CDC (right column) in H&E staining (10x) showing cortex (C), medulla (M), mucosal fold (MF), bursal follicle (BF), epithelium (E) and connective tissue septum (CTS); Scale Bar 180 µm.

4.2.2 Thymus

The thymus of the chicken covered by a thin connective tissue capsule from which numerous fine connective tissue septa originated and divided the organ into many incomplete but distinct lobules. Each lobule was composed of a peripheral cortex which was densely infiltrated with lymphocytes and a central medulla that was enriched with epithelial reticular cells; that's why cortex was more basophilic than medulla. The medulla was pale and diffuse Hassall's corpuscles were found which were arranged in a concentric form **(Figure 4.10)**. The mean length and width of thymic lobule in both SDC and CDC were gradually increased from D_1 to D_{180}. In both SDC and CDC, the highest mean value of length (985.80 ± 28.19 µm and 1001.20 ± 28.96 µm, respectively) and width (566.60 ± 86.39 µm and 608.80 ± 85.40 µm, respectively) of thymic lobules were found at D_{180}. The mean length and width of thymic lobules were higher in all ages of CDC as compared with SDC **(Table 4.2)**.

4.2.3 Spleen

The spleen of deshi chicken was surrounded by a thick capsule and a small number of trabeculi also noticed. The red pulps were less distinct and these were scatteredly distributed within the white pulp **(Figure 4.11)**. The mean length and width of white pulp in both SDC and CDC were gradually increased from D_1 to D_{180}. In both SDC and CDC, the highest mean value of length (362.20 ± 19.43 µm and 399.80 ± 17.17 µm, respectively) and width (167.20 ± 19.09 µm and 190.40 ± 10.53 µm, respectively) of white pulps were observed at D_{180}. The mean length and width of white pulps were higher in all ages of CDC as compared with SDC **(Table 4.2)**.

4.2.4 Cecal tonsil

Histologically cecal tonsil consisted of four layers, i.e. tunica mucosa, submucosa, muscularis and serosa. Their lining epithelium was simple columnar epithelium. The base of the villi was thick and the apex was pointed or rounded in deshi chicken. More diffuse lymphoid tissues and unorganized lymphatic nodules were present both in the mucosa and submucosa **(Figure 4.12)**. The mean length and width of lymphatic nodules in both SDC and CDC were gradually increased from D_1 to D_{180}. In both SDC and CDC, the highest mean value of length (164.20 ± 16.02 µm and 180.20 ± 13.44 µm, respectively)

and width (107.20 ± 15.55 µm and 120.40 ± 18.51 µm, respectively) of lymphatic nodules were found at D_{180}. The mean length and width of lymphatic nodules were higher in all ages of CDC than that of SDC **(Table 4.2)**.

Table 4.2: Histomorphometrical observations of thymus, spleen and cecal tonsil

Lymphoid organ	Parameter	Age group (day)	Chickens group (Mean ± SD)		P-value
			SDC	CDC	
Thymus	LTL (µm)	D_1	265.40 ± 45.75	269.00 ± 39.93	0.89
		D_{30}	547.60 ± 90.56	583.20 ± 102.31	0.05*
		D_{90}	881.00 ± 153.22	913.40 ± 144.00	0.04*
		D_{180}	985.80 ± 28.19	1001.20 ± 28.96	0.01**
	WTL (µm)	D_1	152.00 ± 22.89	152.80 ± 22.19	0.95
		D_{30}	285.60 ± 63.05	328.80 ± 58.78	0.02*
		D_{90}	510.80 ± 174.78	539.20 ± 188.69	0.05*
		D_{180}	566.60 ± 86.39	608.80 ± 85.40	0.015*
Spleen	LWP (µm)	D_1	69.20 ± 6.26	69.60 ± 7.40	0.92
		D_{30}	209.00 ± 39.99	229.40 ± 47.04	0.04*
		D_{90}	279.80 ± 69.02	310.60 ± 82.42	0.05*
		D_{180}	362.20 ± 19.43	399.80 ± 17.17	0.007**
	WWP (µm)	D_1	32.40 ± 5.22	32.60 ± 5.89	0.95
		D_{30}	81.00 ± 10.75	88.20 ± 11.61	0.02*
		D_{90}	140.60 ± 28.51	151.20 ± 31.36	0.05*
		D_{180}	167.20 ± 19.09	190.40 ± 10.53	0.05*
Cecal tonsil	LLN (µm)	D_1	26.60 ± 4.04	27.20 ± 3.89	0.81
		D_{30}	53.40 ± 7.57	61.80 ± 8.26	0.01**
		D_{90}	129.40 ± 22.09	142.80 ± 24.65	0.03*
		D_{180}	164.20 ± 16.02	180.20 ± 13.44	0.01**
	WLN (µm)	D_1	19.40 ± 5.18	19.40 ± 5.03	1.00
		D_{30}	38.60 ± 6.58	49.40 ± 8.14	0.05*
		D_{90}	92.40 ± 16.46	103.80 ± 19.58	0.03*
		D_{180}	107.20 ± 15.55	120.40 ± 18.51	0.05*

Note: N = 40 (n = 5 for each age group); LTL = Length of thymic lobe; WTL = Width of thymic lobe; LWP = Length of white pulp; WWP = Width of white pulp; LLN = Length of lymph nodule; WLN = Width of lymph nodule; µm = micrometer; SD = Standard deviation; Non significant (P>0.05); *Significant ($P \leq 0.05$); **Highly significant ($P \leq 0.01$).

