

Assessment of the Morphological Development of the Thymus in Turkey (*Meleagris gallopavo*)

Nnadozie O*, Nlebedum UC, Agbakwuru I and Ikpegbu E

Department of Veterinary Anatomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Abstract

The post hatch (PH) development of the thymus gland in turkey (*Meleagris gallopavo*) was studied from day (D) 1 post hatch to day 140. The thymus gland composed a bilateral chain of 5-8 irregular elliptical lobes that varied in size from cranial to caudal lobes, and from pale red to pink in color. Histologically, in all ages the thymus was enclosed by a connective tissue capsule from which septa penetrated and divided the stroma into incomplete lobules. Each lobe consisted of a central medulla surrounded by a lobulated cortex. At D 1 PH, lymphocytes and few granulocytes dominated the parenchyma with classical Hassall's corpuscle in the medulla. By D 14, reticular structures were observed in the medulla, and many vacuoles were associated with it by D 28. At D 42, several pink cells and highly basophilic cells dominated the medulla. From D 56, strands of connective tissue fibers formed network in the parenchyma. Encapsulated nodules dominated by lymphocytes with some plasma cells were observed at D 112. At D 140, there was marked depletion of thymic cells, and the distinction of the cortex from the medulla was in apparent.

Keywords: Thymus; Turkey; Morphology; Post-hatch; Development

Introduction

The thymus gland is classified immunologically as a primary lymphoid organ that is responsible for the production of lymphocytes involved in cell-mediated immune responses [1,2]. Its presence has also been found to be essential for the development of peripheral tissues and their associated adaptive immune functions [3]. It is well developed as a functional lymphoid organ at hatch [4], but attains its greatest development in sexually immature birds with involution setting in at the onset of sexual maturity [5]. Though a lot of works have been done on the thymus in various avian species, undoubtedly there is paucity of information on the post hatch morphological development of this organ in turkey. Hence the objective of this study is to assess the age-related morphological changes associated with the growth of the thymus gland in turkey bird.

Materials and Methods

Animal source and sample collection

One hundred apparently healthy day old turkey poult of either sex were purchased from Green Hands Agro-Vet Farms, Umuahia, Abia State, Nigeria. The birds were housed and raised in a deep-litter pen in Michael Okpara University of Agriculture, Umudike, Abia State, and were fed commercially compounded feed (Topfeed®), and water was provided ad libitum. No medication of any kind including vaccination was given throughout the study period. The birds were divided into twenty groups of five birds per group comprising day 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 112, 119, 126, 133 and 140. Based on the above schedule, five randomly selected birds were euthanized by inhalation anaesthesia with chloroform soaked in cotton wool in a lid plastic container. The thymus gland was collected by ventral neck dissection [6], and examined for gross features.

Histological processing and micrography

Slices of the thymus of different ages were fixed in Bouin's fluid and transferred to 70% alcohol after 24 hours. The specimens were processed by placing them in ascending grades of alcohol in the following order, first 95% alcohol for 1 hour and second 95% alcohol for 1¼ hours, first absolute alcohol for 1½ hours and second absolute alcohol for 2 hours to ensure proper dehydration of the tissues. It was then transferred

to mixture of equal volumes of alcohol and xylene where it was left overnight. It was later cleared in two changes of xylene for 1 hour each. It was then infiltrated twice for 1 hour each with molten paraffin wax in the oven at 60°C. The tissues were embedded in paraffin wax, trimmed and mounted on wooden chuck, and then taken to the microtome for sectioning at 5 µm thickness. The sections were floated in floating-out bath from where it was picked with clean albuminized slides. The slides were placed in a staining dish and excess wax was removed by two changes of xylene, hydrated by descending grades of alcohol in the following order - absolute alcohol, 95% alcohol and 70% alcohol for 2 minutes each. The slides were taken to water and then stained by filtered Ehrlich hematoxylin for 15 minutes, and then washed in water for 5 minutes, differentiated in 1% acid alcohol for 3 seconds, and blued in running tap water for 10 minutes. It was then counter stained with filtered eosin for 2 minutes. Excess eosin was removed in ascending grades of alcohol in the following order - 75% alcohol, 95% alcohol and absolute alcohol for 2 minutes each. It was then cleared in two changes of xylene and cover slipped with Depex mountant. The slides were viewed under a light microscope and selected images were captured using moticam 2.0 digital camera attached to a computer.

