Age Related Observations on Gross and Microscopic Changes of Major Lymphatic Organs of Commercial Broiler Chicken

A Thesis

By

DR. MD. SADEQUL ISLAM, DVM

Examination Roll No. 0905095

Semester: March – August, 2010

Registration No. 0905095

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Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200

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Approved as to style and contents by

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Supervisor	**Co-supervisor**

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Examination Committee

August, 2010


DEDICATED TO MY
BELOVED PARENTS

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I wish to acknowledge my debt to my mother for her constant help throughout the period of my study.

Author

LIST OF ABBREVIATIONS AND SYMBOLS

&	and
ANOVA	Analysis of Variance
Cm	centimeter
D	Day
DLS	Department of Livestock services
et al.	and other
FAO	Food and Agricultural Organization
gm	gram
H & E	Hematoxylin and Eosin
i. e.	that is
Mm	millimeter
µm	micrometer

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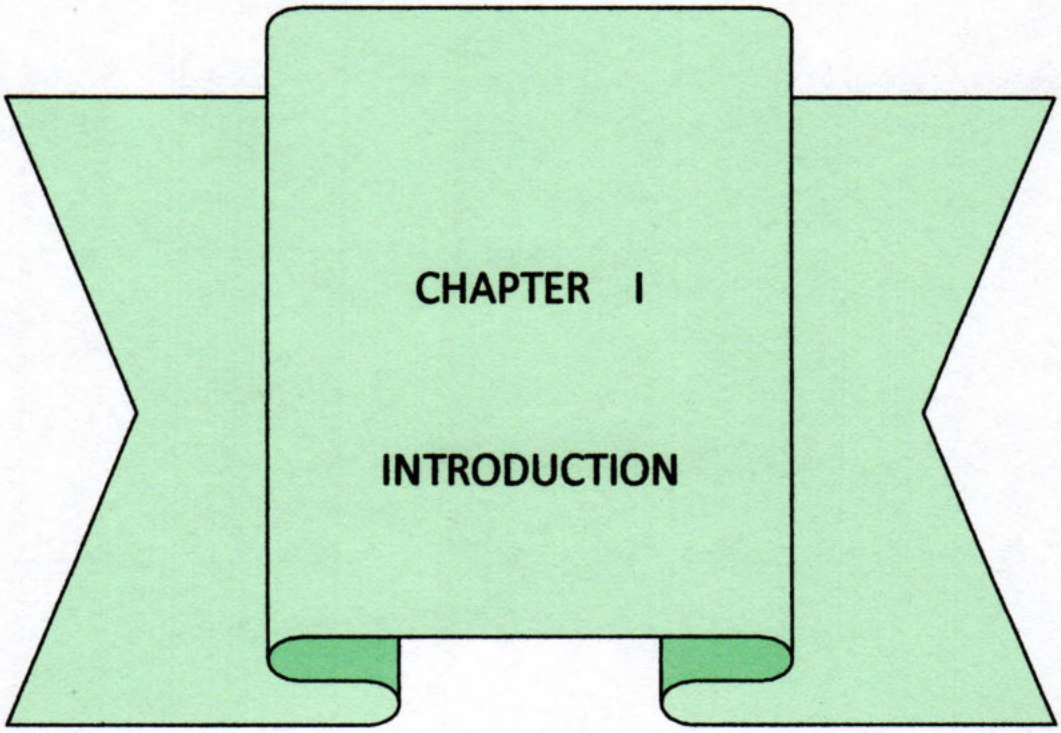
ABSTRACT

The research was designed to study the age related changes of the gross anatomical and histological structures of lymphoid organs of broiler chicken of Bangladesh. The study was conducted in the Department of Anatomy and Histology, HSTU, Dinajpur. The broiler chickens of cobb-500 were grouped into six age groups D_1, D_7, D_{14}, D_{21}, D_{28} and D_{35}. Each age group was consisting of five chickens. Chickens from each groups were killed with cervical subluxation and body weight of each chicken were recorded for the calculation of the relative weight of the lymphoid organs. For the gross study, various parameters including color, weight, length, diameter and number of the lymphoid organs were taken. Routine hematoxylin and eosin (H & E) stain was used to study the histology of the lymphoid organs.

The thymus of the broiler chicken at the D_1 period the lobules were demarcated into cortex and medulla, which becomes larger and well demarcated at next period of life. In the study, Hassall's corpuscles were found during D_1 stage which increased in diameter and number at D_{35} stage of broiler chicken. The capsule and trabeculae becomes thicker with growth and development of the thymus. The single bursa of Fabricius was a dorso-median diverticulum of the proctodeum. Histologically, the bursa of D_1 stage was filled up by plicae and devoid of mucoid substances. The plicae contained few bursal follicles of different sizes. But with increasing age the bursa became larger with the plicae getting taller and thicker, which contained enormous number of large polyhedral primary and secondary lymphoid follicles. The spleen of broiler chicken was variable in size and shape but usually rounded. The color was reddish-brown to pinkish-brown at different period. From D_1 period, spleen was surrounded by connective tissue capsule which became gradually thicker with subsequent growth and development of the chicken. Splenic parenchyma became more distinct with increasing age. Cecal tonsils had four layers, which could be differentiated from D_1. They became more prominent with the growth and development of the chicken. In D_1 the mucosa had few small folds which contains small amount of diffuse lymphatic tissues. But the folds subsequently became larger and broader with large amount of diffuse and nodular lymphoid tissues. Both primary and secondary lymphoid follicles were present from D_1 to D_{35}. The lymphatic nodules were more in the mucosal layer of the cecum.


CHAPTER I
INTRODUCTION

CHAPTER I

INTRODUCTION

The living beings usually manage not only to survive but indeed thrive in potentially hostile milieu, without seeming effort. This freedom from disease is dependent on the existence of a complex and highly sophisticated defense system, called lymphoid system (Cortan, 1989). In Bangladesh chickens are playing a significant role in country economy and reducing poverty by supplying meat, egg and other by-products. However, these birds are affecting seriously by lymphomas and lymphocarcinomas e.g. lymphoid leukosis (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. The human being has also been suffering from different types of crcinomas.

The lymphoid tissues have an independent phylogenetic origin, their function being to react to foreign antigens by producing antibodies, thereby, providing adaptive "Immunity". Adaptive immunity is evidently a very ancient characteristic of vertebrates, first appearing in cyclostomes. It supplements the various non-specific mechanisms of resistance including bactericidal enzymes, interferon and phagocytosis; these constitute an "innate immunity" (King, 1975).The mechanisms of adaptive immunity in birds have two components: one is the bursa of Fabricius with germinal centers and plasma cells in various tissues that are strongly interlinking with humoral immunity. The other is the thymus-dependent system which is the thymus and scattered collections of lymphocytes that are related to cellular immunity.

Furthermore, lymphoid tissue can be divided into "central" and "peripheral" tissues. The former are believed to be primary sites of development of lymphocytes. In birds these are the thymus and bursa as opposed to the thymus alone in mammals. The peripheral or secondary lymphoid tissues apparently depend on the central or primary lymphoid tissue for their origin, development and function. In birds they include lymphoid tissue in the spleen and in the alimentary tract including the cecal tonsils (Getty, 1975; Bach, 1978).

The obvious characteristics of the lymphatic tissues of mammals and birds are densely populated with the lymphocytes. So they are involved with lymphocyte production, immune responses or both of these processes occurring at the same time (Cormack, 1987).The bursa and thymus resemble one another with respect to their epithelial derivation, lymphoid nature, growth during embryonic and early postnatal life and early involution. Due to the fact that these organs begin to involutes before the development of sexual maturity, the account of their structure will be that found in the young chick from hatching to old age (Hodges, 1974).

However, both in birds and animals and even in human beings, lymphomas are not treating in the right way due to lack of proper diagnosis and cost management. Moreover in chemotherapy treated birds, animals and humans, the lymphocytic changes in the major lymphoid organ and lymphoid tissue were not clearly understood.The chicken is an important model for research into the basic features of immunology since its immune system function in a similar way to that of humans and the chicken embryo allows easy experimental access (Battandier, 1980; Vaino, 1995).If there are diseases in the lymphoid organs, irreversible changes are produced in the physiology of the chicken body. So evaluation of the functional state of the lymphoid organ is of immense practical importance, for results of such studies may establish the cause of diseases and determine which type of therapy should be employed.

The present study reveals the microscopic aspect of the lymphoid tissues and in addition attempts were made to bridge the gap between the gross and microscopic anatomy of the lymphoid organs of broiler chickens. The description of the lymphoid organs of chickens had been previously made but the study on the broiler chickens are inadequate and does not contain the particular data of the present study. The great importance of these data is bearing on the functional significance of lymphoid organs of the broiler chicken. It is hoped that the present investigation will be a base line for the study of lymphoid organs of these birds, and also will provide valuable information to poultry immunologist, pathologist, researchers, cell biologist and anatomist.

So the present study investigate the histology of lymphoid organs of broiler chicken and that will add to the knowledge in this field.

Objectives:

1. To examine the initial appearance, distributions and gross developmental changes of the lymphoid tissues (thymus, bursa of Fabricius, spleen and cecal tonsil) of broiler chicken during various period of life.

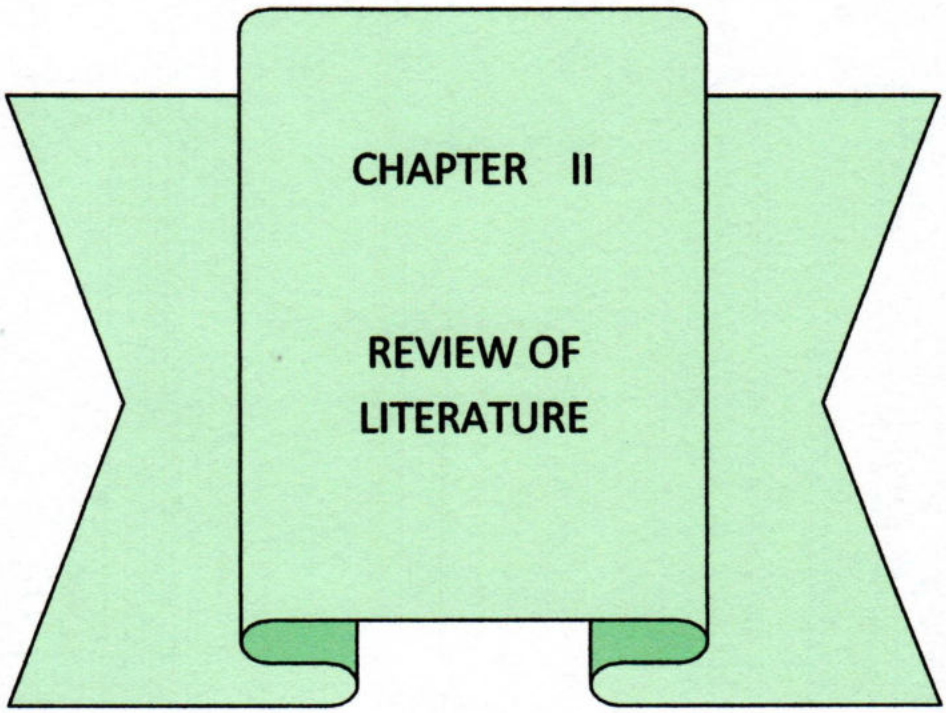
This study also includes

a) anatomical position of lymphoid organs,

b) topographical relations with adjoining structures,

c) length , diameter and weight of these organs, and

2. To study the histological structure of the different components of the lymphoid organs during different stages of their development.


CHAPTER II
REVIEW OF
LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the previous research conducted so far in relation to the present study, the number of works directly related to the present study were scanty. Detailed anatomical study of the lymphoid organs of birds had been studied previously by (Bradley 1960; Cooper, 1966; Payne, 1971; Hodges, 1974; King, 1975 ;)

2.1 Gross anatomy of thymus

King (1975) reported that the thymus is an organ of fetal and early baby chicken hood. It is an important lymphoid organ responsible for the mechanisms rejecting foreign tissues. With growth of the bird it soon undergoes retrogressive changes into fat and connective tissue. Getty (1975) observed that, the appearance of the thymus varies considerably with age. The paired thymus of the chicken consists of a series of separate pale red or yellowish irregular lobes. In the adult there were three or eight such lobes, of varying size and shape, extending along each jugular vein as far as the thyroid gland. Thymic tissue sometimes penetrates the thyroid and parathyroid gland. Upper fifth of the neck of chicken was devoid of lobes. In white Rock males the maximum total weight (the left and right thymus together) of 15.76 gm was reached at 17 weeks of age. Involution was then progressive, so that in males at 19 months of age it is about 2.2 gm and in females of similar ages about 0.6 gm, but remnants of all the lobes, including the most cranial ones, persists in birds at least up to 16 months of age.

2.2 Histology of thymus

Hodges (1974) stated that the chicken thymus was enclosed by a connective tissue capsule. This was comparatively thin and is composed mainly of course collagen fibers together with a few, fine elastic fibers. External to the capsule are considerable amount of loose connective tissue and adipose tissue. Passing inwards from the capsule were numerous fine septa which divide the mass of the gland into lobules. Branching out from the main septa were normally numerous small, short septules into segments.

Hodges (1974) reported that the lobules were divided into, indistinct cortex and the medulla. The underlying stroma of both cortex and medulla consists of a network of reticular cells and their fibers. These cells possess a round, oval or elongated nucleus with a few fine chromatin granules and one or two nucleoli. In the cortex there were packed large numbers of lymphocytes, mainly small lymphocytes, although medium-sized lymphocytes can also be seen. In normal 2-month-old chickens the cortical lymphocyte population fairly uniform in consistency. The cells being about 3.5-4.0 micron in diameter.

Bach (1978) reported that, subcapsular cortex of the thymus of white leghorn chicken consisting of large lymphocytes which were less numerous, however, in this zone, the stem cells penetrate and divide, explaining the high incidence of mitosis. He further reported that, the deep cortex stores small lymphocytes. A large number of cortical lymphocytes also die in the deep cortex.

Bach (1978) further observed in his study, that, the medulla contains few lymphocytes with pale cytoplasm and Hassall's corpuscles. Hassall's corpuscles consist of epithelial cell piled and coiled on top of one another. Their size is variable, and they may be cystic, with a central eosinophilic substance plus pyknotic cells. The cells are linked together by desmosomes and show cytoplasmic tonofilaments The functional significance of Hassall's corpuscles in chicken remains obscure They could play a role in transferring the humoral products of thymus to the circulation.

The blood supply with in the medulla is more abundant than that in the cortex. It consists of a well-developed network of arterioles, capillaries and small veins. Other cells which can be seen within the medulla of chicken thymus were eosinophils, haemocytoblasts and plasma cells. The germinal centers are normally not found, however, present in occasional cases in the cortex at 14 weeks old birds (Greenwood,1930; Bradly, 1960; Ham,1961;Hodges,1974;Copenhaver,1975; Dellmom,1976;Leeson,1976 ;Cormack, 1987, Arey, 1994; Khan 1996, Junqueira, 1998).