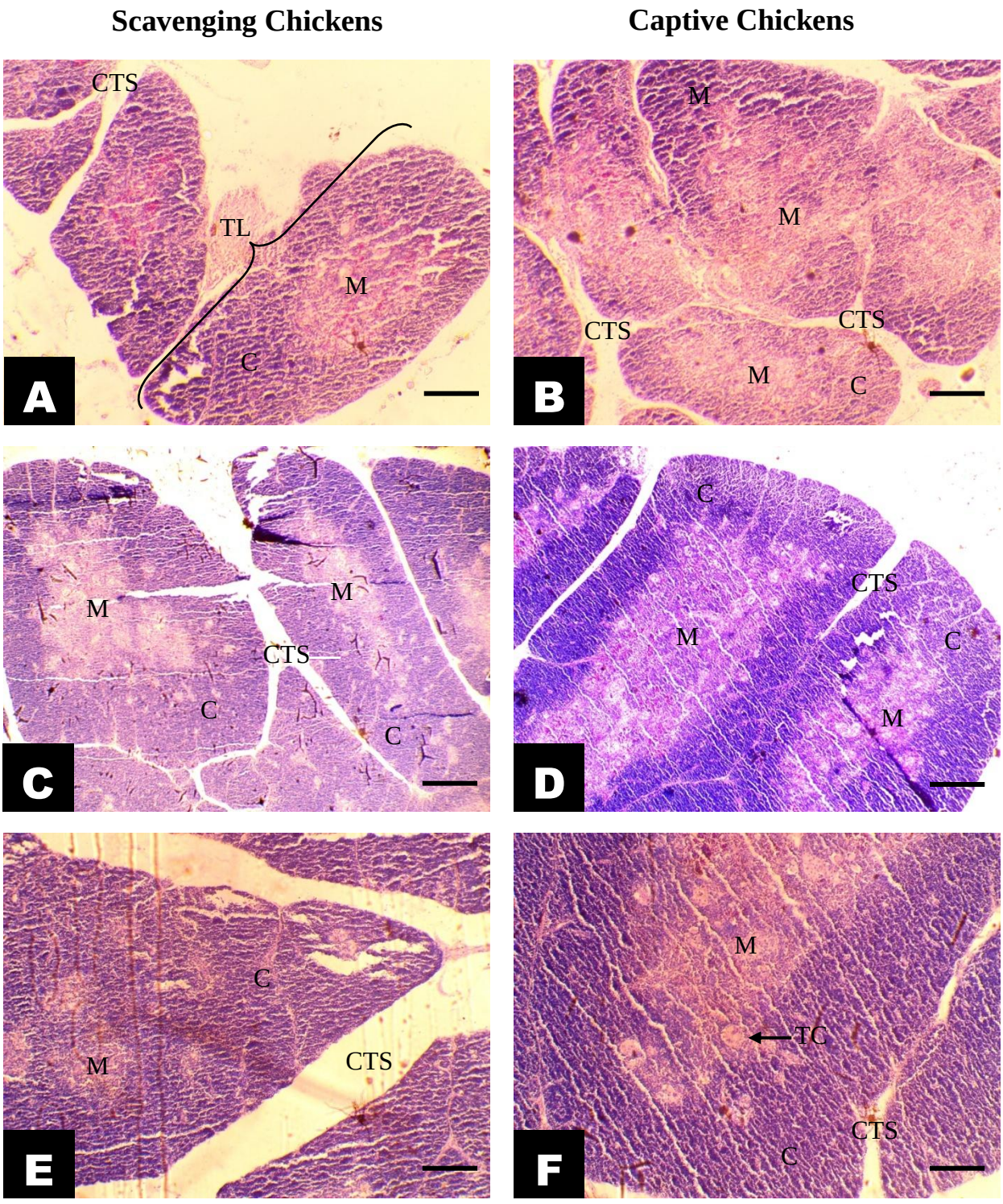

Figure 4.10: Histological structure of 30 days (A, B), 90 days (C, D) and 180 days (E, F) old thymus in both SDC (left column) and CDC (right column) in H&E staining (10x) showing cortex (C), medulla (M), thymic lobule (TL), thymic corpuscle (TC), connective tissue septum (CTS); Scale Bar 180 µm.