Result

The thymus gland composed a bilateral chain of 5-8 irregular elliptical lobes that varied in size from cranial to caudal lobes, and from pale red to pink in color. The left thymus was more cranially located than the right, which extended more into the thoracic region. Lobation was not quite distinct until about day 14 post hatch.

***Corresponding author:** Nnadozie O, Department of Veterinary Anatomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, Tel: +2347085987809; E-mail: drdozie15@gmail.com

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Histologically, at day 1 post hatch, the thymus had a capsule of fine connective tissue from which septa emerged and divided the parenchyma into incomplete lobules of varying sizes. These septa ended at the cortico-medullary border therefore, the parenchyma consisted of a centrally located medulla surrounded by the lobulated cortex (Figure 1).

The medulla was composed of lymphocytes with scattered eosinophilic epithelial reticular cells whose nuclei varied from round to oval with prominent nucleoli. The Hassall's corpuscles were already well developed and consisted of concentrically arranged keratinized structure surrounded by flattened acidophilic reticular cells (Figure 2). Also in the medulla was the diffused form of the Hassall's corpuscles known as the reticular structures which were composed of irregular masses of reticular cells with vesicles that contained degenerating cells. The cortex consisted of dense populations of lymphocytes, and less prominent reticular cells.

By day 7 post hatch, the cortex had increased in dimension and the interlobular connective tissue trabeculae in transverse section were observed in deeper cortical structures, and in the subcapsular region was localized accumulation of adipose tissue. The medulla was obviously less densely populated with lymphocytes, but showed more prominently the distribution of the epithelial reticular cells (Figure 3).

By day 14, the trabeculae had increased in thickness and the

blood vessels showed obvious increase in luminal diameter and wall thickness. Heterochromatic small lymphocytes still dominated the cellular composition of the thymus with classical Hassall's corpuscles populated with reticular cells and few vacuoles in the medulla.

By day 35, the distinction between the cortex and medulla still remained clearly defined. Many strands of connective tissue trabeculae had traversed the parenchyma and formed a network within, while adipose tissue accumulations were observed in the sub capsular regions (Figure 4). The medulla and cortex remained densely populated with lymphocytes, while plasma cells were observed in large numbers in the medulla.

By day 49, the medulla showed predominance of basophilic small lymphocytes and some spherical pink cells thought to be also lymphocytes probably undergoing some transformations (Figure 5). The cortex equally showed marked increase in outline, but its distinction from the medulla was fairly apparent.

By day 56, numerous connective tissue fibers had traversed the thymic parenchyma and formed more network of trabecular tissue within. The cortex appeared prominently denser than the medulla, suggesting an increase in cortical cell density. At the corticomedullary border was a Hassall's corpuscle with accumulations of giant reticular cells. The cortex showed dense accumulations of lymphocytes around

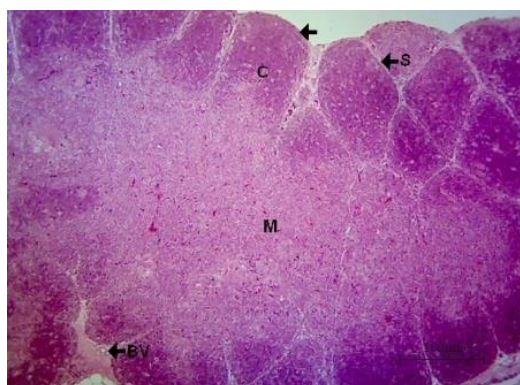


Figure 1: Transverse Section of the thymus gland at day 1 post hatch showing the thymic parenchyma. Note the pale stained M: Central medulla; and the lobulated more basophilic outer C: Cortex; S: septum; Arrow: capsule; BV: blood vessel X100.

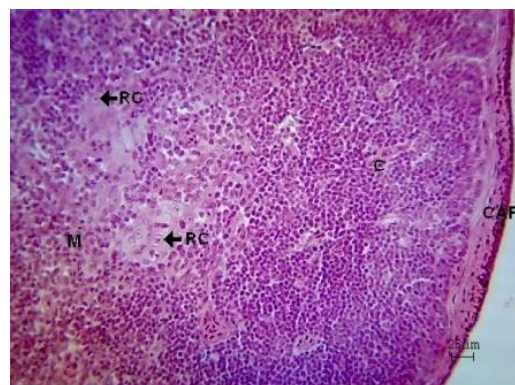


Figure 3: Transverse Section of the thymus gland at day 7 showing the parenchyma. Note the disparity in lymphocytic densities of the C: Cortex; M: Medulla; RC: Reticular cell; CAP: Capsule. X400.