2.3 Gross anatomy of the bursa of Fabricius (Cloacal bursa)

The bursa of Fabricius was an asymmetric medial, lymphoepithelial organ which was peculiar to birds. King (1975) reported that it was the second primary lymphoid organ, after the thymus in birds. It was a blind, round to oval, sac like, dorsal diverticulum of the proctodeal wall of the cloaca. The central lumen of the organ was to a great extent obscured by the presence of about 12 plicae, long folds of the mucous membrane of the bursa) wall, which resemble villous projections.

Bach (1978) reported that it is primary lymphoid organ whose development was independent of exogenous antigenic stimulation. It was the second lymphoid organ, after the thymus, to appear in birds. It developed from a proliferation of the endodermal epithelium of the dorsal caudal region of the embryonic cloaca at about fifth days of incubation. By the tenth day of incubation the bursa has developed a lumen and formation of the plicae begins. The lumen was lined with a layer of cuboidal to columnar cells about three cells thick. About the twelfth day of incubation the primitive undifferentiated epithelial cells lining the lumen gave rise to points of epithelial proliferation.

Hodges (1974) reported that, at the time of hatching the bursa of the white leghorn chicken was well developed and was growing rapidly. On the day of hatch it weighs about 0.04gm. There after growth was rapid, the weight increasing much faster, in proportion, than that of the body weight until the fourth week. At this point it was about 0.42% of the body weight and from then on the rate of growth begins to fall slowly until at 10 weeks. When bursa was about 0.3%

of the body weight, being about 4.25 gm. After 10 weeks there was a decline in bursa weight as the organ begins to involutes by 23 weeks of age the bursa was reduced to a remnant. The actual time of involution varies according to strains and although it was originally thought to occur at about 4 months, recent figures give a time of around 2-3 months. Bursa) regression was related to increases in testosterone or estrogen levels at puberty. The bursa of Fabricius plays a central role in the chicken B cell development.

The bursa was colonized between days 8 and 14 of embryonic development by precursor cells which migrate across the epithelial basement membrane form the epithelial buds which ultimately develop into the lymphoid follicles of the bursa (Cooper, 1965; Glick, 1956, 1977; Befus, 1980; Michael, 1994).

2.4 Histology of The bursa of Fabricius (Cloacal bursa)

Bach (1978) reported that, the mucosa, the musculosa, and the serosa of the bursa are in continuity with the corresponding tissues of the intestine. The epithelial surface, like that of the intestine, consists of cylindrical cells, but the bursa has no mucous cells. lymphoepithelial nodules are present in the lamina propria directly under the epithelium . These nodules contain a light medulla and a dark cortex. The medulla contains epithelial cells that form a continuous area in the periphery, which projects into the epithelial coating. The center of the meddulla was less structured; it contains in addition epithelial cells and various other types of cells, including large lymphocytes, plasma cells, reticular cells, macrophages and granulocytes. Cortex is seperated from the medulla by basement membrane. This membrane permits exchange between the two zones of the lymphoepithelial nodule. The cortex consists mostly of small lymphocytes and plasma cells (Ackerman, 1959; Bradly, 1960; Papermaster, 1962; Gilmore, 1977; Bach, 1978; Odend'hal, 1979; Battandier, 1980; Dolfi, 1988; Michael, 1994; Paramithiotis, 1994 and Khan, 1996).

2.5 Gross anatomy of the Spleen

Hodges (1974) observed in White leghorn chicken, that, the spleen were soft, friable, highly vascular, rounded and reddish-brown organ which lies close to right side of the junction between the proventriculus and the gizzard. He further reported that, the spleen was rather variable in size and shape but may be as much as 2 cm in diameter in the adult chicken. Small accessory spleens have been reported as occurring frequently in fowls. At 10 weeks of age the spleen weighed 2.65 gm or 0.2% of the body weight, there after its weight tended to fluctuate. The spleen is at its maximum size at 3-4 month of age of white leg horn. The size and weight of the spleen vary at different ages in different breed and in the same breed under different conditions.

The spleens are briefly erythropoietic in the embryo and are lymphopoietic through out adult life. It facilitates immune responses to antigens that have gained access to the circulating blood and to dispose of defective blood cells. (King, 1975; Cormack, 1987 ;).

2.6 Histology of the Spleen

King (1975) stated that the spleen was enclosed by a capsule, comprising collagen fibers, elastic fibers, a few muscle fibers and fibroblasts. He also observed that, the internal architecture of the spleen in general resembles that of the mammal. About 85 percent of the substance was equally divided into the red and white pulps. The connective tissue trabeculae were relatively indistinct. The splenic tissue consists of a network of reticular cells and reticular fibers. Superimposed upon this frame work were the two types of splenic tissue, the white pulp and the red pulp. The dominant cell type of the whole spleen was the small to medium lymphocyte. The chicken spleen does not have a marginal zone with marginal zone macrophages and marginal metallophil (Bradly, 1960; Hodges,1974; Jeurissen, *et al.*,1992;).

The red pulp was a loose spongy tissue composed of ramifying cellular cords surrounded by venous sinuses. The cell cords between the sinuses were composed basically of reticular cells, lymphocytes, macrophages and all types of circulating blood cells. Plasma cells may be seen occasionally in the normal spleen but tend to become more abundant in experimental and infective conditions (Hodges, 1974).

Bach (1976) reported, the red pulp plays a role in the capture of antigens that were initially bound to macrophages in the marginal zone and the rest of the red pulp.

The white pulp

Payne (1971) reported that, the white pulp consists of two types of tissue, diffuse lymphoid tissue enveloping the central arteries and their branches, and germinal centers near the central arteries. The diffuse lymphoid tissue of the white pulp was evidently thymus dependent, where as the germinal centers and the plasma cells around them were bursa dependent (Dransfield,1945; Stiles, 1956; Hoshi, 1972;Dellmom, 1976; Ogata, 1981; Fukuta, 1987; Nicader, 1993 Junqueira, 1998).

2.7 Gross anatomy of the Cecal tonsil

Lymphoid foci occur irregularly in the lamina propria or submucosa of the alimentary tract and they are called tonsils. The majority of mucosa-associated lymphoid tissues (MALT), develop in various ways and at various rates. Cecal tonsils were always the most immunologically mature lymphoid organ. In birds, the ceca commonly arise bilaterally at the junction of small and large intestines.

King (1975) the ceca of the white leghorn chicken average 16 - 18 cm in length. They were large in calibre toward the blind extremity and are constricted near their origin. The parietal coats are continued from the small intestine. The cecum extend towards the liver and later were doubled on themselves. The mesentery runs between the ceca and passes on the ileum. King(1975) described about lymphoid foci situated irregularly in the lamina propria or submucosa of the alimentry tract and in some regions these have been called tonsils. The most prominent of these are the two "Cecal tonsils", each of which is an enlargement on the medial wall of the cecum near the ileocecocolic junction.

Mclelland (1989) stated that the ceca may be classified according to length into long, intermediate, short and vestigial categories. As in most birds, the chicken cecum was long and simple.King 1975 reported that the galliform caeca were generally subdivided into a proximal light red portion or base, a middle portion, the body which is bluish green to gray green, is wider and the wall is thinner and the blind tip or apex with fairly thick walls of light red colour (Cormack 1987, Mclelland, 1989).

King (1975); and McLoed (1939) reported that both the body and apex occupy a major part of the cecum, preserving a unique environment which continuously regulates the proliferation of specific microflora and to prevent the infection by foreign organisms. These intestinal lymphoid tissues appear to be dependent partly on the thymus and partly on the bursa. The cecal tonsils are an important source of antibody, besides sharing the general immune responses, the cecal tonsils and other intestinal lymphoid foci evidently have a local immunological function in relation to bacteria and other antigenic substances in the gut.

2.8 Histology of the Cecal tonsil

Hodges (1974); Del Cacho *et al.* (1993) stated that the gut-associated lymphoid tissue (GALT) must be richly populated at the cecal mucosa. However, no detailed distribution of lymphoid nodules has been reported in the mucosa of the body and apex, whereas well developed accumulated lymphoid nodules, termed the cecal tonsil, and distal lymphoid nodules have both been reported in the base. Each consists of a diffuse mass of dense lymphoid tissue in which small lymphocytes predominate and many plasma cells are present together with numerous large circumscribed germinal centers. In the mucosa, the linning epithelium is composed of columnar and goblet cells. Both paneth's cells and Russell's bodies were plentiful. The lamina propria is formed by the connective tissue fibres. The mucose forms folds and crypts. Between the folds, glandular crypts dip into the corium. The submucosa was separated from the mucosa by the muscularis mucosa, which was thin at the cecal apex. The muscular coats were an outer longitudinal, 2 middle circular and an in inner longitudinal. The apex shows a great thickening of all three layers of muscle.

Elastic tissue was confined to the tunica media of the blood vessels. The argentophil reticulum was as profuse as in any other part of the digestive tract. The serous coat has many nerve plexuse (Looper, 1929; Dransfield, 1945; Hoshi, 1973; Hodges, 1974; Burns, 1982; Jeurissen,1989;Calhoun, 1993; Del Cacho, 1993; Kitagawa, 1996;).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Animal: Broiler Chicken (diseases free live bird).

3.2 Experimental area and research period:

The broiler chicken were collected from five selected broiler farm where vaccination and other managemental program were performed properly in Dinajpur sadar thana near the University Hajee Mohammed Danesh Science and Technology, Dinajpur-5200, Bangladesh skillfully from February to July 2010.

3.3 Collection of diseases free live bird and sacrificed for gross and microscopic study:

The broiler chickens of cobb-500 were grouped into six age groups D_1, D_7, D_{14}, D_{21}, D_{28} and D_{35}. Each age group was consisting of five chickens. Chickens from each group were killed with cervical subluxation. Food and water was with held two hours before killing and body weight of each chicken were recorded for the calculation of the relative weight of the lymphoid organs.

Just after killing of the chickens- the thymus, bursa of Fabricius, spleen and cecal tonsil were collected both for gross and histological studies.

3.4 Gross anatomical Study:

The gross study included the color, length, weight, diameter and thickness of lymphoid organs. Relative weights of the lymphoid organs were also calculated (Tables 1-4) adopting following formula according to Federova (1987).

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

.

3.5 Microscopic Study:

For the purpose of histological and histochemical studies the tissues of different lymphoid organs (thymus, spleen, bursa of Fabricius and cecal tonsil) were collected and fixed in the 10 % formalin for few days. 10 % formalin was prepared as follows-

37 % formaldehyde	10 ml
Distilled water	90 ml

Then tissues were taken from the formalin and 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 clean 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.

Hematoxylin and Eosin (H & E) Stain

Preparation of Harris hematoxylin solution:

Hematoxylin crystal	5 gm
Alcohol, absolute	50 cc
Potassium alum	100 gm
Distilled water	1000 cc
Mercuric oxide (red)	2.5 gm

Hematoxylin was dissolved in alcohol, the alum in water by the aid of heat. After mixing the solutions it was brought to heat. After removal from heat, the mercuric oxide was added slowly. Reheated and was removed immediately and the vessel was plunged into basin of cold water until cool. 4cc glacial acetic acid per 100cc of solution was added. The solution was filtrated before use.

Preparation of counter stain for hematoxylin stain

Stock 1% aqueous eosin solution:	
Eosin Y, water soluble	10 g
Distilled water	1000 cc
Glacial acetic acid	2 cc
Working eosin solution:	
Eosin, 1% stock solution	1 part
Alcohol, 80 %	3 part

Staining procedure

Xylene-1	2 minutes
Xylene-2	2 minutes
Absolute alcohol-1	2 minutes
Absolute alcohol-2	2 minutes
70 % alcohol	2 minutes
Running water	2 minutes
Hematoxylin	5 minutes
Running water	15 minutes
Distilled water	5 minutes
Eosin	1 minute
70 Alcohol	5 seconds
80 % alcohol	2 minutes
90 % alcohol	2 minutes
95 % alcohol	2 minutes

Absolute alcohol-1	2 minutes
Absolute alcohol-2	2 minutes
Xylene-1	2 minutes
Xylene-2	2 minutes
Mounting in Canada balsam	

The sections were stained in order to study:-

i. The structure of thymus, bursa of Fabricius, spleen and cecal tonsil.
ii. Histological development during various period of life.
iii. To study the distribution of lymphocytes.

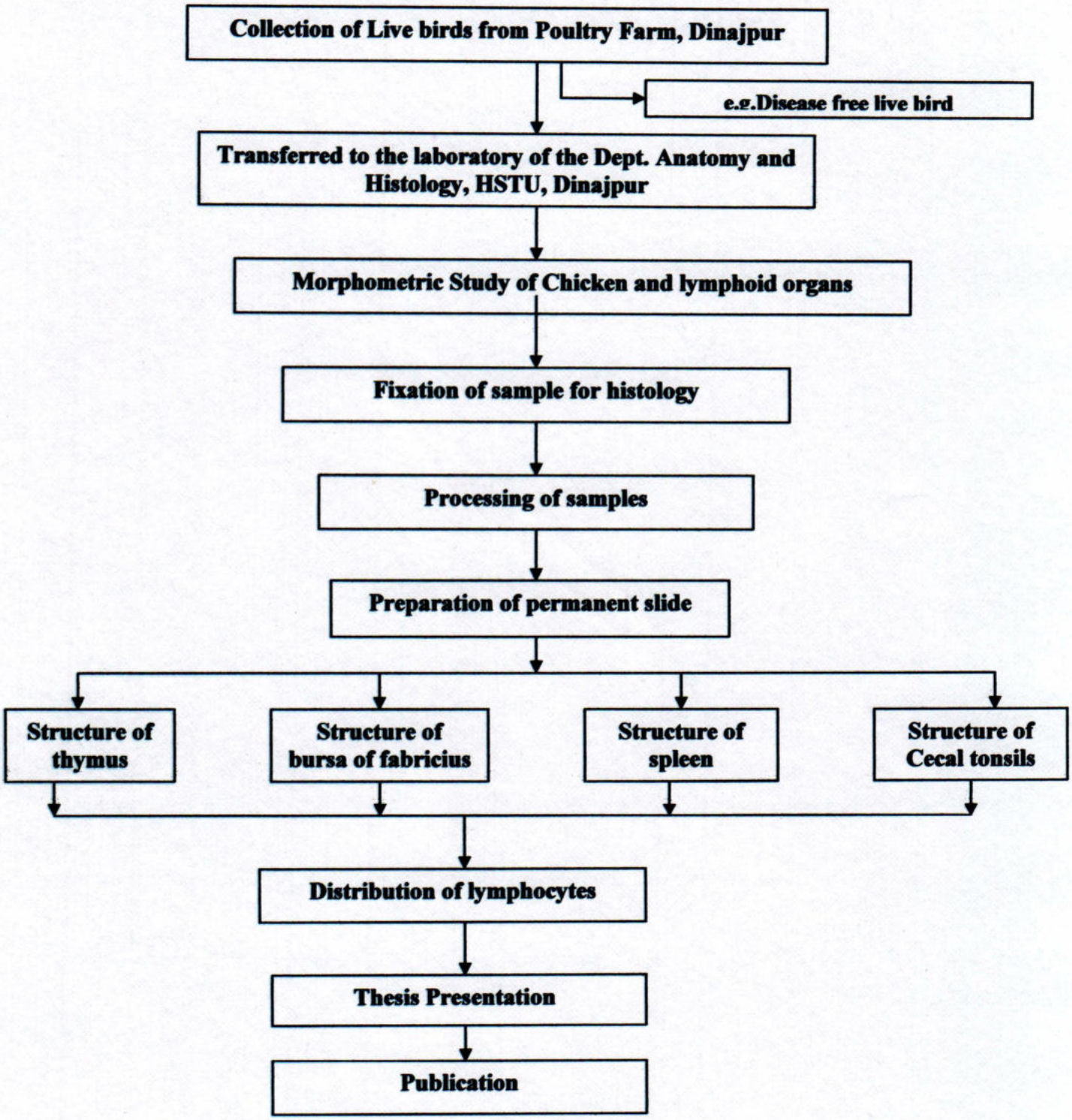
3.6 Statistical analysis:

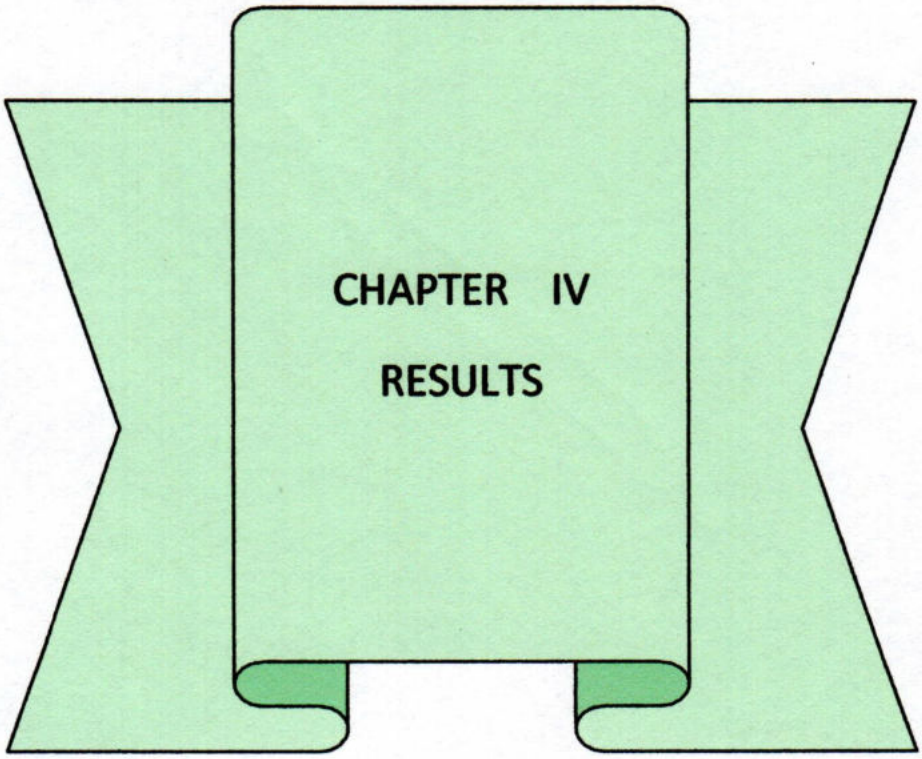
The raw data were decoded, entered and sorted using the MS Excel from various parameters in present study was subjected to statistical analysis. The data were calculated by one factor randomized complete block design using analysis of variance table. Initially the data were sorted and cross cheeked for duplication and/or missing value. The missing values for each variable were recorded (numeric) as to be excluded in the analysis.

3.7 Photography:

The microphotographs made from the selected areas of tissues (with the help of computer connected with microscope) under 40x and 10x microscopic objectives were placed in this thesis for better illustration of the result.

3.8 Experimental Design




CHAPTER IV
RESULTS

CHAPTER IV

RESULTS

4.1 GROSS ANATOMY OF THE LYMPHOID ORGANS OF BROILER CHICKEN

4.1.1 Thymus

The thymus of the broiler chicken was a paired lobulated gland, one half of which was located on either side of the neck. Each half consist of six to eight, flattened ovoid pale white to yellowish white lobes of varying size and shape of lymphoid tissue lying in the subdermal connective tissue of the neck (Fig.1). The cranial end of each string of lobes was found at the level of the third cervical vertebra and extends caudally to about the region of the thyroid gland within the thoracic cavity, so that upper one fifth of the neck was devoid of the lobes. The gland was closely associated with the jugular vein and the vagus nerve. The lobes of the thymus were surrounded by considerable amounts of loose connective tissue and adipose tissue. The growth of the thymus was observed in the present study along with the various ages of the chickens. The length of the neck occupied by the thymus, it's weight and diameter was given at Table -1.

The length of the neck occupied by the thymus of the chicken at day-1 (D_1) was measured 25.63 ± 0.26 mm and at D_{35} it was 64.84 ± 0.20 mm (Table-1). Their growth was found to be greater at D_{35} (Fig. 4). It was observed that the difference between each stage from D_1 to D_{35} were statistically significant ($P < 0.01$) (ANOVA - 1).

The diameter of the lobes of the chicken's thymus at D1 was found to be 3.61± 0.09 mm and at D_{35} it was 8.12 ± 0.03 mm (Table-1). The thymic diameter was observed to be maximum at D_{35} of the chicken (Fig.5). It was observed that the difference of diameter of thymus between each stage from D_1 to D_{35} were statistically significant ($P< 0.01$) (ANOVA - 2).

Similarly, the weight of the thymus was 0.31 ± 0.01 gm at D_1 and at D_{35} was 6.17 ± 0.03 gm (Table-1). The growth rate was maximum at D35 (Fig. 6). It was observed that the difference of weight of thymus between each stage from D_1 to D_{35} were statistically significant ($P< 0.01$) (ANOVA -3).

4.1.2 Bursa of Fabricius

The bursa of Fabricius (Fig. 2) of broiler chicken is a single lympho-epithelial organ which is peculiar to birds. The organ appears as a dorsal median diverticulum of the proctodeum, being smooth and globular in shape and yellowish white in color. When fully developed it consists of a wall surrounding a small, axial, main cavity. The main cavity gives off small diverticula, and also leads into the cloaca through a small median opening in the dorsal wall of the proctodeum. The central lumen of the organ is to a great extent obscured by the presence of about 12 plicae, long folds of the mucous membrane of the bursal wall, which resemble villous projections. The length of the bursa of Fabricius, its maximum diameter and weight was given at Table -2.

The length of the bursa of broiler chicken at D_1 was measured 4.04 ± 0.01 mm and at D_{35} it was 16.90 ± 0.20 mm (Table -2). There growth was found to be greater at D_{35} (Fig. 4). It was observed that the difference of length of bursa between each stage from D_1 to D_{35} were statistically significant ($P<0.01$) (ANOVA -4).

The maximum diameter of the bursa of Fabricius of the broiler chicken at D_{35} it was 12.27 ± 0.21 mm and at D_1 was found to be 2.71 ± 0.02 mm (Table - 2). The growth rate of the bursal diameter was observed to be maximum at D_{35} of the growth and development (Fig. 5). It was observed that the difference of diameter of bursa between each stage from D_1 to D35 were statistically significant ($P<0.01$) (ANOVA -5).

Similarly, the weight of the bursa was 0.02 ± 0.001 gm at D_1 and at D_{35} 1.53 ± 0.02 gm (Table-2). The growth rate of bursa was maximum at D_{35} (Fig. 6). It was observed that the difference of weight of bursa between each stage from D_1 to D_{35} stages were statistically significant ($P< 0.01$) (ANOVA -6).

4.1.3 Spleen

The spleen of broiler chicken was a lymphoid organ with variable in size and shape and usually rounded, reddish - brown to pinkish brown in color. It lies close to the right side of the junction between the proventriculus and the gizzard (Fig. 2). The diameter and weight of the spleen of broiler chicken was given at Table - 3. The diameter of the spleen of the chicken embryo at D_1 was measured 2.67 ± 0.04 mm and D_{35} it was 10.38 ± 0.02 mm (Table -3). There growth was found to be greater at D_{35} (Fig. 5). It was observed that the difference of diameter of spleen between each stage from D_1 to D_{35} were statistically significant ($P< 0.01$) (ANOVA - 7).

In the same way, the weight of the spleen was 0.01 ± 0.000 gm at D_1 and at D_{35} was 1.44 ± 0.00 gm (Table - 3). The growth rate became maximum from D_1 to D_{35} (Fig. 6). It was observed that the difference of weight of spleen between each stage from D_1 to D_{35} were statistically significant ($P<0.01$) (ANOVA - 8).

4.1.4 Cecal Tonsil

The cecal tonsils of broiler chicken arose bilateraly at the junction of small and large intestines (Fig. 2, 3). The cecal tonsils consists of mucosa associated lymphoid tissue (MALT) found at the proximal part or base of the two cecum. It was two tubular structures, yellowish white in color and its surface was smooth due to the covering of peritoneum. At the junction between proximal and middle part of the cecum there was an angular bending which indicates the distal end of the cecal tonsils. In broiler chicken they are long and simple. The length of the cecal tonsil, its diameter and weight was given at Table-4.

The length of the cecal tonsil of the chicken at D_1 to be measured 13.99 ± 0.19 mm and at D_{35} it was 95.44 ± 0.39 mm (Table -4). There growth was found to be greater at D_{35}. It was observed that the difference between each stage from D_1 to D_{35} were statistically significant ($p < 0.01$) (ANOVA - 9).

The.diameter of the cecal tonsil at D_1 was found to be 2.19 ± 0.02 mm and at D_{35} it was 5.32 ± 0.01mm (Table-4). The cecal tonsil diameter was observed to be maximum at D_{35} of the growth and development of broiler chicken. It was observed that the difference of diameter of cecal tonsil between each stage from D_1 to D_{35} were statistically significant ($P< 0.01$) (ANOVA - 10).

Similarly, the weight of the cecal tonsil was 0.030 ± 0.00 gm at D_1 and at D_{35} was 1.43 ± 0.01 gm (Table-4). The growth rate was maximum at D_{35} (Fig. 6). It was observed that the difference of weight of cecal tonsil between each stage from D_1 to D_{35} were statistically significant ($P< 0.01$) (ANOVA -11).

4.2 HISTOLOGICAL STUDIES OF THE LYMPHOID ORGANS OF BROILER CHICKEN

4.2.1 Thymus

D_1

The fibrous capsule of the thymus at birth of the broiler chickens were well demarcated and connective tissue septum were very thin and divides the gland into lobes and lobules (Fig. 7a).

Each lobule consists of an external dark staining dense and highly cellular cortex and an internal light staining less dense medulla. Inside the pale staining area of the medulla paler rounded areas were found which was considered to be a diffuse form of a Hassall's corpuscle (Fig. 8a). Hassall's corpuscles consisted of diffuse groups of reticular cells and scattered vesicles.

D_{21}

Properly developed thymuses were found. The capsule was very thin from which septum arises and divides the thymus into lobules. Each lobule has cortex and medulla. Inside the medulla pale staining diffuse Hassall's corpuscles where present, which were Characteristics of the region (Fig.8b).

D_{28}

All the components of the thymus were well developed at this stage of growth and development. The capsule was very thin from which septums arise and divides the thymus into lobules (Fig. 7b). Each lobule has cortex and medulla. The cortex takes more stain and medulla takes lighter stain. Some of the medulla almost occupies whole of the lobules except a small margin of cortex. Inside the medulla pale staining diffuse Hassall's corpuscles were present, which were characteristic of this region.

Using high power objective, presence of cords of lymphocytes were observed both in cortex and medulla. However the numbers of lymphocytes were more in the cortex. The cortex was composed of an extensive population of lymphocytes, dispersed epithelial reticular cells and few macrophages. The medulla has less cell population (Fig.7b). Nucleons of the lymphocytes were round or oval and they occupy almost whole of the cell. Fine capillaries were also found inside the lobules.

D_{35}

With increasing the age amount of fat cell and fine septa increase. At this stage well developed lobulated thymus surrounded by a fibrous capsule was observed (Fig. 7c). However, capsule was comparatively thin. From the capsule numerous fine septa were passing inwards and divide the gland into lobules. In general, each lobule contains a relatively pale medullary core surrounded by a densely cellular dark heavily stained cortex. In the cortex were packed large numbers of lymphocytes (Fig. 7c), mainly small lymphocytes, although medium-sized lymphocytes can also be seen. Due to the preponderance of this cell type the cortical region stains deeply basophilic and all other cell types, including the reticular cells, are almost completely obscured. The pale areas throughout the medullary regions of the lobules were called reticular structures and were considered a diffuse form of a Hassall's corpuscle. It was consisted of diffused groups of reticular cells and scattered vesicles which takes eosinophilic stain. The medulla contains far fewer lymphocytes than the cortex and was consequently much more pale staining (Fig. 7c). Other cells which can be seen within the medulla were eosinophils, haemocytoblasts and plasma cells.

Using high power objective it was observed that the cortex was composed of an extensive population of small lymphocytes. In contrast, the medulla was lightly staining and contineous with adjacent medulla. Large numbers of Hassall's corpuscels were found which takes eosinophilic stain (Fig.8c). The nucleons in the cells of the Hassall's corpuscles were pale, large and elongated. Thymic capillaries were also present.

4.2.2 Bursa of Fabricius

D_1

The bursa has developed plicae at this stage (Fig. 9a). The lumen did not contain mucoid substance. In some bursa the middle region of the plicae was thicker than the base and apical part. In other cases of the plicae, the base was thinner and apex was broad and rounded.

The lymphatic follicles were increased in size and there shape was polyhydral. Cortex and medulla were not well differentiated in all the lymphatic follicles (Fig. 9a). The medulla was very small in some follicles and it contains germinal centre which indicates there active stage of lymphopoeisis.

Amount of connective tissue was very minimum inside the core of plicae. Lamina propria contains minimum amount of connective tissue. Wall of the bursa was very thin with small amount of muscle fibers.

Under high power objective, it was observed that the lymphatic follicles were largely packed with lymphocytes with prominent nucleous. Some of the nucleous were darker and others were paler. Below the lining epithelium a layer of lymphocytes were present. And the mucosa was lined by columnar epithelium.