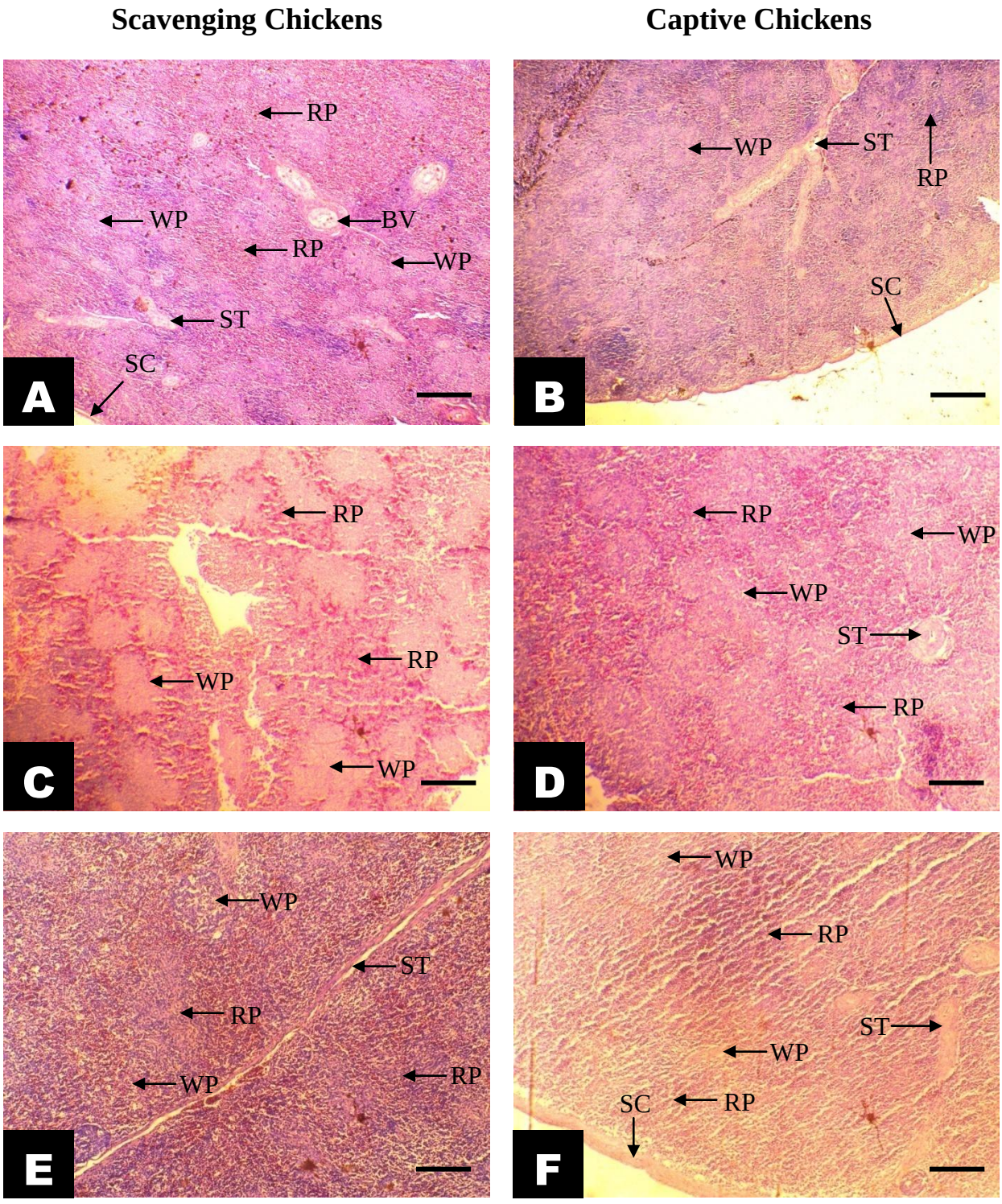

Figure 4.11: Histological structure of 30 days (A, B), 90 days (C, D) and 180 days (E, F) old spleen in both SDC (left column) and CDC (right column) in H&E staining (10x) showing white pulp (WP), red pulp (RP), splenic capsule (SC), splenic trabeculae (ST), blood vessel (BV); Scale Bar 180 µm.

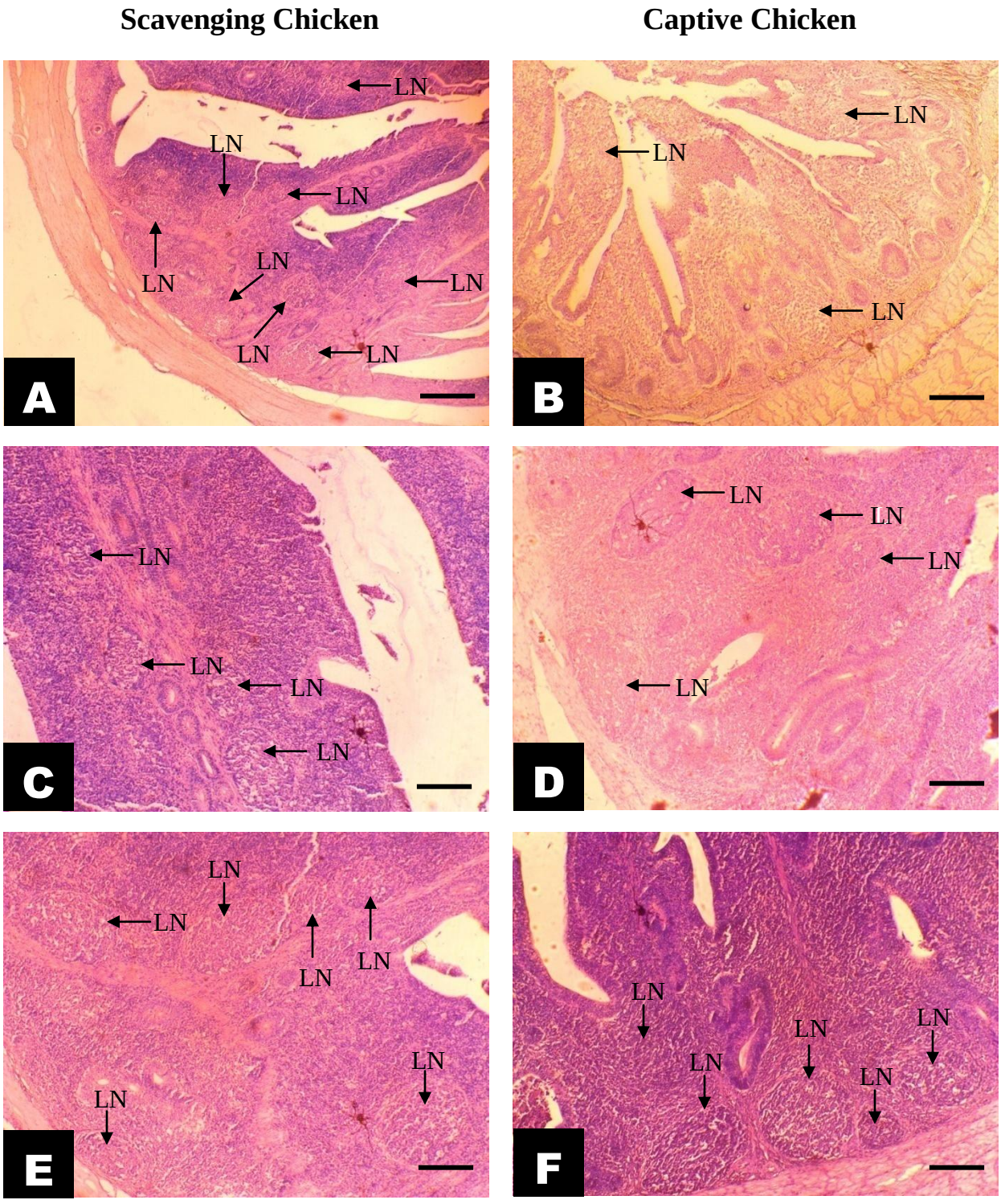

Figure 4.12: Histological structure of 30 days (A, B), 90 days (C, D) and 180 days (E, F) old cecal tonsil in both SDC (left column) and CDC (right column) in H&E staining (10x) showing lymphatic nodule (LN); Scale Bar 180 µm.