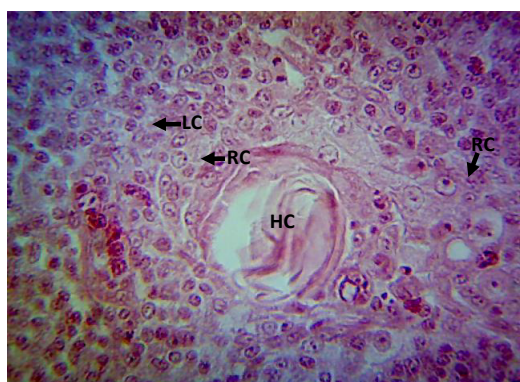


Figure 2: Section of thymic medulla at day 1 post hatch showing the HC: Hassall's corpuscle; Note the concentric arrangement of structures within the figure and the surrounding RC: Reticular cells; LC: lymphocyte. X1000.

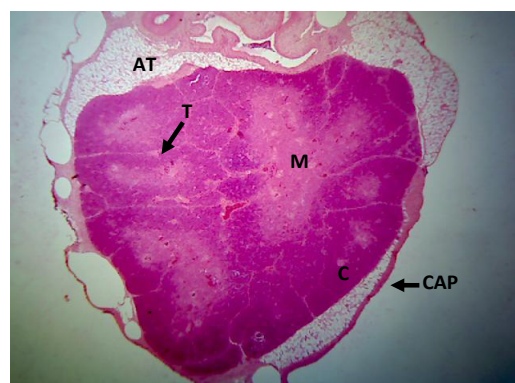


Figure 4: Transverse Section of thymus gland at day 35 showing the parenchyma. Note the network of fine T: Connective tissue trabeculae; within the parenchyma and the subcapsular deposits of AT: Adipose tissue; C: cortex; M: Medulla; CAP: Capsule. X40.

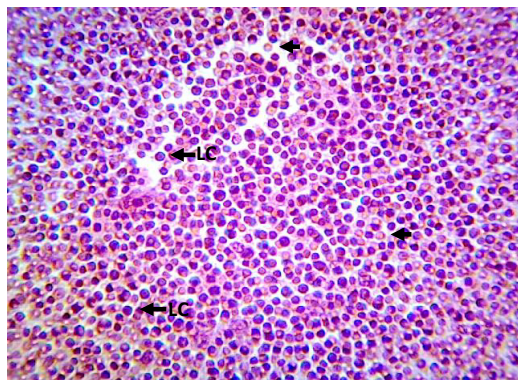


Figure 5: Section of the thymus at day 49 showing the cellular components of the medulla. Note the abundance of the basophilic LC: lymphocytes and the Arrow: Pink cells. X1000.

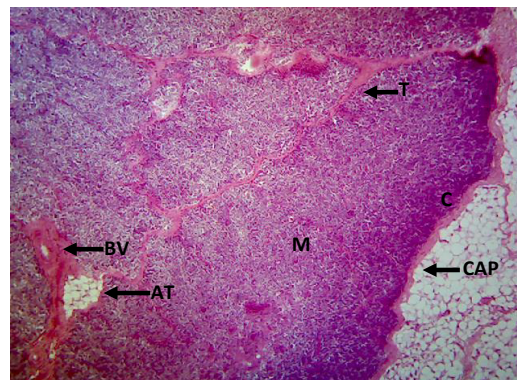


Figure 7: Transverse Section of thymus gland at day 84 showing the parenchyma. Note the T: Trabeculae; BV: Blood Vessel and AT: Adipose Tissue; C: Cortex; M: Medulla; CAP: Capsule. X100.

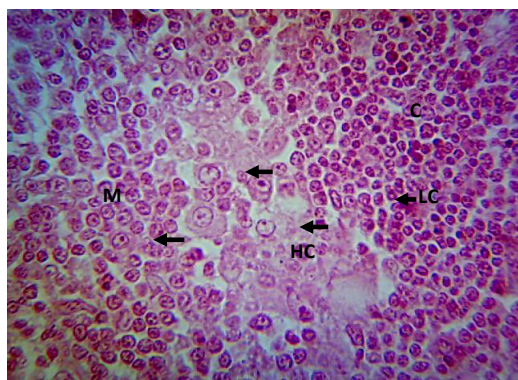


Figure 6: Section of thymus gland at day 56 showing the C: Cortex; and M: Medulla, Note the HC: Hassall's corpuscle; at the cortico-medullary border. Arrow: Reticular cells; LC: lymphocytes. X1000.