D_{21}

At the D_{21} the bursa was increasing in size with well developed plicae which was lined by pseudostratified and columnar epithelium (Fig. 9b). The plicae were tall with uniform thickness. All the lymphatic follicles were of not the same size and shape (Fig.9b). 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. 10a). The germinal center also called medulla contains lymphoblast.

It was clearly observed under high power objective that smooth muscle fibers present in the wall. The muscle fibers were arranged inner circularly and outer thinner longitudinally. Lymphoid follicles were round and oval in shape. They contain lymphoblast and lymphocytes.

D_{28}

The bursa was increasing in size with well developed plicae which was lined by pseudostratified and columnar epithelium (Fig.9c). The plicae were very tall with uniform thickness. All the lymphatic follicles were of not the same size and shape (Fig.9c). Some of the follicles were quite large with prominent lymphocytes. These prominant lymphatic follicles had clear cut margin and they were separeted from the adjacent lymphoid tissue by connective tissue fibers and cells and some space (Fig. 9c). 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. 10b). The germinal center also called medulla contains lymphoblast. There were precise margin between cortex and medulla.

In high power field it was observed that smooth muscle fibers present in the wall. The muscle fibers were arranged inner circularly and outer thinner longitudinally. Lymphoid follicles were round and oval in shape. They contain lymphoblast and lymphocytes. Below the lumenal .surface a layer of lymphoblast and lymphocytes were present. Outer most covering of the bursa were made up of serosa.

D_{35}

The bursa at this stage of growth and development were characterized by the presence of tall and thick plicae which was lined by pseudostratified columnar epithelium, (Fig. 9d, 10c). The numbers of the plicae were found to be more at this stage. Each plicae consist mainly of large number of polyhedral, prominent elongated and square shaped follicles which were closely packed together and were separated little bit with very small amounts of connective tissue. The follicles consist of outer cortex and an inner medulla (Fig. 10c). The germinal centers were very large indicating an active functional state of the bursa. The cortex was composed mostly of many small lymphocytes. The inter follicular connective tissue was composed of numerous reticular fibers. The lumen of the bursa in this stage of growth was devoid of mucoid substance indicating good communication with the cloaca. Its wall was found to be made up with circularly arranged smooth muscles, connective tissue and outer most serous membranes.

4.2.3 Spleen

D_1

With the growth of the chicken significant histological development of the spleen was observed. The histological field of spleen at this stage was developed. However, the capsule was very thin, which was composed of collagen fibers and smooth muscle fibers and the trabeculae was present in some cases. The red and white pulp was distinct at this stage (Fig. 11a). Using hematoxylin and eosin (H & E) stain, it was observed that the nucleus occupied the whole of the cytoplasm of lymphocytes and the rounded nucleus stained very darkly.

D_{21}

Indistinct demarcation between red and white pulp and white pulp was diffusely scattered through out the spleen (Fig.11b).Central arteriole slightly visualized. There was a gap around the central arteriole which may be due to shrinkage of blood vessels.

D_{28}

The thick splenic capsule was present without any trabeculae (Fig. 11c) at this stage. Indistinct, demarcation between red and white pulp was observed and white pulp was diffusely scattered through out the spleen. Central arteriole can be visualized even in low power magnification in the white pulp (Fig. 11c). There was a gap around the central arteriole which may be due to the shrinkage of the blood vessels. Some large blood vessels were also seen. Central arteriole was surrounded by the periarterial lymphatic sheath.

D_{35}

The spleen of the broiler chicken was covered at this stage by a thick fibrous capsule with very few trabeculae (Fig. 11cd). The areas of red and white pulp were distinct and white pulp was diffusely scattered throughout the spleen (Fig.11d) and was composed of network of reticular cells within which small medium and large size lymphocytes were diffusely located. It contains sheath arteries and occasionally, lymphatic nodules. Red pulp was formed from venous sinuses and was consisting of anastomosing cords of reticular cells, macrophages, lymphocytes and blood cells. The framework of the splenic tissue consists of a

network of reticular cells and reticular fibres. Over 80% of the substance of the spleen was divided about equally between red and white pulp. The collagen and reticular fibers were confined with in the arteriolar system of the spleen (Fig. 11d).

4.2.4 Cecal tonsil

D_1

All the histological layer were present with collagenous fibers in the cecal tonsil just after birth at one-day old. The mucosal folds and the lining epithelium were formed by a simple columnnar epithelium. Mucosa and submucosa contained diffuse lymphocytes and many lymphatic nodules (Fig. 12a). The muscular wall was thick and formed by the smooth muscles. The outer serosa was thin and the lymphatic nodules were abundantly present in the submucosa (Fig. 13a).

D_{21}

The histological field of ceacal tonsil at D_{21} consisting of all the layers and more well developed structures than D_1. Mucosa, tunica muscularis and serosa were present. The lymphatic nodules were abundantly present in the submucosa in comparison to the diffuse lymphocytes (Fig. 12b). The serosa was lined by mesothelium and the collagenous fibers was found to observed in all the layers and in high power object lymphatic nodules were observed (Fig. 13b).

D_{28}

At this stages of growth all the components of the cecal tonsil e.g. mucosa, tunica muscularis and serosa were present. The lining epithelium was simple columnar type. The bases of the mucosal folds were thick and the apexes were pointed. Diffuse lymphoid tissue and lymphoid nodules were present both in the mucosa and submucosa (Fig. 12c). The lymphatic nodules were abundantly present in the submucosa in comparison to the diffuse lymphocytes (Fig. 13c). In this stage of development primary lymphoid follicles with no clear central zone and secondary lymphoid follicles with germinal center was visible under light microscope.

The muscular coat was thick and consisting of smooth muscle and blood vessels. The serosa was lined by mesothelium as usual. The collagenous fibers were found to observe in all the layers.

D_{35}

In this stage of growth and development of the broiler chicken the villi were well developed both in length and breadth (Fig. 12d). Within the villi and at the base of the villi abundant lymphocytes and lymphoid nodules with cortex and medulla were observed (Fig. 13d). Nodular lymphatic tissues were found to be observed also in the submucosa of this organ. The collagenous fibers in the mucosa was originated from the muscularis mucosae, (Fig.12d).The muscularis externa and serosa were as usually well developed with collagenous materials at this stage of the development (Fig.12d).

Table 1: Standard Mean Error (SEM) of the Thymus from day-1 to day-35 at a regular interval. (n=5)

Age group	Length of the neck(mm) occupied by thymus	Diameter (mm)	Weight (gm)	Relative Weight (gm)
Day-1	25.63±0.26	3.61±0.09	0.31±0.01	0.85±0.02
Day-7	34.02±0.32	4.26±0.03	0.42±0.01	0.45±0.01
Day-14	43.01±0.55	5.09±0.02	0.51±0.01	0.26±0.01
Day-21	50.82±0.57	5.61±0.11	1.56±0.01	0.33±0.001
Day-28	61.40±0.24	6.60±0.09	4.14±0.01	0.40±0.01
Day-35	64.84±0.20	8.12±0.03	6.17±0.03	0.37±0.01

Table 2: Standard Mean Error (SEM) of the Bursa of Fabricius from day-1 to day-35 at a regular interval. (n=5)

Age group	Length (mm)	Diameter(mm)	Weight (gm)	Relative Weight (gm)
Day-1	4.04±0.01	2.71±0.02	0.02±0.001	0.06±0.01
Day-7	5.30±0.01	5.40±0.03	0.13±0.01	0.14±0.01
Day-14	10.86±0.22	7.41±0.02	0.27±0.01	0.14±0.00
Day-21	14.89±0.19	9.27±0.01	0.95±0.01	0.20±0.002
Day-28	16.01±0.20	10.18±0.03	1.32±0.01	0.13±0.002
Day-35	16.90±0.20	12.27±0.21	1.53±0.02	0.09±0.001

Table 3: Standard Mean Error (SEM) of the spleen from day-1 to day-35 at a regular interval. (n=5)

Age group	Diameter(mm)	Weight (gm)	Relative Weight (gm)
Day-1	2.67±0.04	0.01±0.00	0.03±0.001
Day-7	5.09±0.02	0.04±0.00	0.05±0.001
Day-14	5.49±0.04	0.16±0.00	0.08±0.001
Day-21	8.13±0.20	0.66±0.00	0.14±0.001
Day-28	9.11±0.02	1.22±0.02	0.12±0.001
Day-35	10.38±0.02	1.44±0.00	0.09±0.001

Table 4: Standard Mean Error (SEM) of the Cecal tonsils from day-1 to day-35 at a regular interval. (n=5)

Age group	Length (mm)	Diameter(mm)	Weight (gm)	Relative Weight (gm)
Day-1	13.99±0.19	2.19±0.02	0.03±0.00	0.08±0.001
Day-7	21.89±0.19	2.69±0.03	0.07±0.00	0.07±0.001
Day-14	35.00±0.20	3.27±0.01	0.39±0.00	0.20±0.01
Day-21	48.85±0.21	3.61±0.01	1.10±0.01	0.23±0.001
Day-28	57.61±0.40	3.73±0.01	1.23±0.01	0.12±0.001
Day-35	95.44±0.39	5.32±0.01	1.43±0.01	0.08±0.001

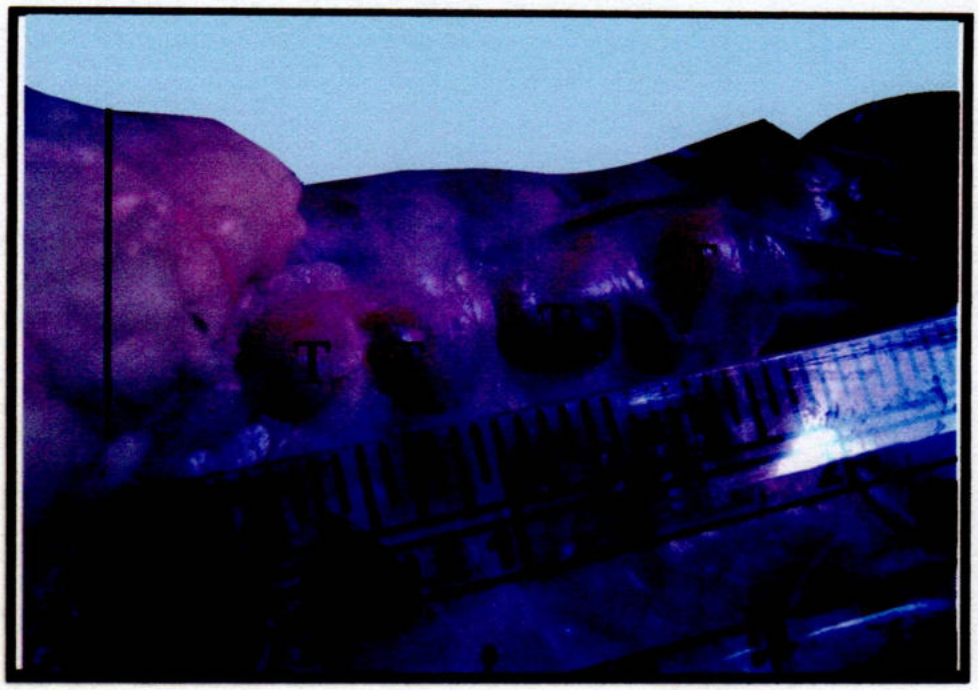


Figure 1.Gross photograph of thymus (T) of broiler chicken.

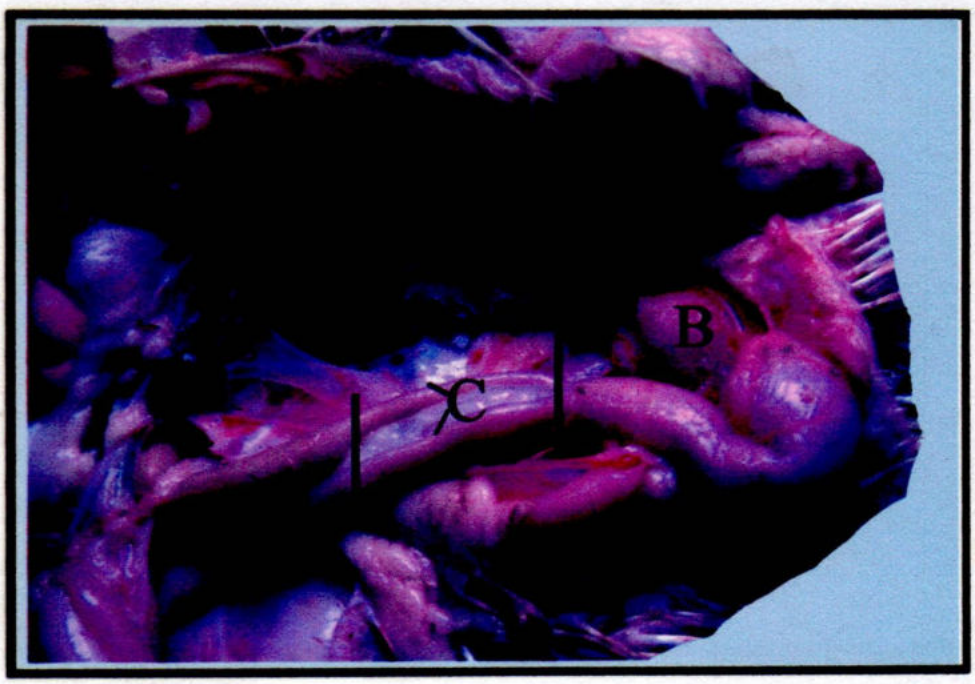


Figure 2: Gross photographic of spleen (S), cecal tonsil (C) and bursa of Fabricius (B) of broiler chicken. These lymphoid organs were increased in length, diameter and weight during their growth period.

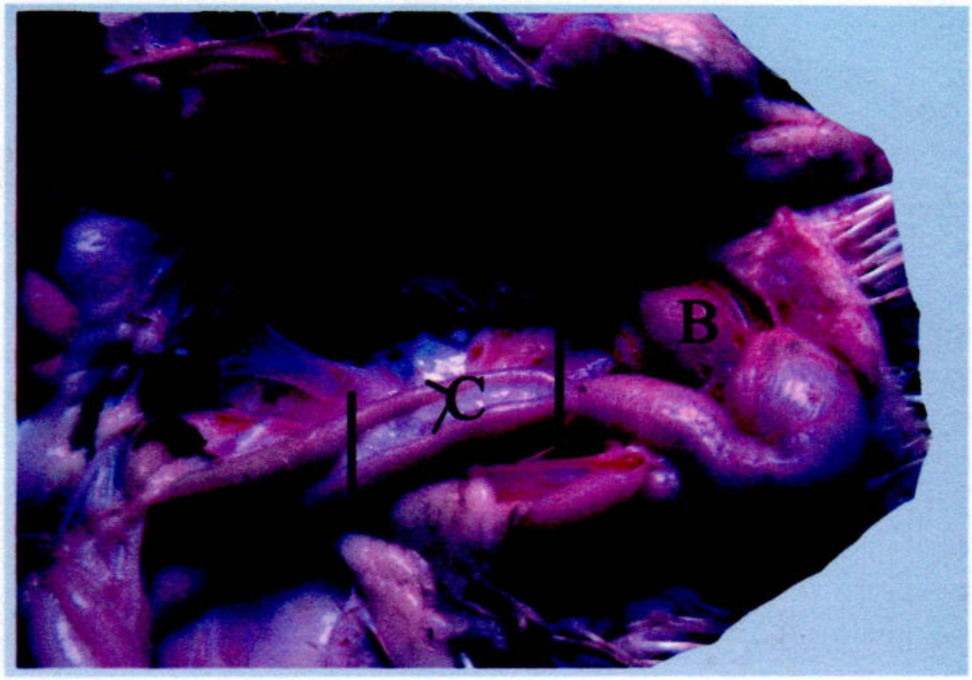


Figure 3: Gross photographic of cecal tonsil (C) of broiler chicken. This lymphoid organ increased in length, diameter and weight during their growth period.