Chapter-5: Discussion

5.1 General morphology of lymphoid organs

In present study the bursa of all age groups in both scavenging and captive deshi chickens were globular in shape and whitish cream in color as reported previously in Deshi chicken (Akter *et al.*, 2006) and Hybrid chicken (Hodges, 1974). It was located at the dorsal surface of proctodeum. These findings were similar to the previous reports in Hybrid chicken (Getty, 1975; Dellmann and Eurell, 1998). Khan *et al.* (2014) observed bursa was antero-posteriorly compressed in broiler chicken but in deshi chicken, it was slightly dorso-ventrally compressed which may be due to their different breed characteristics. In contrast, no differences were found in the color, shape and location of bursa in between the SDC and CDC because of their similar breed characteristics. Histologically, the bursa of Fabricius in both SDC and CDC was composed of tunica mucosa, submucosa, tunica muscularis and tunica serosa which was supported the previous findings in Deshi chicken (Akter *et al.*, 2006) and Hybrid chicken (Hodges, 1974; Dellmann and Eurell, 1998; Bacha Jr and Bacha, 2012). The bursa was consisting of long thick mucosal folds which were projected into the lumen and numerous follicles filled the lamina propria of each fold. They were separated from the adjacent lymphoid tissue by connective tissue fibers, cells and intercellular spaces. These similar observations also observed in Hybrid chicken (Naukkarinen and Sorvari, 1984; Dellmann and Eurell, 1998), Broiler chicken (Khan *et al.*, 2014) and Deshi chicken (Khalil *et al.*, 2002). Each bursal follicle was consisting of a peripheral cortex and a central medulla as noticed by Dellmann and Eurell (1998). In the present study, the darkly stained cortex composed of many closely packed small lymphocytes but the paler medulla contained various sizes smaller number of lymphocytes. These results agreed with previous report in Deshi chicken (Khalil *et al.*, 2002), in Hybrid chicken (Dellmann and Eurell, 1998) and in Broiler chicken (Hussan *et al.*, 2009; Khan *et al.*, 2014). The mucosal fold of the bursa in both SDC and CDC was lined by pseudostratified columnar epithelium except at the apex of each follicle which was lined by a simple columnar epithelium. These results of present study were agreed with previous findings in Broiler chicken (Akter *et al.*, 2006; Khan *et al.*, 2014) and Hybrid chickens (Hodges, 1974; Dellmann and Eurell,

1998). On the other hand, no differences were found in the overall general histology of bursa in between the SDC and CDC which may be due to their similar breed characteristics. This similar reasons were reported by Jain *et al.* (2010) in CARI Shyama and Vanaraja chickens in India.

The thymus of all age groups in both SDC and CDC were a paired organ, located on parallel to the vagus nerve and internal jugular vein, extended from the anterior cervical region to the thoracic region on either side of the neck. These findings were consistent with the earlier reports in White Leghorn chicken (Cooper *et al.*, 1965; Akers and Denbow, 2008), Deshi chicken (Khalil *et al.*, 2003) and in Broiler chicken (Karim *et al.*, 2005). Each lobe was well separated from other and they were connected as a chain and lying in the sub-dermal connective tissue of the neck region. These analogous findings were reported in Hybrid chicken (Getty, 1975) and in Deshi chicken (Akter *et al.*, 2006; Khan *et al.*, 2014). The thymus was yellowish or pale white color in Broiler (Akter *et al.*, 2006; Khan *et al.*, 2014), yellowish or pale white color in Aseel chicken (Haseeb *et al.*, 2014) but in this study, the color of the thymus of deshi chicken was pale red to yellowish red. This color variation may be due to the variation of species, breed and their food habits. Histologically, the thymus of this study chicken was enclosed by a thin connective tissue capsule which was strongly supports the earlier findings in chickens (Cooper *et al.*, 1965; Dellmann and Eurell, 1998). Numerous fine septa of connective tissue originated from this capsule and divided the organ into incompletely separated lobules. Khalil *et al.* (2003) also observed this similar findings in deshi chicken. Each lobule organized into a peripheral cortex and a central medulla as reported in Hybrid chicken (Cooper *et al.*, 1966; Dellmann and Eurell, 1998). The cortex stained more deeply basophilic than that of medulla due to high concentration of cortical cells which observations consistent with the earlier findings in Hybrid chicken (Day and Schultz, 2014). A few number of Hassall's corpuscles were found in the medulla which were arranged in a concentric form. These general histological features of the thymus in the present study were consistent to the earlier findings in White Leghorn chicken (Khan *et al.*, 1998), Vancobb chicken (Karim *et al.*, 2005) and Broiler chicken (Akter *et al.*, 2006; Khan *et al.*, 2014) but differ in some points. They have found large number of Hassall's corpuscles in the medulla of thymus.

The spleen was brownish red colored and somewhat rounded shape which was located to the right side of the junction between the proventriculus and gizzard as observed previously in Broiler chicken (Akter *et al.*, 2006; Khan *et al.*, 2014) and in White Leghorn chicken (Getty, 1975). Histologically, the spleen of the deshi chicken was surrounded by a thick capsule and there was a small number of trabeculi. These analogous results also noticed in Hybrid chicken (Dellmann and Eurell, 1998; Seres *et al.*, 2013). In this study, the red pulps were less distinct and these were scatteredly distributed within the white pulps as noticed in Broiler chicken (Hussan *et al.*, 2009; Khan *et al.*, 2014) and Hybrid chicken (Brisbin *et al.*, 2010). The white pulp composed of network of reticular cells and fibers within which lymphocytes and plasma cells were diffusely distributed. It contained sheathed arteries and lymphatic nodules. These general histological features of spleen in deshi chickens were similar to the previous findings in Native chicken in Iran (Mobini, 2012), in Hybrid chicken (Jeurissen *et al.*, 1992) and in Broiler chicken in Bangladesh (Akter *et al.*, 2006; Khan *et al.*, 2014).