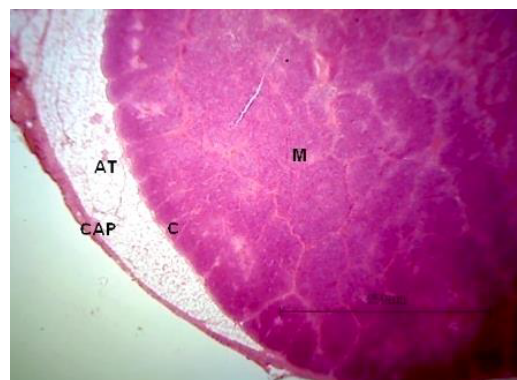


Figure 8: Transverse Section of the thymus gland at day 98 showing the parenchyma. Note the network of T: Connective tissue; in the parenchyma. C: Cortex; M: Medulla; AT: Adipose Tissue; CAP: Capsule. X100.

the corpuscle, while the medulla was composed predominantly of reticular cells (Figure 6).

At day 70, the cortex appeared reduced in profile and the trabeculae had penetrated deeper into the parenchyma transecting the thymic structures in some areas. Lymphocyte densities in both the cortex and medulla remained apparently high but showed predominance of medium-sized lymphocytes. Active macrophages were as well observed in greater population in the medulla.

By day 84, adipose tissue accumulations were observed within the thymic substance around the thick trabeculae whose blood vessels equally showed prominent thick walls and wide lumen (Figure 7). Cellular composition of the thymus remained predominantly small and medium-sized lymphocytes with few granulocytes and plasma cells.

At day 98, the cortex appeared to have regressed further to the periphery but its distinction from the medulla was still apparent. Subcapsular adipose tissue accumulation had remarkably increased, while lobulation was still clearly defined. Fine connective tissue fibers had formed irregular polygonal structures in the parenchyma (Figure 8). Lymphocyte density remained high, but plasma cells were few.

By day 112, discrete spherical nodules arranged in a semi circle were found in the medulla. These nodules were lined by thin connective tissue fiber that was surrounded by cells arranged end to end in a

unique formation. Each of these encapsulated nodules contained dense population of lymphocytes with numerous plasma cells and few mitotic figures (Figure 9). The cortex remained distinguishable from the medulla, and the connective tissue fibers that traversed the thymic parenchyma were still obvious.

By day 133, the parenchyma was still differentiated into cortex and medulla, and the capsule had become very thick in appearance. Both the cortex and medulla remained densely populated with lymphocytes, but the deep basophilic lymphocytes and the pink cell counterparts dominated the medulla.

At day 140, the cortex was inapparent, hence the entire parenchyma stained uniformly, but the capsule remained thick in appearance and the subcapsular adipose tissue accumulation was massive. There was evidence of cell depletion in the parenchyma marked by accumulation and dominance of fibrous tissue in some regions (Figure 10). Also, vacuolated reticular cells dominated some areas and most blood vessels were of thick walls with fibrotic tunica media that was composed of loose strands of smooth muscle fibers (Figure 11).

Discussion

The location of the thymus gland on the lateral aspect of both sides of the neck in close association with the jugular vein in turkey is similar to those of chicken [7], quail [8] and guinea fowl [9]. The irregular elliptical lobes that varied in color from pale red to pink as observed in

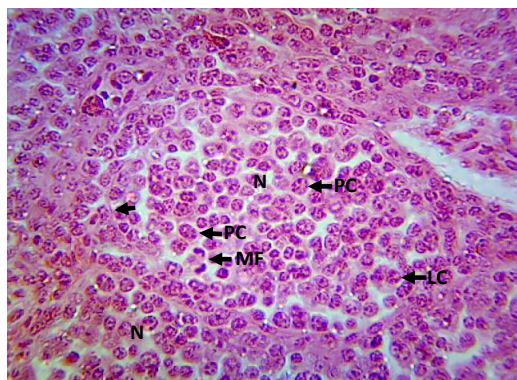


Figure 9: Section of thymus gland at day 112 showing the N: Nodules; lined by a thin strand of Arrow: Connective tissue; Note the cellular composition of the nodules, LC: lymphocyte; PC: Plasma cell; MF: Mitotic figure MF. X1000.