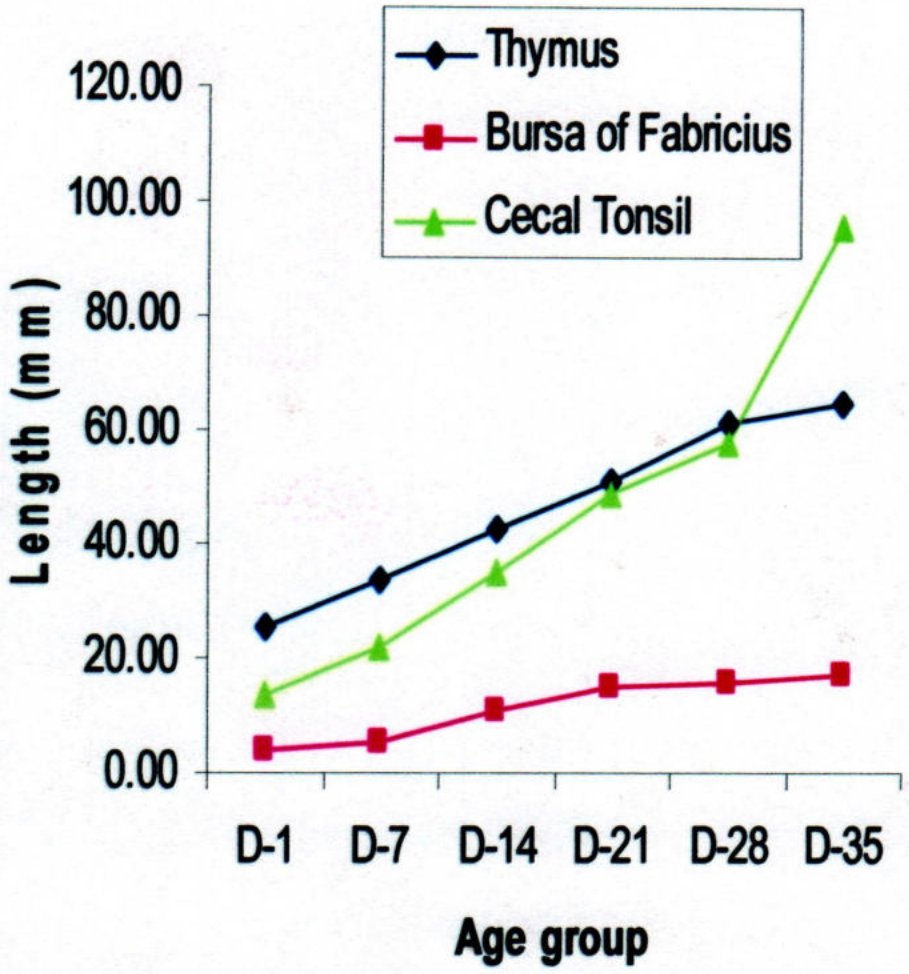


Figure 4: Length of lymphoid organs of broiler chicken at different stages of growth and development. The thymus, bursa and cecal tonsil were very small in length at D_1, and with the advanced of age they attained a maximum growth at D_{35} (n=5).

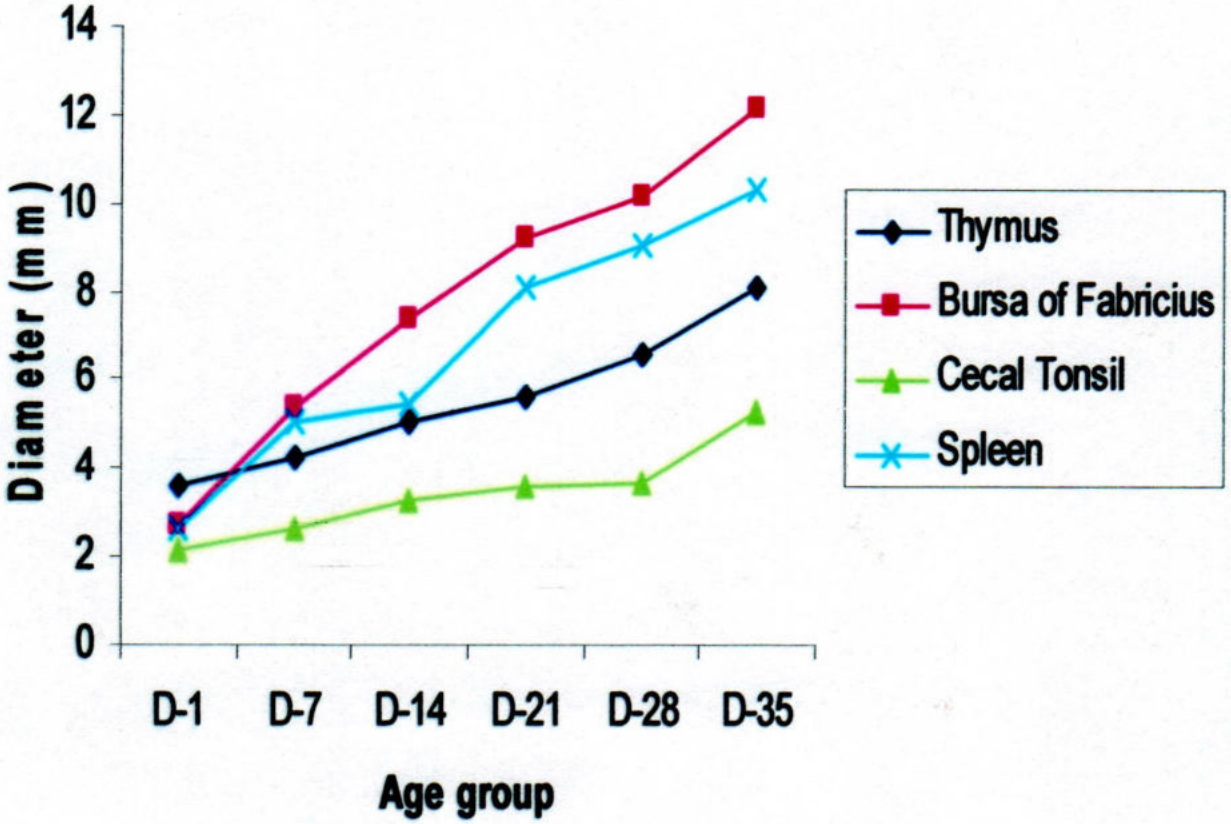


Figure 5: Diameter of lymphoid organs of broiler chicken at different stages of growth and development (n=5).

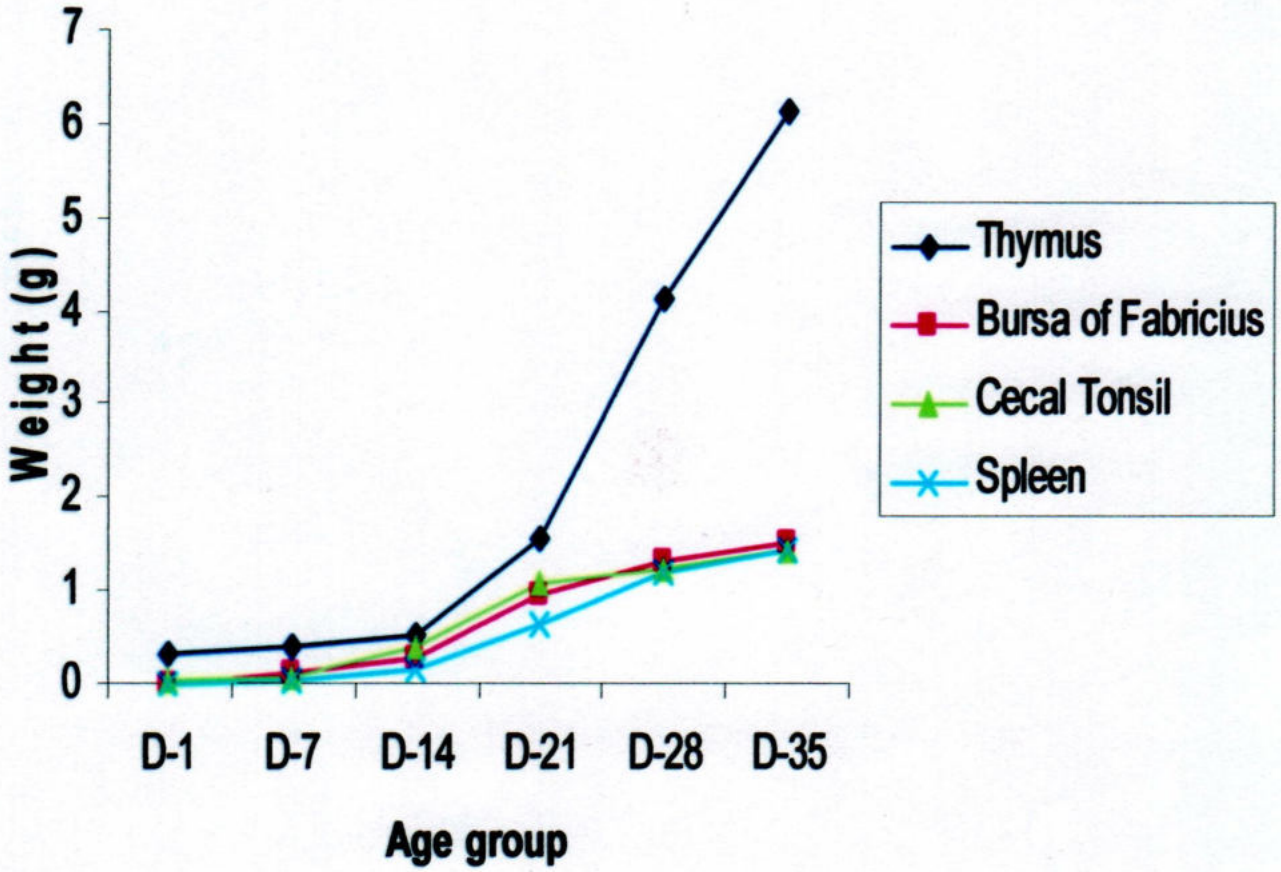


Figure 6: Weight of lymphoid organs of broiler chicken at different stages of growth and development (n=5).

Table 5: Tabular form of histological characteristics of thymus of broiler chicken

Thymus

Age	Histological features
D_1	❖ Connective tissue septum were very thin and divides the gland into lobes and lobules (Fig. 7a). ❖ Each lobule consists of an external dark staining dense and highly cellular cortex and an internal light staining less dense medulla. ❖ Inside the pale staining area of the medulla paler rounded areas were found which was considered to be a diffuse form of a Hassall's corpuscle (Fig. 8a).
D_{21}	❖ The thymuses were properly developed. ❖ Each lobule has cortex and medulla. Inside the medulla pale staining diffuse Hassall's corpuscles where present, which were Charecteristic of the region (Fig.8b).
D_{28}	❖ All the components of the thymus were well developed at this stage of growth and development. The capsule was very thin from which septums arose and divided the thymus into lobules (Fig. 7b). ❖ The cortex was composed of an extensive population of lymphocytes and the medulla has less cell population (Fig.7b).
D_{35}	❖ The cortex were packed large numbers of lymphocytes (Fig. 7c), ❖ The medulla contains far fewer lymphocytes than the cortex and was consequently much more pale staining (Fig. 7c). ❖ Large numbers of Hassall's corpuscles were found which takes eosinophilic stain (Fig.8c).

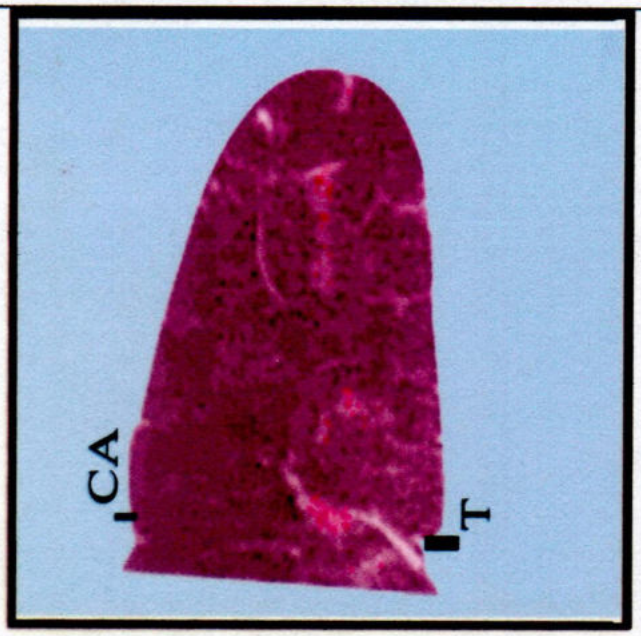


Figure 7a: The thymus of broiler chicken at day-1 (D1) of growth and development showing capsul (CA), trabeculae (T). H & E stain. X 10.

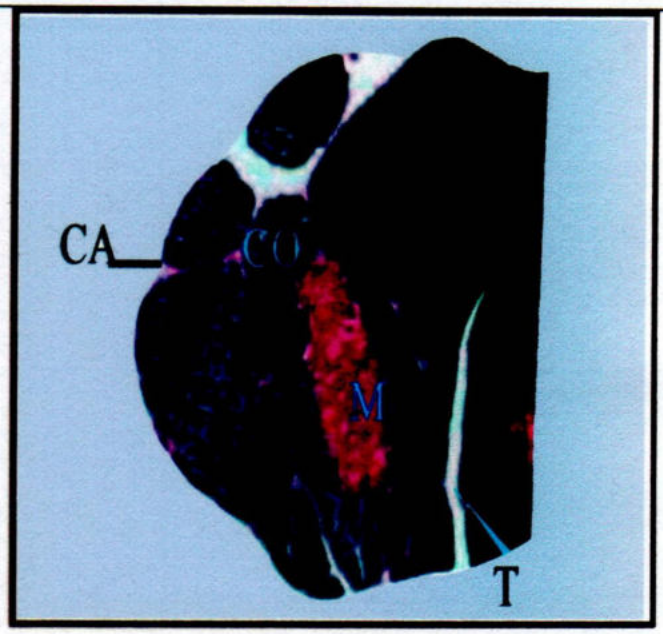


Figure 7b: The thymus of broiler chicken at day-28 (D28) of growth and development showing capsul (CA), trabeculae (T), Cortex (CO) and medulla (M). H & E stain. X 10.