The broad tubular shape cecal tonsil of all age groups in both SDC and CDC were in the proximal one third of the paired tubular cecum, which lies along each side of the large intestine. These observations were strongly agreed with the previous observations in Broiler chicken (Akter *et al.*, 2006; Khan *et al.*, 2014), in Native chicken in Iran (Mobini, 2012), in Hybrid chicken (Brisbin *et al.*, 2010) and in White Leghorn chicken (Getty, 1975). In histological observations, there was four layers in cecal tonsil i.e., tunica mucosa, submucosa, muscularis and serosa which is in agreement with the results reported by Dellmann and Eurell (1998) in Hybrid chicken, Peng *et al.* (2014) and Hussan *et al.* (2009) in Broiler chickens. The lining epithelium of both chickens were simple columnar which concords with the findings of Dellmann and Eurell (1998) in Hybrid chicken, Sultana *et al.* (2012) in Duck of Bangladesh and Gulmez and Aslan (1999) in Geese. The base of the villi was thick but the apex was pointed to rounded in Deshi chicken as observed previously in Native chicken of Bangladesh (Khan *et al.*, 1998) and Native chicken of Iran (Mobini, 2012). More diffuse lymphoid tissue and unorganized lymphatic nodules were also noticed in both the mucosa and submucosa. Similar findings were pointed out previously in Broiler chicken (Akter *et al.*, 2006; Khan *et al.*, 2014).

5.2 Gross morphometry of lymphoid organs

In the present study gross morphometric parameters of bursa (mean weight, height and width) in both scavenging and captive deshi chickens were gradually increased from D_1 to D_{90} and the highest mean values of those parameters (2.04 ± 0.11 gm, 1.48 ± 0.08 cm and 1.04 ± 0.11 cm, respectively) were found at 90 days old CDC. The highest weight, height and width of bursa was 3.0 gm, 3.0 cm and 2.0 cm, respectively at 120-150 days old Hybrid chicken (Getty, 1975), 1.95 gm, 0.6 cm and 0.31cm, respectively at 70-80 days old Broiler chicken (Khenenou *et al.*, 2012) and 3.8 gm, 5.0 cm and 0.7 cm, respectively at 180 days old Duck (Getty, 1975; Sultana *et al.*, 2012). It also found that the mean weight, height and width of bursa were significantly higher in all ages of CDC except day old chicks as compared with SDC. This higher weight, height and width of bursa in CDC may be due to their higher growth rate in intensive farming system as compared with SDC in free range scavenging system. Jain *et al.* (2010) pointed out that the size and shape of bursa depends on the species, breed even farming condition of birds. At day old (D_1) chicks, the bursa in both CDC and SDC were analogous because their body weights were relatively equal in both farming conditions. On the other hand, the mean weight, height and width of bursa decreased at D_{180} in both SDC and CDC which indicates that involution of bursa was found at that age level. Previously reported that involution of bursa was started at D_{140} in Broiler chicken (Khenenou *et al.*, 2012), >150 days in White Leghorn chicken and >180 days in Duck (Getty, 1975). So, the size and involution of bursa in both SDC and CDC may be depending on the variation of species and breed.

The present study reveals that gross morphometric parameters of thymus includes the number of thymic lobes, weight and length in both SDC and CDC were gradually increased with the advancement of age from D_1 to D_{180}. These results were partially differ with the reports of Getty (1975) who reported the number of thymic lobes, weight and length of thymus in White Leghorn chickens increased up to D_{120}. This dissimilarity may be due to their breed difference. In both SDC and CDC, the number of thymic lobes was variable according to the different age group although no difference was found in the same age group. The average mean number of thymic lobes on each side of neck was 4.40 to 6.40 in both SDC and CDC which was partially agreed with some previous

reports where they reported 3-8 on each side of neck in Domestic fowl (Hodges, 1974; King and McLelland, 1984), 6-7 in Guinea fowl (Onyeanusi *et al.*, 1994), 5-9 in Geese (Gulmez and Aslan, 1999). This partial discrepancy is may be due to the variation of species. Contrarily, the mean weight and length of thymus were significantly ($P \leq 0.05$) higher in all age group (except day old chicks) of CDC as compared with SDC. May be the cause of this inconsistency with the advancement of age was their specific rearing environment. Comparatively similar weight and length of thymus was found at D_1 chicks in both SDC and CDC as their body weights were relatively equal in both scavenging and intensive farming conditions.

The mean weight, length and width of spleen and cecal tonsil in both SDC and CDC were also increased from D_1 to D_{180} which were agreed with the study of Getty (1975) who described that the weight, length and width of spleen and cecal tonsil in White Leghorn chicken were also increased with the advancement of age. In present study also found that the mean weight, length and width of spleen and cecal tonsil were significantly ($P \leq 0.05$) higher in all age group (except D_1 chicks) in CDC as compared with SDC. Captive deshi chickens were reared in intensive farming condition where sufficient amount of feed and water has been supplied thus, their growth rates were higher than scavenging deshi chickens. For that reason the mean weight, length and width of spleen and cecal tonsil were higher in CDC than that of SDC. Ciriaco *et al.* (2003) reported that the mean weight, length and width of lymphoid organs of Hybrid chickens depend on the farming condition as well as their weight gain. In case of day old (D_1) chicks in both SDC and CDC, the mean weight, length and width of spleen and cecal tonsils were relatively similar since, their body weights were relatively equal in both scavenging and intensive farming conditions. On the other hand, maximum mean values of weight, length and width of spleen (3.06 ± 0.18 gm, 2.26 ± 0.16 cm and 1.62 ± 0.08 cm, respectively) were found at 180 days old CDC. Previously reported that the highest mean weight, length and width was 3.0-4.5 gm, 2.0-3.0 cm and 1.0-2.0 cm, respectively at 120-150 days old Hybrid chicken (Getty, 1975), 1.974 ± 0.04 gm, 18.93 ± 0.39 mm and 13.45 ± 0.34 mm, respectively at 28 days old Broiler chicken in Bangladesh (Khan *et al.*, 2014) and 0.18 gm, 0.94 cm and 0.68 cm, respectively at 90-120 days old indigenous Duckling in Bangladesh (Sultana *et al.*, 2012). These types of dissimilarity may be due to the different

species, breed and age as reported by Jain *et al.* (2010) who noticed that the color, weight, length and width of lymphoid organs of Hybrid chicken depend on the breed, age, sex as well as their rearing environment.