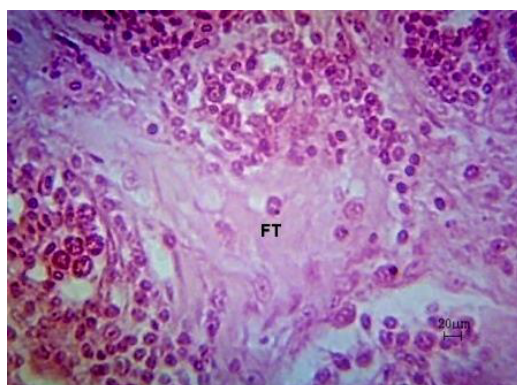


Figure 10: Section of thymus at day 140 showing the subcapsular region of the parenchyma. Note the level of displacement of thymic cells by FT: Fibrous connective tissue. X1000.



Figure 11: Section of the thymus at day 140 showing the parenchyma. Note the BV: Blood vessels, whose media consisted of Arrow: Prominent smooth muscle fibers; V: vesicle. X400.

this study was similar to the observation made by Muthukumaran et al. [3] in turkey. In general there had been species variations in number of lobes of the thymus gland. There were 5-8 lobes on each side of the neck in turkey as observed from this study. Hodges recorded 7 lobes on each side of the neck in domestic fowl, and 3-8 lobes [7].

Onyeonusi et al. found 7 lobes on the right and 6 lobes on the left

in guinea fowl, while in geese there were 6-9 lobes on the right and 5-9 lobes on the left [10].

The histology of the thymus in the turkey is similar to those described for the goose [11,12], guinea fowl [10] and chicken [13]. At day 1 post hatch, our investigation showed that the thymus gland in turkey had developed all structures of avian thymus. Lobulation and differentiation of lobules into cortex and medulla were very pronounced at hatch as was also reported for chicken, but still remained extremely poor or even completely lacking in some lobes in the guinea fowl [10].

Although the pre-hatch period was not investigated in this work, differentiation of the thymic parenchyma into cortex and medulla was found to have started as early as day 13 and 20 of incubation in the chicken [14] and guinea fowl [10] respectively. Therefore, considering the extent of development of the thymus at hatching in the turkey, differentiation of the thymic tissue into cortex and medulla may have started earlier prenatally, but further investigations on pre-hatch developmental changes are necessary to ascertain the exact time this embryonic process commenced in turkey.

The classical histological structure in the thymic medulla, the Hassall's corpuscle had well developed in turkey at day 1 post hatch. This figure consisted of concentric arrangement of keratinized structures that showed evidence of lamination and cornification as described in the goose by Gulmez and Aslan [11], but Hodges, King and McLelland [7] stated that lamination and cornification of Hassall's corpuscle were rare and absent in birds respectively. Though the true function of the Hassall's corpuscle remained an enigma, it had been proposed that the turnover of the epithelial reticular cells and degeneration of thymocytes resulted in the Hassall's corpuscle [13].

Previous works on avian thymus had not frequently mentioned the existence of lymphatic nodules or its equivalent in the thymus gland. At day 112 in turkey, we observed some spherical encapsulated nodule-like structures that contained predominantly lymphocytes with some plasma cells and mitotic figures in the medulla.

The thymic cortex in turkey was found to regress, though inconsistently with age as had been reported for some avian species, and by day 140, the clear distinction between the cortex and medulla was completely lost with apparent reduction in thymic cell density. Large areas of the subcapsular regions were occupied by fibrous tissue, and clusters of vacuolated cells were widespread in the medulla. Hoffmann-Fezer [15] reported that in some chicken the cortex decreased with age and became absent by day 224, but in the guinea fowl the cortex remained an integral part of the parenchyma even at 224 days [10]. Several histological changes have been associated with thymic involution in avian species. According to Franchini and Ottaviani [16] such changes include the disappearance of the clear distinction between the cortex and medulla and proliferation of non-epithelial cells. Aita et al. [17] and Ciriaco et al. [18] equally reported progressive reduction of cortical area and in the number of cortical and medullary epithelial cells. Therefore, our observations from this study suggested that involution of thymus gland in turkey commenced at about day 133 of age.

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