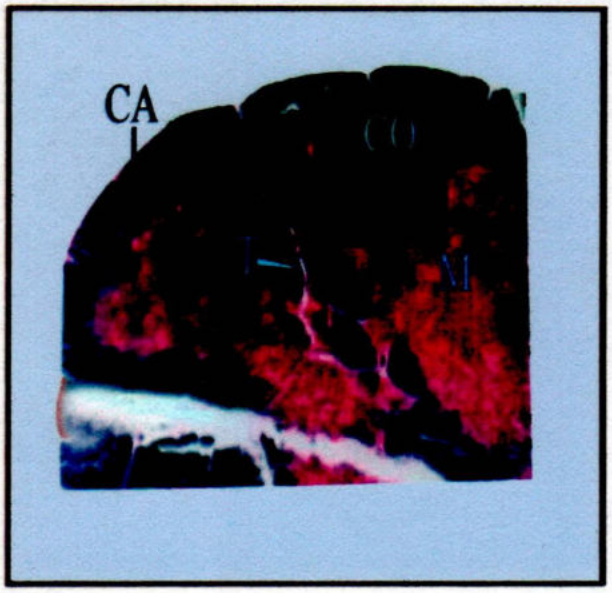


Figure 7c: The thymus of broiler chicken at day-35 (D35) of growth and development showing capsul (CA), trabecula (T), Cortex (CO) and medulla (M). H & E stain. X 10.

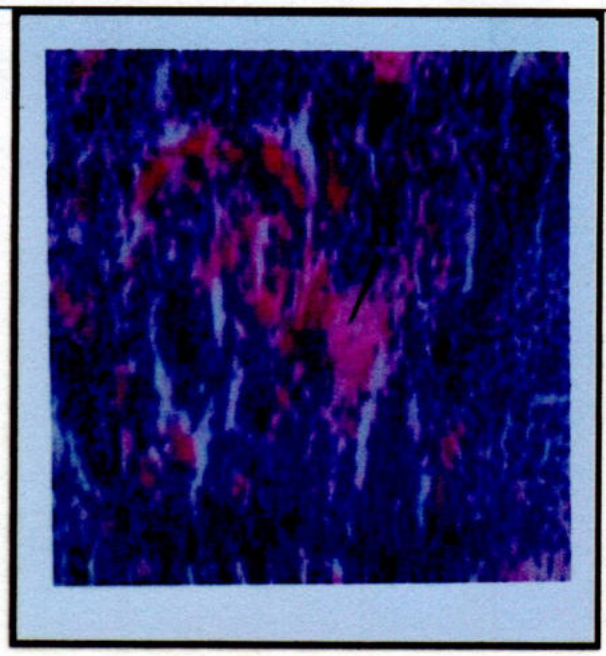


Figure 8a: The thymus of broiler chicken at day-1 (D1) of growth and development showing Hassal's corpuscles (H). H & E stain. X 40.

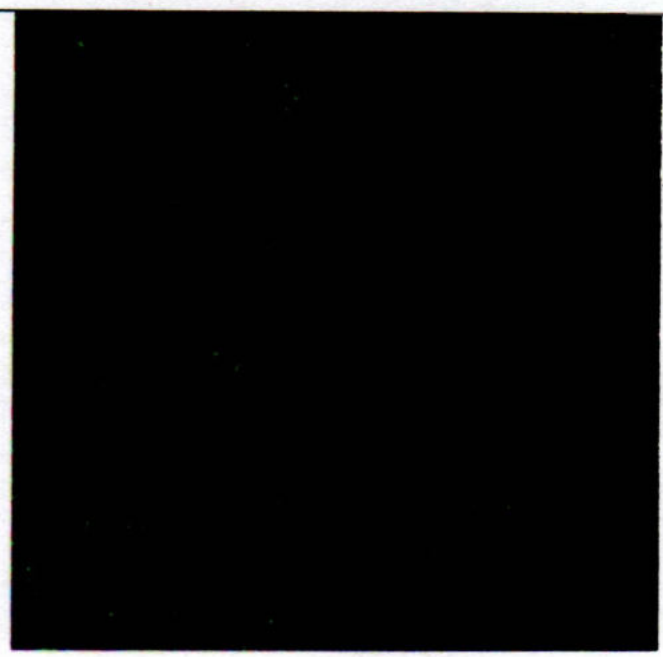


Figure 8b: The thymus of broiler chicken at day-21(D21) of growth and development showing Hassal's corpuscles (H). H & E stain. X40.

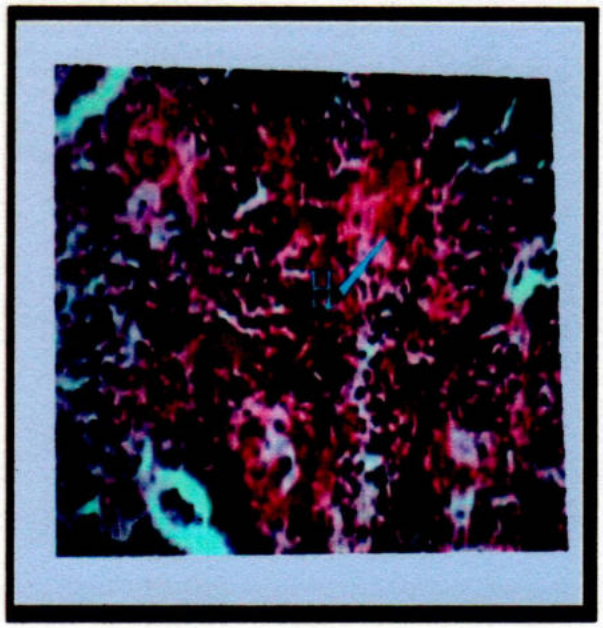


Figure 8c: The thymus of broiler chicken at day-35 (D35) of growth and development showing Hassal's corpuscles (H). H & E stain. X 40.

Table 6: Tabular form of histological characteristics of bursa of Fabricius of broiler chicken

Bursa of Fabricius

Age	Histological features
D_1	❖ Plicae are not well developed. ❖ Cortex and medulla were not well differentiated in all the lymphatic follicles (Fig. 9a). ❖ Small number of lymphatic follicle.
D_{21}	❖ The bursa was increasing in size with well developed plicae which was lined by pseudostratified and columnar epithelium (Fig.9b). ❖ All the lymphatic follicles were of not the same size and shape and increased the filtration of lymphocytes. (Fig.9b). ❖ 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. 10a).
D_{28}	❖ The bursa was increasing in size with well developed plicae which was lined by pseudostratified and columnar epithelium (Fig.9c). ❖ 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. 10b).
D_{35}	❖ The bursas were characterized by the presence of tall and thick plicae which was lined by pseudostratified columnar epithelium, (Fig. 9d, 10c). ❖ Each plicae consist mainly of large number of polyhedral, prominent elongated and square shaped follicles which were closely packed together and were separated little bit with very small amounts of connective tissue. The follicles consist of outer cortex and an inner medulla (Fig. 10c).

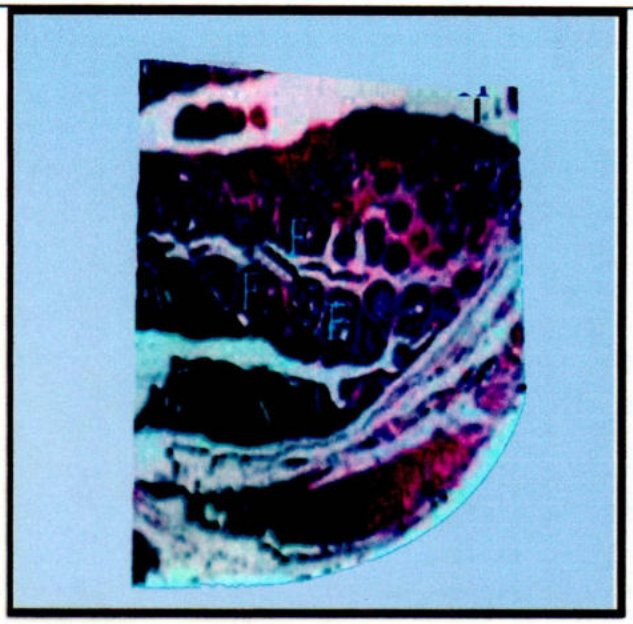


Figure 9a: The bursa of broiler chicken at day-1 (D1) of growth and development showing tall plicae (TP). Which contain many lymphoid follicles (F). The lumen was devoid of lymphoid substance. H & E stain. X 10.

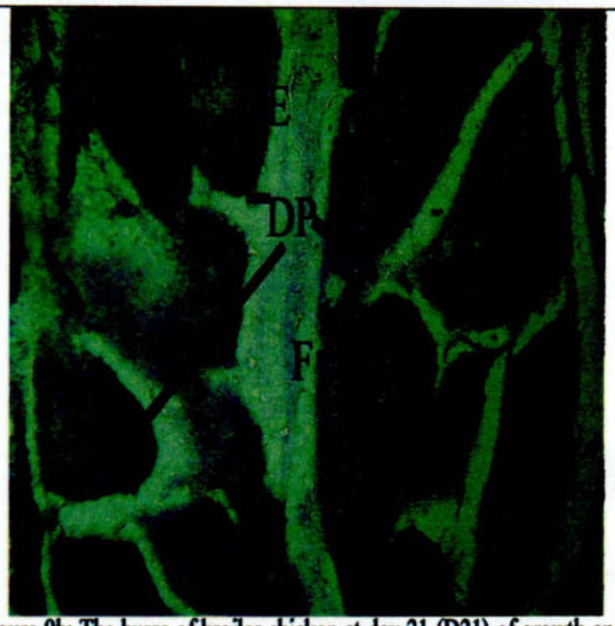


Figure 9b: The bursa of broiler chicken at day-21 (D21) of growth and development showing developed plicae (DP) which was lined by pseudostratified and columnar epithelium (E). The plicae were tall with uniform thickness. All the lymphatic follicles (F) were of not the same size and shape. H & E stain. X 10.

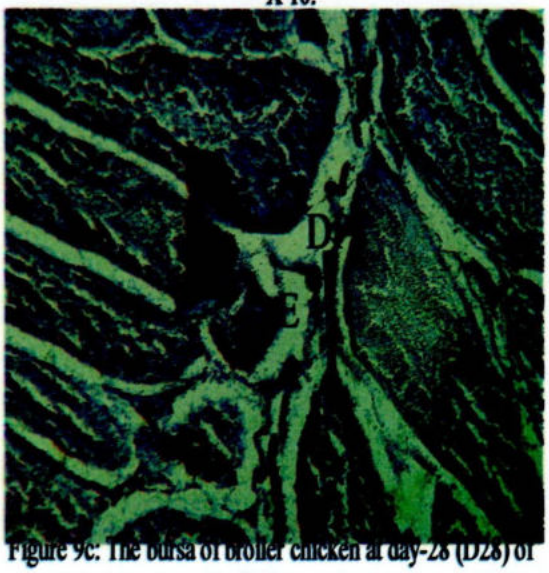


Figure 9c: The bursa of broiler chicken at day-28 (D28) of growth and development showing developed plicae (DP) which was lined by pseudostratified and columnar epithelium (E). The plicae are tall with uniform thickness. All the lymphatic follicles were of not the same size and shape. H & E stain. X 10.

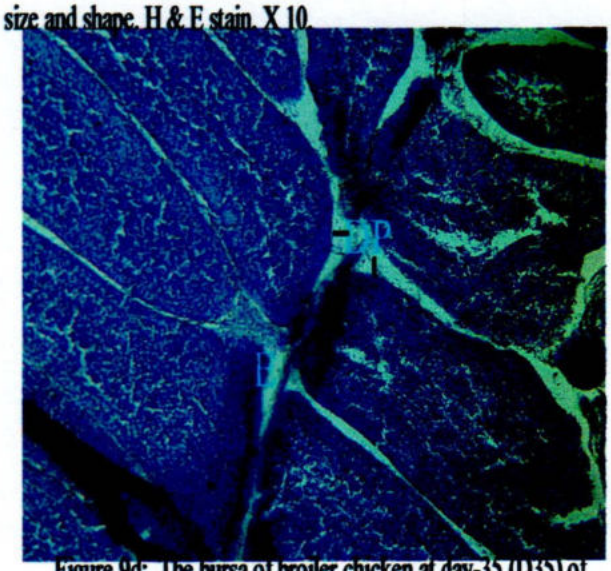


Figure 9d: .The bursa of broiler chicken at day-35 (D35) of growth and development showing developed plicae (DP) which was lined by pseudostratified and columnar epithelium (E). The plicae were tall with uniform thickness. All the lymphatic follicles were of not the same size and shape. H & E stain. X 10.

Figure 10a: 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.

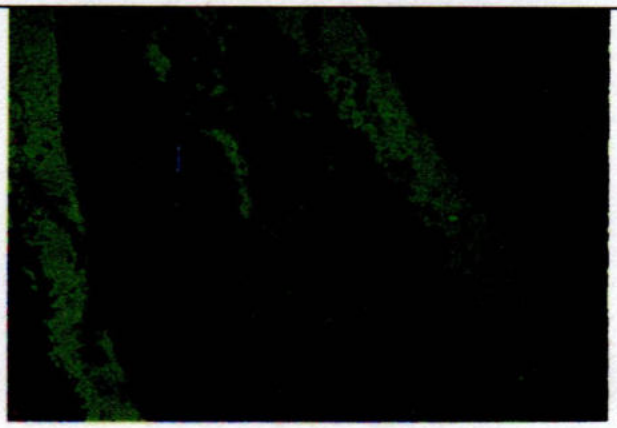


Figure 10b.The bursa of broiler chicken at day-28(D28) of growth and development showing lymphoid follicles (F) contain lymphoblast and lymphocytes. H & E stain. X 40.