5.3 Histomorphometry of lymphoid organs

The histomorphometrical observations of bursa of Fabricius in deshi chickens were similar to many other birds stated in literature with little differences. The number of mucosal folds in the present study was varied from 6-12, while the number of mucosal folds has been reported about 12 in Domestic fowl (Hodges, 1974; King and McLelland, 1984), 12-14 in Helmeted Guinea fowl (Onyeanusi *et al.*, 1993), 11-13 in Geese (Gulmez and Aslan, 1999), 8-16 in both CARI Shyama and Vanaraja breeds of poultry (Jain *et al.*, 2010) and 11-13 in Hybrid chicken (Betti *et al.*, 1991). It was also noticed that the mucosal fold of the bursa was covered by pseudostratified columnar epithelium except at the apex which was covered by a simple columnar epithelium. Some authors have reported that the surfaces of mucosal folds were covered by pseudostratified columnar epithelium (Hodges, 1974; Onyeanusi *et al.*, 1994) but the others have reported, they were covered by both simple columnar and pseudostratified columnar epithelium (Jain *et al.*, 2010). Jain *et al.* (2010) have seen that the highest mean value of height of epithelium in CARI Shyama and Vanaraja breeds of poultry were 54.24 ± 1.76 µm, 42.56 ± 2.40 µm, respectively; the height of mucosal folds were 2200± 40.60 µm, 2280 ± 45.00 µm, respectively and the number of follicles per fold were 25.14 ± 0.05, 18.98 ± 2.34, respectively. So, the number of mucosal folds, number of follicles per fold, height of mucosal folds and covering epithelium of bursa may vary within different breed and species. In this study found that the height of epithelium, number of mucosal folds, number of follicles per mucosal folds and height of mucosal folds were increased with the advancement of age from D_1 to D_{90} in both SDC and CDC but all those parameters were decreased at D_{180} in both SDC and CDC. These histomorphometrical results indicating involution of bursa which was found at D_{180} in both SDC and CDC. In contrast, all those histomorphometrical parameters of bursa were higher in all age group in CDC as compared with SDC. The possible causes of these higher results in CDC may be due to their higher growth rate in intensive farming system as compared with SDC in free range scavenging system but other factors may be also involved. Kannan *et al.*

(2012) have seen that the histological structure of lymphoid organs in Hybrid chicken depend on the breed, age, sex as well as their rearing environment. In case of day old (D_1) chicks in both SDC and CDC, the mean values of all histomorphometrical parameters of bursa were relatively similar as their body weights were relatively equal in both scavenging and intensive farming conditions.

The present study showed that the mean length and width of thymic lobules, white pulps of spleen and lymphatic nodules of cecal tonsil in both SDC and CDC were increased with the advancement of age from D_1 to D_{180}. These similar findings were also noticed in Broiler chicken by Akter *et al.* (2006) and Khan *et al.* (2014) and in Aseel chicken by Haseeb *et al.* (2014). On the other hand, the mean length and width of thymic lobules, white pulps of spleen and lymphatic nodules of cecal tonsils were higher in all age group in CDC as compared with SDC. The body weights of CDC were higher as they reared in intensive farming condition with sufficient amount of feed and water. In contrast, the SDC being reared in free range scavenging system where they usually take kitchen waste, seeds and grains, insects, green grasses and all other human refusal in nature as a result their body weight gain were comparatively slower than CDC. So, the length and width of thymic lobules, white pulps of spleen and lymphatic nodules of cecal tonsils were higher in all age group in CDC. Some authors previously have seen that the histological structures of lymphoid organs in Hybrid chicken depend on the breed, age, sex as well as their rearing environment (Jain *et al.*, 2010; Kannan *et al.*, 2012).

Chapter-6: Conclusions

The gross and histomorphometrical parameters of thymus, spleen and cecal tonsils were gradually increased with the advancement of ages from D_1 to D_{180} in both SDC and CDC but the gross and histomorphometrical parameters of bursa of Fabricius were increased up to D_{90} and then these parameters were decreased that indicate involution of bursa was found at D_{180}. All those gross and histomorphometrical parameters of bursa of Fabricius, thymus, spleen and cecal tonsils were higher in all age groups of CDC (except D_1 chicks) as compared with SDC. In contrast, at D_1 chicks, the gross and histomorphometrical parameters of bursa of Fabricius, thymus, spleen and cecal tonsils in both SDC and CDC were more or less similar as their body weight gain were comparatively equal in both scavenging and intensive rearing system. Therefore, we would like to conclude that the higher gross and histomorphometrical parameters of lymphoid organs and tissues in CDC than that of SDC were due to their different pattern of rearing system. This present work showed the comparative gross and histomorphometrical study of the postnatal lymphoid organs and tissues of deshi chicken being reared in scavenging and intensive system in Bangladesh. So, it is expected that this result will contribute as a guideline for further studies on comparative postnatal growth and development of lymphoid organs and tissues of scavenging and captive deshi chickens in Bangladesh.

Chapter-7: Recommendations and Future Perspectives

The present work regarding postnatal growth and development of lymphoid organs and tissues of deshi chickens in Bangladesh rearing in scavenging and intensive farming systems revealed novel data, therefore, the following recommendations are given for future investigations:

1. Immunohistochemical study can be done to determine the immunoglobulin levels of the plasma cells.
2. Avidin-Biotin complex staining method can be done to know the lymphocyte subpopulations with their receptor typing.