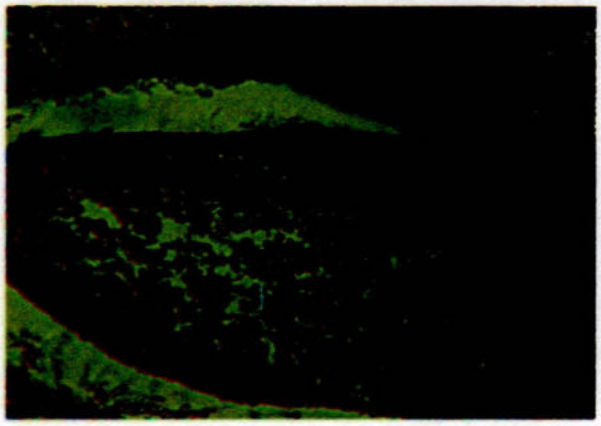


Figure 10c: The bursa of broiler chicken at day-35 (D35) of growth and development showing lymphoid follicles (F) contain lymphoblast and lymphocytes. H & E stain. X 40.

Table7: Tabular form of histological characteristics of spleen of broiler chicken

Spleen

Age	Histological features
D_1	❖ The capsule was very thin, which was composed of collagen fibers and smooth muscle fibers. The red and white pulp was distinct at this stage (Fig. 11a).
D_{21}	❖ There was no distinct demarcation between red and white pulp (Fig.11b).
D_{28}	❖ The thick splenic capsule was present without any trabeculae (Fig. 11c) at this stage. ❖ Indistinct, demarcation between red and white pulp was observed and white pulp was diffusely scattered through out the spleen.
D_{35}	❖ The areas of red and white pulp were distinct and white pulp was diffusely scattered throughout the spleen (Fig.11d) and was composed of network of reticular cells within which small medium and large size lymphocytes were diffusely located.

Figure 11a: The spleen of broiler chicken at day-1 (D1) of growth and development showing the red (R) and white pulp (W) were distinct and very thin capsul (CA). H & E stain. X 10.

Figure 11b: The spleen of broiler chicken at day-21(D21) of growth and development showing there were no distinct demarcation between the red (R) and white pulp (W) and white pulp was diffusely scattered throughout the spleen. H & E stain. X 10.

Figure 11c: The spleen of broiler chicken at day-28(D28) of growth and development showing thick splenic capsule (CA) is present without any trabeculae at this stage. Indistinct, demarcation between red (R) and white pulp (W) were observed and white pulp was diffusely scattered throughout the spleen. Central arteriole (CEN) can be visualized even in low power magnification in the white pulp (W). H & E stain. X 10.

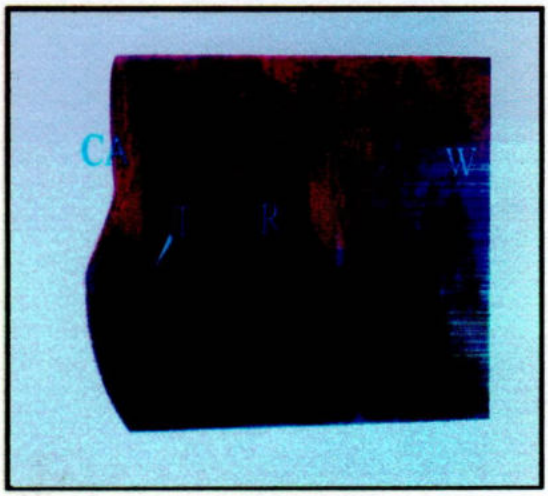


Figure 11d.The spleen of broiler chicken at day-35(D35) of growth and development showing thick splenic capsule (CA) and trabeculae (T). The red (R) and white pulp (W) was observed. H & E stain. X 10.

Table 8: Tabular form of histological characteristics of Cecal tonsil of broiler chicken

Cecal tonsil

Age	Histological features
D_1	❖ Mucosa and submucosa contained diffuse lymphocytes and many lymphatic nodules (Fig. 12a).
D_{21}	❖ It was consisting of all the layers and more well developed structures than D_1. ❖ The lymphatic nodules were abundantly present in the submucosa in comparison to the diffuse lymphocytes (Fig. 13b).
D_{28}	❖ All the components of the cecal tonsil e.g: mucosa, tunica muscularis and serosa were present. ❖ Diffuse lymphoid tissue and lymphoid nodules were present both in the mucosa and submucosa (Fig. 12c). ❖ The lymphatic nodules were abundantly present in the submucosa in comparison to the diffuse lymphocytes (Fig. 13c).
D_{35}	❖ The Mucosal fold (MF) were well developed both in length and breadth (Fig. 12d). ❖ Within the Mucosal fold and at the base of the mucosal fold abundant lymphocytes and lymphoid nodules with cortex and medulla were observed (Fig. 13d).

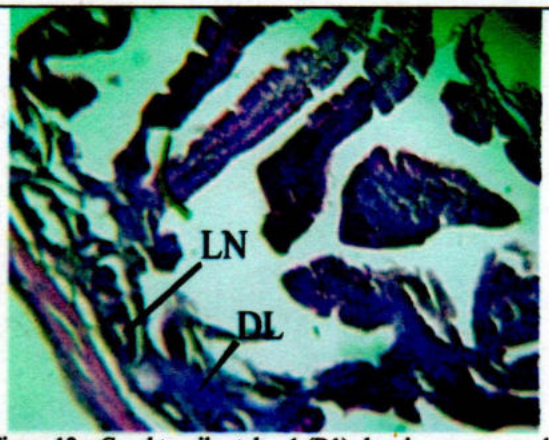


Figure 12a: Cecal tonsils at day-1 (D1) showing mucosa and submucosa contained diffuse lymphocytes (DL) and many lymphatic nodules (LN). The muscular wall is thick and formed by the smooth muscles and the outer serosa is thin. H & E stain. X 10.

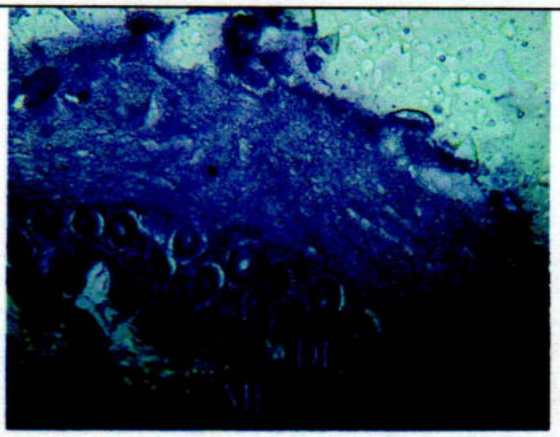


Figure 12b: Cecal tonsils at day-21 (D21) showing mucosal folds (MF) and submucosa contained diffuse lymphocytes (DL). H & E stain. X 10.

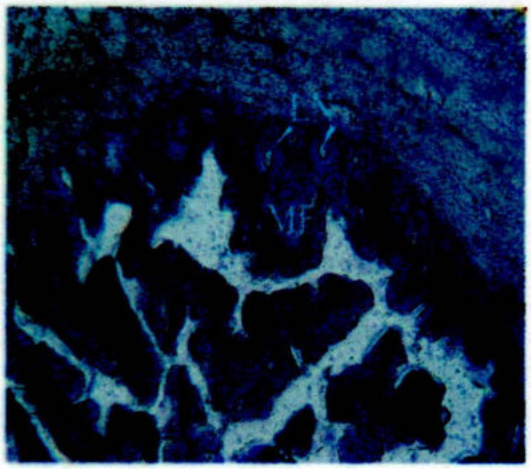


Figure 12c: Cecal tonsils at day-28 (D28) showing diffuse lymphoid tissue and lymphoid nodules (LN) are present both in the mucosa and submucosa .H & E stain.X 10.

Figure 12d: Cecal tonsils at day-35 (D35) showing wide and broad Mucosal fold (MF) having lymphatic nodule (LN). H & E stain. X 10.

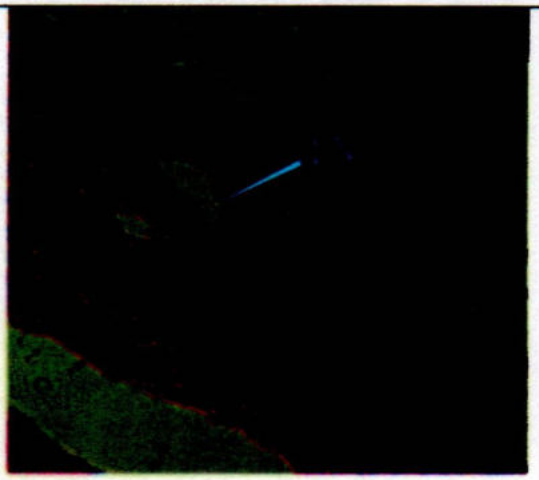


Figure 13a: Cecal tonsils at day-1(D1) showing lymphatic nodules (LN). H & E stain. X 40.

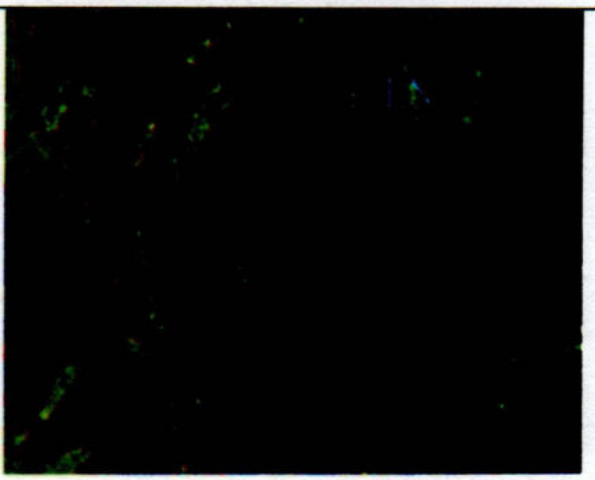


Figure 13b: Cecal tonsils at day-21(D21) showing lymphatic nodules (LN). H & E stain. X 40.

Figure 13c: Cecal tonsils at day-28(D28) showing lymphoid nodules (LN) are present. H & E stain. X 40.

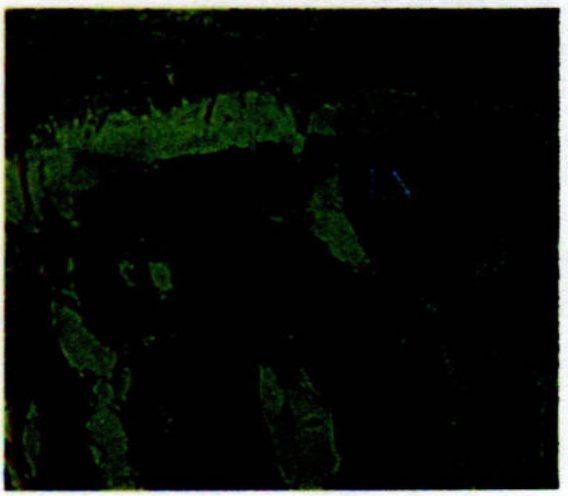


Figure 13d: Cecal tonsils at day-35(D35) showing lymphatic nodule (LN) having cortex (C) and medulla (M). H & E stain. X 40.

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

In the present study, observation of gross and histological structure has been carried out to identify the different structures and development of the thymus, bursa of Fabricius, cecal tonsil and spleen of broiler chicken. Similarities and dissimilarities has been observed in these lymphoid organs of broiler chicken in comparison to deshi chicken and other previous study related to my study and the results were discussed very briefly under the following sub-heading.

5.1 Thymus

In the present study it was revealed that the lobes of the thymus were ovoid at D_1 and becomes slightly elongated at D_{35} of the broiler chickens. These findings were similar to that of the hybrid chicken (Hodges, 1974; King, 1975) and deshi chicken (Mohsin khalil, 2001).

In the present study, the thymus were symmetrically placed on either side of the neck with close contact with the jugular vein. In case of D_1 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. But in case of adult stages of thymus of the broiler chicken, the findings which agreed to the study made by the Hodges (1974), Bach (1978) and King (1995), and for deshi chicken Mohsin khalil (2001).

. The present study revealed that the thymus were pale white at D_1 period of the broiler chicken but becomes yellowish white at D_{35}. But in case of adult stages the color of the thymus of the hybrid chickens were more yellowish then the study and the deshi cehicken (King, 1975; Hodges, 1974; and Bach 1978).

In the present study, 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 D_1 of life but then it gradually decreases.

In this context Getty (1975) reported that the greatest weight of the hybrid chicken was 15.76 gm at 17 weeks of age and Mohsin Khalil (2001) showed that the relative weight was greatest at embryonic stage of prenatal life (ED_1) of deshi chicken.

The result of the present study showed that the mean length of the neck occupied by the thymus of the broiler chicken at D_1 was 25.63 ± 0.26 mm and at D_{35} it reached up to 64.84 ± 0.20 mm. For deshi chicken at D_1 was 23.00 ± 0.948 mm and at D_{90} it reached up to 63.84 ± 0.969 mm Mohsin khalil (2001).

The mean diameter of the thymus of broiler chicken at D_1 was 3.61 ± 0.09 mm at D_{35} it reaches up to 8.12 ± 0.03 mm. The result of the present study showed that the diameter gradually increases with the increase of age of the broiler chicken and similar with Mohsin khalil, (2001) for deshi chicken.

. The numbers of lobes of the thymus on each side of the neck were usually between 4 to 8 in numbers. But in case of hybrid chickens they varied between 3 to 8 in numbers (King, 1995; Hodges, 1974; and Bach,1978).So the variance was similar in case of broiler chicken but Mohsin khalil, (2001) for deshi chicken showed the number between 6 to 7.

In the present study it was found that the thymus of broiler chicken was surrounded by connective tissue and adipose tissue from D_1 to D_{35} of life of broiler chicken. 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 (D_1). Capillaries were found in the parenchyma at this stage. Groups of lymphocytes were present with large nucleus. However, reports in these regard agreed with Mohsin khalil, (2001) for deshi chicken. Hassall's corpuscles became larger and there number increases at the period in the medulla of the thymus (D_{35}). Gradually all the components of the thymus were developed at these periods. The lobules become well developed with advancing age.

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 present findings of broiler chicken were similar to the adult hybrid chicken (Bach, 1978; Hodges, 1974; and King, 1995).

5.2 bursa of Fabricius

The shapes of bursas were globular with slight anterio-posterior compression in shape. The surface was smooth with shining serous covering. It was attached to the dorsal aspect of the proctodeum and becomes gradually large and more ovoid. At D_1 the central lumen devoid of mucoid substance.

In the present study, the bursa of Fabricius appeared as a dorso-median diverticulum from the proctodeal part of the cloaca which was similar to the previous report of Hodges (1974) in case of hybrid chicken.

The color of the bursa of Fabricius of the broiler chicken was whitish at D_1 and becomes yellowish white at D_{35}. The color was same as that of the hybrid chicken (King, 1975; Hodges, 1974) and for deshi chicken Mohsin khalil (2001).

The mean weight of the bursa of Fabricius gradually increased with the age of the broiler chicken but in case of relative weight it was highest at D_{21} of life but then it gradually decreases. The greatest weight of bursa of the hybrid chicken was 4.25 gm at 10 weeks (Hodges, 1974) but Mohsin khalil, (2001) for deshi chicken showed that relative weight it was highest a ED_{18} of prenatal life .