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Appendix-I: Questionnaire

Questionnaire for the Postnatal Growth and Development of Lymphoid Organs and Tissues of Deshi Chickens *(Gallus domesticus)* in Bangladesh

1. Sample number: ……..
2. Rearing method of chickens: (i) Scavenging system (ii) Intensive system
3. Age: (i) D_1 (ii) D_{30} (iii) D_{90} (iv) D_{180}
4. Sex: (i) Male (ii) Female
5. Body weight: ……..
6. Gross measurement:

Name of lymphoid organ	Weight (gm)	Length (gm)	Width (gm)	Height (gm)	Number of thymic lobes
Bursa of Fabricius					
Thymus					
Spleen					
Cecal tonsil					

7. Histological measurement:

Bursa of Fabricius			
Height of epithelium (µm)	No. of mucosal fold	No. of follicle per fold	Height of mucosal fold (µm)
Spleen			
Length of white pulp (µm)	Width of white pulp (µm)	Length of red pulp (µm)	Width of red pulp (µm)
Thymus		Cecal tonsil	
Length of thymic lobule (µm)	Width of thymic lobule (µm)	Length of lymphatic nodule (µm)	Width of lymphatic nodule (µm)

Appendix-II: Gross morphometrical data

Bursa of Fabricius							
Age (day)	Sample number	Scavenging deshi chicken			Captive deshi chicken		
		Weight (gm)	Height (cm)	Width (cm)	Weight (gm)	Height (cm)	Width (cm)
D_1	1	0.1	0.4	0.2	0.1	0.4	0.2
	2	0.2	0.6	0.1	0.3	0.6	0.1
	3	0.5	0.5	0.2	0.5	0.5	0.3
	4	0.09	0.3	0.3	0.09	0.3	0.3
	5	0.4	0.4	0.1	0.2	0.6	0.2
D_{30}	1	1.3	1.0	0.5	1.4	1.2	0.8
	2	1.4	0.8	0.5	1.6	0.8	0.6
	3	1.4	0.9	0.6	1.3	1.1	0.8
	4	1.0	1.1	0.6	1.1	1.1	0.7
	5	1.1	0.7	0.4	1.5	0.9	0.7
D_{90}	1	2.0	1.3	0.7	2.0	1.5	1.1
	2	1.8	1.1	0.8	2.2	1.4	1.0
	3	2.0	1.4	0.7	1.9	1.6	1.0
	4	1.9	1.3	0.7	2.1	1.5	1.2
	5	1.7	1.2	0.9	2.0	1.4	0.9
D_{180}	1	0.3	0.4	0.2	0.5	0.9	0.6
	2	0.4	0.3	0.1	0.3	0.7	0.7
	3	0.2	0.5	0.1	0.4	0.8	0.4
	4	0.3	0.4	0.2	0.3	0.8	0.5
	5	0.4	0.3	0.2	0.5	0.6	0.6
Spleen							
Age (day)	Sample number	Scavenging deshi chicken			Captive deshi chicken		
		Weight (gm)	Length (cm)	Width (cm)	Weight (gm)	Length (cm)	Width (cm)
D_1	1	0.1	0.3	0.3	0.2	0.2	0.3
	2	0.4	0.2	0.1	0.4	0.3	0.1
	3	0.3	0.5	0.3	0.3	0.5	0.3
	4	0.6	0.3	0.2	0.6	0.3	0.1
	5	0.4	0.4	0.2	0.4	0.5	0.2
D_{30}	1	1.1	0.9	0.6	1.5	1.2	0.7
	2	1.3	0.7	0.4	1.8	1.3	0.6
	3	0.9	1.1	0.5	1.7	1.1	0.5
	4	1.5	0.9	0.4	1.4	1.2	0.6
	5	1.0	1.0	0.5	1.3	1.3	0.7
D_{90}	1	1.9	1.4	1.1	2.4	1.6	1.3
	2	2.1	1.6	0.9	2.3	1.8	1.2
	3	2.2	1.5	1.0	2.4	1.7	1.3
	4	2.0	1.3	0.9	2.1	1.6	1.1

Continuing……..

	5	1.8	1.4	1.1	2.4	1.4	1.0
D_{180}	1	2.7	2.0	1.5	3.2	2.2	1.7
	2	2.9	2.1	1.4	3.3	2.4	1.5
	3	3.0	2.0	1.5	3.0	2.4	1.6
	4	2.6	1.9	1.3	2.9	2.3	1.6
	5	2.7	1.9	1.4	2.9	2.0	1.7
Cecal Tonsil							
Age (day)	Sample number	Scavenging deshi chicken			Captive deshi chicken		
		Weight (gm)	Length (cm)	Width (cm)	Weight (gm)	Length (cm)	Width (cm)
D_1	1	0.7	0.2	0.1	0.7	0.2	0.1
	2	0.6	0.3	0.2	0.5	0.3	0.2
	3	0.8	0.4	0.1	0.8	0.4	0.1
	4	0.7	0.2	0.2	0.6	0.2	0.1
	5	0.5	0.3	0.2	0.5	0.3	0.2
D_{30}	1	1.3	0.7	0.4	1.4	0.9	0.7
	2	1.1	0.8	0.5	1.5	1.0	0.5
	3	1.3	0.9	0.4	1.3	0.8	0.6
	4	1.1	0.7	0.4	1.4	0.9	0.5
	5	1.0	0.8	0.3	1.5	0.8	0.7
D_{90}	1	1.9	1.2	0.7	2.3	1.4	0.9
	2	2.0	1.3	0.9	2.4	1.3	1.1
	3	2.2	1.4	0.8	2.3	1.5	1.0
	4	2.1	1.3	0.9	2.4	1.4	1.0
	5	2.0	1.2	0.8	2.3	1.2	0.9
D_{180}	1	2.7	1.7	1.2	2.7	1.8	1.2
	2	2.5	1.8	1.3	2.8	1.9	1.4
	3	2.6	1.8	1.2	2.6	1.7	1.3
	4	2.5	1.6	1.1	2.7	1.8	1.2
	5	2.7	1.6	1.1	2.8	1.9	1.4
Thymus							
Age (day)	Sample number	Scavenging deshi chicken			Captive deshi chicken		
		NTL /side	Weight (gm)	Length (cm)	NTL /side	Weight (gm)	Length (cm)
D_1	1	4.0	0.06	1.3	4.0	0.07	1.3
	2	4.0	0.08	1.4	4.0	0.06	1.2
	3	5.0	0.09	1.2	5.0	0.09	1.2
	4	5.0	0.07	1.3	5.0	0.07	1.3
	5	4.0	0.08	1.4	4.0	0.08	1.1
D_{30}	1	5.0	0.1	2.3	5.0	0.3	2.6
	2	5.0	0.3	2.5	5.0	0.4	2.5
	3	6.0	0.2	2.4	6.0	0.2	2.4
	4	6.0	0.3	2.2	6.0	0.3	2.6
	5	5.0	0.2	2.3	5.0	0.5	2.4

Continuing……..