The mean length of the bursa of Fabricius of the broiler chicken at D_1 was 4.04 ± 0.01 mm and at D_{35} it reached up to 16.90 ± 0.20 mm. The study of Mohsin khalil, 2001 for deshi chicken showed that the mean length of the bursa of Fabricius of the deshi chicken at ED_{15} was 2.80 ± 0.122 mm and at D_{90} it reached up to 11.00 ± 0.158 mm.

The result of the present study showed that the mean diameter at the base of the bursa of the broiler chicken at D_1 was 2.71 ± 0.02 mm and at D_{35} it reaches up to 12.27 ± 0.21 mm. The result of the present study showed that the diameter gradually increases with the increase of age of the broiler. In the study Mohsin khalil, 2001 for deshi chicken revealed that deshi chicken at $ED_{1\ 5}$ was 2.20 ± 0.122 mm and at D90 it reaches upto 8.40 ± 0.187 mm.

The bursa was surrounded by mesothelium, connective tissue and smooth muscles. Though all the layers of the bursal wall were present, they were not well developed. In the stage of D_{35} all the components of the bursa were well developed, specially the plicae which were taller and branched with large number of lymphatic tissue in the lamina propria. The lining epithelium of the plicae was pseudo-stratified columnar epithelium. The findings of the broiler chickens were similar to that of the adult hybrid chicken. (Hodges,1974; King, 1995; Bach, 1978) and for deshi chicken (Mohsin Khalil, 2001).

5.3 Spleen

In the present study, it was observed that the shape of the spleen was rounded with slightly flattened at D_1 and becomes rounded at D_{35}. In case of postnatal period, the shapes of the spleen of the broiler chicken were similar to that of the hybrid chicken (Hodges, 1974).

The spleen lies close to the right side of the junction between the proventriculus and the gizzard. But in case of adult hybrid chicken, the positions were similar to the study (Hodges, 1974; King, 1975).

The spleen was pinkish brown to brownish red in the adult period (D_{35}). In case of the adult stages, the color of the spleen of the hybrid chicken, were same as that of the study (King 1975; Hodges 1974; and Bach 1978).

The mean weight of the spleen gradually increased with age of the broiler chicken (weight at D1 was 0.01±0.00 gm and D35 it was 1.44 ± 0.00 gm). But in case of relative weight it was highest at D21 of prenatal life but then gradually decreases. According to Hodges (1974), at 10 weeks of hybrid chicken spleen's weight was 2.65 gm or 0.2% of the body weight. He stated that the weight varies in different breed and in the same breed under different conditions.

The mean diameter of the spleen of the broiler chicken at D_1 was 2.67 ± 0.04 mm and at D_{35} it reaches up to 10.38 ± 0.02 mm which increased gradually and agreed with the study for deshi chicken (Mohsin khalil, 2001).

In the present study it was found that the spleen of broiler chicken was surrounded by thin capsule at early life, which gradually became thicker from D_1 to D_{35}. Isolated connective tissue septums arising from the capsule with blood vessels were found in some cases. The lymphocytes were found both in red and white pulps. The red pulp also contains erythrocytes. Central arterioles were found when white pulps become distinct. Outside the splenic capsule peritoneal mesothelium were present in all the stages. The histological structure of spleen in case of adult broiler chicken was similar to the previous study as reported by Hodges (1974), King (1995), and Bach (1978), except the presence of connective tissue trabeculae.

5.4 Cecal tonsils

In the present study, it was found that the cecal tonsils were found in the proximal one third of the paired tubular cecum, which lies along each side of the large intestine. There was a slight bend or angulation at the junction between proximal one third and distal two third of the cecum. This angulation was less prominant at D_1 but more prominant at D_{35}. The shape of cecal tonsils of the broiler chicken were similar to that of the hybrid chicken (King, 1975; Hodges, 1974) and deshi chicken (Mohsin khalil, 2001).

In the present study, the cecal tonsils were symmetrically placed on either side of the large intestine and they arise bilaterally at the junction of small and large intestines. In case of D_1 and that of the D_{35} periods, their positions were same those were similar similar to the study made by the Kitagawa (1969); Hodges (1974), King (1975); Bach (1978) and.The cecal tonsils were yellowish white in the D_1 of the broiler chicken but becomes greenish brown in the D_{35}.

In case of adult stages, the colour of the cecal tonsils were similar findings with the hybrid chicken (Hodges 1974, King 1975, Bach 1978).

The mean weight of the cecal tonsils gradually increased with the age of the broiler chicken at (D_1) 0.030 ± 0.00 gm to (D_{35}) 1.43 ± 0.01 gm) but in case of relative weight at D_{21} of life it was greatest but then it gradually decreases up to D_{35}. In the study of Mohsin khalil, 2001 revealed that the mean weight of the cecal tonsils gradually increased with the age of the deshi chicken $_{(ED15)}$ 0.030 ± 0.000 gm to D90 0.811 ± 0.000 gm.

The result of the present study revealed that the mean length of the cecal tonsils of the broiler chicken at D_1 was 13.99 ± 0.19 mm and at D_{35} it reached up to 95.44 ± 0.19 mm.

The mean diameter of the cecal tonsils of broiler chicken at D_1 was 2.19± 0.02 mm and at D_{35} it reaches up to 5.32 ± 0.01 mm. The result of the present study showed that the diameter gradually increases with the increase of age of the broiler chicken. The study of Mohsin khalil, (2001) revealed showed that the mean diameter of the cecal tonsils of deshi chicken at ED_{15} was 1.20 ± 0.122 mm and at D_{90} it reaches up to 4.20 ± 0.122 mm.

In the present study it was found that the lumen of the two cecal tonsils were of unequal diameters and the lumen becomes larger without any mucoid substances. All the histological layers of the cecum were present from D_1 to D_{35} of the present study but gradually they becomes thicker and more prominant with the advancement of the ages.

The mucosal folds were very few and small at day $_{D1}$ and gradually they became prominant. These mucosal folds or villi fill up the lumen and they contain diffuse and nodular lymphoid tissue. The lymphoid nodules with cortex and medulla were observed in the mucosa and submucosa of the cecal walls. The histological information were found about the hybrid adult chicken which were similar to present study (Hodges,1974; King, 1975; and Bach,1978).

CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

The thymus of the broiler chicken was a paired lobulated gland, flattened ovoid pale white to yellowish white lobes of varying size and shape of lymphoid tissue lying in the subdermal connective tissue of the neck. The mean weight, diameter and the extent of the neck occupied by the thymus of broiler chicken were significantly higher ($P<0.01$) in between each stages of development. Histologically, the organ was surrounded by connective tissue capsule from which septa arises and incompletely divides the glands into lobules. So that medullas were continuous with each other. Since the D_1 period the lobules were demarcated into cortex and medulla, which becomes larger and well demarcated. In the present study, Hassall's corpuscles were found during D_1 stage which increased in diameter and number at D_{35} stage of broiler chicken. The capsule and trabeculae becomes thicker with growth and development of the thymus.

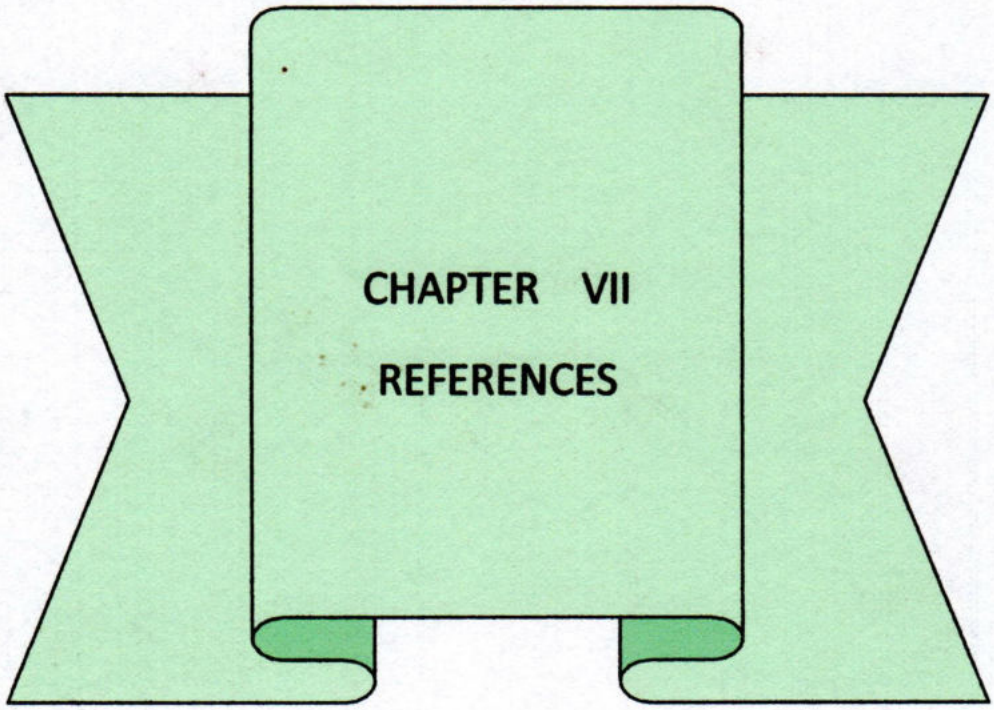
The single bursa of Fabricius was a dorso-median diverticulum of the proctodeum. It was smooth and globular in shape and yellowish white in colour at all the stages of development. It had a cavity which leads into the cloaca through a median opening in the dorsal wall of the proctodeum. The cavity becomes larger with subsequent growth of the bursa. The mean length, diameter and weight of the bursa of Fabricius of broiler chicken were significantly higher ($P<0.01$) in between each stages of development. Histologically, the bursa of D_1 stage was filled up by plicae and devoid of mucoid substances. The plicae contained few bursal follicles of different sizes. But with increasing age the bursa became larger with the plicae getting taller and thicker, which contained enormous number of large polyhedral primary and secondary lymphoid follicles. Thin wall of the bursa of D_1 stage became thicker at different periods (D_{21}, D_{28} and D_{35}).

The spleen of broiler chicken was variable in size and shape but usually rounded. The color was reddish-brown to pinkish-brown at different period. It was found on the right side of the junction between the proventriculus and gizzard. The mean diameter and weight of the spleen of broiler chicken were significantly higher ($P<0.01$) in between each stages of development. Histologically, from D_1 stage spleen was surrounded by connective tissue capsule which became gradually thicker with subsequent growth and development of the chicken. Splenic parenchyma became more distinct with increasing age.

The two cecal tonsils were present on either side of the large intestine and they arose bilaterally at the junction of small and large intestine and the position was similar at different stages. The color was yellowish white to greenish brown in the developing period. The mean length, diameter and weight of the cecal tonsil of broiler chicken were significantly higher ($P<0.01$) in between each stages of development. Histologically, cecal tonsils had four layers, which could be differentiated from D_1. They became more prominent with the growth and development of the chicken. In D_1 the mucosa had few small folds which contains small amount of diffuse lymphatic tissues. But the folds subsequently became larger and broader with large amount of diffuse and nodular lymphoid tissues.

The study may act as a guide line for the advance research on immunological aspect of major lymphatic organs. It may also plays as a base line for the anatomist, microbiologist and histopathologist. The age from 21st to 28th days is more critical for bursa related diseases that may be a clue for diagnosis. Further study will provide for the focusing of the lymphatic organs related diseases of human and similarities or dissimilarities with chicken.

In conclusion, the present study showed that the growth and development of the major lymphoid organs in the broiler chickens started from early age of life and continuous up to D_{35} in the present study and the maximum development was observed at the period of day thirty five (D_{35}). So it can be summarized that the growth and development of the major lymphoid organs of a broiler chicken was age-related.


CHAPTER VII
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APPENDICES

Anova-1: Analysis of variance for length of Thymus of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	2.657	0.664	0.8777
2	Factor A	5	5901.433	1180.287	1559.817**
3	Error	20	15.134	0.757	
	Total	29	5919.223		

**Significant at 1% level of probability ($p<0.01$)

Anova-2: Analysis of variance for diameter of Thymus of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.044	0.011	0.3897
2	Factor A	5	66.54	13.308	472.011**
3	Error	20	0.564	0.028	
	Total	29	67.148		

**Significant at 1% level of probability ($p<0.01$)

Anova-3: Analysis of variance for weight of Thymus of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.002	0.001	0.549
2	Factor A	5	147.661	29.532	27403.77**
3	Error	20	0.022	0.001	
	Total	29	147.685		

**Significant at 1% level of probability ($p<0.01$)

Anova-4: Analysis of variance for length of bursa of Fabricius of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.731	0.183	1.4008
2	Factor A	5	776.677	155.335	1191.091**
3	Error	20	2.608	0.13	
	Total	29	780.016		

**Significant at 1% level of probability ($p<0.01$)

Anova-5: Analysis of variance for diameter of bursa of Fabricius of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.131	0.033	0.8013
2	Factor A	5	297.864	59.573	1455.219**
3	Error	20	0.819	0.041	
	Total	29	298.814		

**Significant at 1% level of probability ($p<0.01$)

Anova-6: Analysis of variance for weight of bursa of Fabricius of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.007	0.002	6.572
2	Factor A	5	10.491	2.098	8314.915**
3	Error	20	0.005	0.0001	
	Total	29	10.502		

**Significant at 1% level of probability ($p<0.01$)

Anova-7: Analysis of variance for diameter of spleen of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.195	0.049	1.4993
2	Factor A	5	208.321	41.664	1279.285**
3	Error	20	0.651	0.033	
	Total	29	209.168		

**Significant at 1% level of probability ($p<0.01$)

Anova-8: Analysis of variance for weight of spleen of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.002	0.001	1.3562
2	Factor A	5	9.739	1.948	5981.164**
3	Error	20	0.007	0.0001	
	Total	29	9.748		

**Significant at 1% level of probability ($p<0.01$)

Anova-9: Analysis of variance for length of Cecal tonsils of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	1.406	0.351	0.8785
2	Factor A	5	21560.67	4312.135	10781.13**
3	Error	20	7.999	0.4	
	Total	29	21570.08		

**Significant at 1% level of probability ($p<0.01$)

Anova-10: Analysis of variance for diameter of Cecal tonsils of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.012	0.003	2.9871
2	Factor A	5	28.895	5.779	5894.894**
3	Error	20	0.02	0.001	
	Total	29	28.926		

**Significant at 1% level of probability ($p<0.01$)

Anova-11: Analysis of variance for weight of Cecal tonsils of broiler chicken from day-1 to day-35 at a definite interval of growth and development.

K Value	Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Calculated F Value
1	Replication	4	0.001	0.001	1.0273
2	Factor A	5	9.604	1.921	6364.313**
3	Error	20	0.006	0.0001	
	Total	29	9.611		

**Significant at 1% level of probability ($p<0.01$)