D_{90}	1	6.0	0.5	3.1	6.0	0.8	3.6
	2	7.0	0.9	3.5	7.0	0.9	3.4
	3	7.0	0.7	3.2	7.0	1.0	3.2
	4	6.0	0.8	3.1	6.0	0.9	3.6
	5	6.0	0.6	3.4	6.0	0.9	3.3
D_{180}	1	6.0	1.0	3.7	6.0	1.3	3.8
	2	7.0	1.1	3.8	7.0	1.3	3.8
	3	7.0	0.9	3.7	7.0	1.2	3.7
	4	6.0	0.9	3.6	6.0	1.1	3.9
	5	6.0	1.0	3.6	6.0	1.4	3.7

Appendix-III: Histomorphometrical data

Bursa of Fabricius									
Age (day)	Sample number	Scavenging deshi chicken				Captive deshi chicken			
		HE (µm)	NMF (µm)	NFPMF (µm)	HMF (µm)	HE (µm)	NMF (µm)	NFPMF (µm)	HMF (µm)
D_1	1	30	07	18	610	30	06	18	615
	2	20	08	12	420	25	08	11	432
	3	30	07	16	520	30	07	16	513
	4	35	07	14	460	35	07	14	464
	5	30	06	10	330	30	06	12	334
D_{30}	1	40	09	36	1040	47	10	38	1105
	2	35	08	22	780	51	09	25	807
	3	45	07	30	920	53	08	32	933
	4	50	09	28	805	50	10	33	856
	5	47	06	18	660	57	07	23	698
D_{90}	1	50	09	68	1220	75	11	72	1312
	2	60	10	54	948	72	10	57	1005
	3	65	12	65	1050	65	14	69	1097
	4	55	11	62	1010	68	12	65	1078
	5	57	12	50	857	63	13	54	890
D_{180}	1	40	06	28	710	45	07	29	755
	2	37	08	22	543	42	09	25	587
	3	45	09	26	590	55	10	31	643
	4	43	06	24	534	43	07	26	589
	5	39	07	20	427	50	08	23	468

Thymus					
Age (day)	Sample number	Scavenging deshi chicken		Captive deshi chicken	
		Length of lobules (µm)	Width of lobules (µm)	Length of lobules (µm)	Width of lobules (µm)
D_1	1	230	175	235	175
	2	292	167	290	170
	3	320	143	320	143
	4	208	158	223	156
	5	277	117	277	120
D_{30}	1	550	372	577	397
	2	582	313	598	378
	3	634	240	720	292
	4	395	292	432	321
	5	577	211	589	256
D_{90}	1	853	474	888	498
	2	1004	391	1035	424
	3	1022	812	1046	867
	4	640	387	690	402
	5	886	490	908	505
D_{180}	1	967	489	1022	504
	2	1002	578	958	613
	3	978	467	991	543
	4	1026	645	1003	691
	5	956	654	1032	693

Spleen					
Age (day)	Sample number	Scavenging deshi chicken		Captive deshi chicken	
		Length of white pulp (µm)	Width of white pulp (µm)	Length of white pulp (µm)	Width of white pulp (µm)
D_1	1	65	32	64	32
	2	68	26	68	26
	3	79	29	82	28
	4	63	36	64	37
	5	71	39	70	40
D_{30}	1	182	84	198	93
	2	173	67	189	73
	3	268	73	301	79
	4	190	88	206	96
	5	232	93	253	100
D_{90}	1	230	140	243	145
	2	212	102	223	112
	3	285	132	295	144
	4	320	148	389	156
	5	352	181	403	199
D_{180}	1	335	154	390	189
	2	379	187	410	187
	3	350	147	379	207
	4	367	188	423	178
	5	380	160	397	191

Cecal Tonsil					
Age (day)	Sample number	Scavenging deshi chicken		Captive deshi chicken	
		Length of lymph nodule (µm)	Width of lymph nodule (µm)	Length of lymph nodule (µm)	Width of lymph nodule (µm)
D_1	1	21	12	21	12
	2	28	22	29	23
	3	32	26	31	25
	4	25	18	26	18
	5	27	19	29	19
D_{30}	1	42	31	48	38
	2	55	35	62	46
	3	63	48	70	59
	4	52	42	64	55
	5	55	37	65	49
D_{90}	1	92	66	102	73
	2	148	98	165	108
	3	140	102	156	118
	4	128	88	139	98
	5	139	108	152	122
D_{180}	1	143	169	88	125
	2	165	189	97	103
	3	154	164	106	99
	4	178	183	119	136
	5	181	196	126	139

Brief Biography

DR. Md. Shahriar Hasan Sohel was born on March 27, 1991 in Palashtali village of Tangail district, to Md. Shahjahan Miah, a businessman, and Hasina Begam. He is the youngest of five children. He passed the Secondary School Certificate (SSC) examination as a student of Nagbari Hasina Chowdhury High School, Tangail in 2006. He completed Higher Secondary Certificate (HSC) from Major General Mahmudul Hasan Adarsha College, Tangail in 2008. Then he enrolled at Chittagong Veterinary and Animal Sciences University (CVASU) in Doctors of Veterinary Medicine (DVM) and received DVM degree with CGPA 3.78 (in a scale of 4.00). Now, he is a candidate for the degree of MS in Anatomy under the Department of Anatomy and Histology, Faculty of Veterinary Medicine, CVASU. He has published five research articles in international journals. His favorite hobby is reading Al-Quran, books and research articles. He has immense interest to work in Immunohistochemistry and Molecular biology of domestic animal.