

AGE RELATED HISTOLOGICAL CHANGES IN THE THYMUS OF TANUVAS ASEEL BIRDS

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ABSTRACT

The present study was undertaken to investigate the age-related histological changes in the thymus of TANUVAS Aseel chickens. Healthy birds were selected from five different age groups, namely day-old, 4, 8, 18 and 32 weeks, with six birds representing each group. Thymic samples were collected from birds maintained at the Poultry Research Station, Madhavaram Milk Colony, Chennai. The tissues were thoroughly rinsed in normal saline and fixed in 10 per cent neutral buffered formalin, Bouin's fluid and Zenker's fluid for histological evaluation. Following fixation, the tissues were processed by routine dehydration, clearing and paraffin embedding. Sections of 4–5 µm thickness were prepared and stained with Haematoxylin and Eosin for general histological examination and Masson's Trichrome for demonstration of connective tissue fibres. The thymus of day-old chicks was characterized by a thin connective tissue capsule and delicate septa, with well-defined cortical and medullary regions. The cortex contained a greater number of lymphoblasts, whereas the medulla showed a higher occurrence of reticuloepithelial cells and Hassall's corpuscles. At four weeks, the capsule and septa showed slight thickening, while the general cortical and medullary proportions remained comparable to those of day-old chicks. By eight weeks, connective tissue thickening and more distinct lobular compartmentalization were evident, accompanied by a slight increase in the medullary component. Hassall's corpuscles of Types I–III were identified in the medulla, with occasional Types I and II in the cortex. At 18 weeks, progressive changes became apparent, including increased thickness of the capsule and septa, lymphoid depletion, reduced lymphocyte size, degeneration of reticuloepithelial cells and vacuolation. Numerous Hassall's corpuscles of Types I–V were observed in the medulla. By 32 weeks, marked thymic involution was evident, characterized by collagen-rich capsular thickening, cortical lymphocyte depletion, connective tissue invasion and cyst formation. The findings demonstrate progressive structural maturation followed by age-associated involution of the thymus in TANUVAS Aseel chickens.

Keywords: Age related changes, histology, thymus, lymphoblast, reticuloepithelial cells, Hassall's corpuscles

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INTRODUCTION

Thymus is a primary lymphoid organ in birds, initiating their development during the embryonic stage (Hayashi *et al.*, 2013). The organ shares similarities in terms of its epithelial origin, lymphoid characteristics, and growth during embryonic and early postnatal stages. Notably, the thymus start to involute before reaching sexual maturity (Gulmez and Aslan, 1999).

The thymus gland is considered as immunologically the primary lymphoid organ that supports the differentiation of lymphoid progenitor cells along the T-cell pathway. T-cells are the main component of the adaptive immune system which is capable of responding to infectious agents and to increase defensive capabilities with each successive exposure to antigens (Kannan *et al.*, 2015).

Thymic involution is characterized by several age-related changes, including decreased thymic size, a reduction in thymocyte population, and a decline in the organ's ability to generate new T-cells (Zinkernagel, 2002). Thymic involution, beginning in early adulthood, accelerates with advancing age, leading to significant functional impairment in the thymus. This decline correlates with immunosenescence, as reduced thymic output compromises the immune system's ability to respond to novel pathogens and maintain immune tolerance (Zinkernagel, 2015).

The thymus is a paired, lobulated endocrine glands, situated along the sides of the neck, extending from the beginning of the cervical region to the entrance of the thoracic region. Thymic lobes are positioned parallel to the common carotid artery, jugular vein, trachea and esophagus (Ali, 2017; Trautmann and Fiebtger, 2015).

In the poultry industry, the main reason to do vaccination in the early post-hatch life is the presence of active immune cells during this period which can recognize any antigenic stimulation and do the needful. The comparative study of chicken lymphoid organs in relation to age will unveil their histomorphogenesis as well as provide a clear understanding to study the arrangement of immune cells in lymphoid organs (Ayman *et al.*, 2020).

MATERIALS AND METHODS

Healthy TANUVAS Aseel chicken were selected, with six birds chosen from each age group: day-old, four, eight, eighteen, and thirty-two weeks were obtained from the Poultry Research Station, Madhavaram Milk Colony, Chennai – 51.

Samples of thymus were collected from the aforementioned age groups, thoroughly rinsed in normal saline, and then fixed using various fixatives such as 10 per cent Neutral buffered formalin, Bouin's fluid, Zenker's fluid. These fixatives were employed to facilitate histological investigation.

In the histological examination the fixed tissues underwent a process involving dehydration in ascending grades of alcohol, clearing in xylene and embedding in paraffin wax. Subsequently, paraffin tissue blocks were made, and microtome sections with a thickness of 4-5µm were obtained. The sections are subjected to staining such as Haematoxylin and Eosin method for routine histological observations (Bancroft and Stevens, 1996) and Masson's Trichrome method (Luna, 1968) for connective tissue fibres.

RESULTS AND DISCUSSION

In thymus of day-old TANUVAS Aseel Chicks, the connective tissue capsule and septa were observed to be very thin. This observation is consistent with the findings reported by Mishra *et al.* (2010) in chicken thymus. The thymic lobes exhibited an outer darker cortex and an inner paler medulla. Notably, the proportion of cortex was more pronounced in day-old chicks, which intermingled with medulla and with advancing age, the medullary component became more prominent (Fig.1).

In day-old thymic parenchyma, there was an increased population of lymphoblasts compared to lymphocytes. Additionally, a few erythrocytes were noted in the medulla. Reticuloepithelial cells were found to be more prevalent in the medulla. Interestingly, Hassall's corpuscles were present in the day-old thymus (Fig.6), primarily restricted to the medulla, and encompassing Types

I and II. This finding is in line with the observations made by Kannan *et al.* (2018) in Nandanam chicken.

At 4 weeks of age, there was a slight increase in the thickness of the capsule and septa. The proportion of cortex and medulla remained similar to that observed in day-old thymus (Fig.2). In terms of cellular population, there was still a higher number of lymphoblasts compared to lymphocytes. Lymphoblasts are immature and appear as larger cells with visible nucleoli, whereas lymphocytes appear smaller without clear nucleoli (Fig.7). All three types of reticuloepithelial cells were observed (Fig.8). Additionally, Hassall's corpuscles of Type I and II were noticed (Fig.7). These findings are entirely consistent with the observations made by Kannan *et al.* (2015) in Nandanam chicken, Islam *et al.* (2019) in commercial broiler chicken, and Ayman *et al.* (2020) in Sonali chicken.

In the thymus of 8-week-old chickens, there was a relative thickening of the connective tissue capsule and septa. The compartmentalization of the cortex by intralobular septa became more prominent. The proportion of medulla was slightly increased, as illustrated in (Fig.3).

Regarding cellular composition, the medulla consisted mainly of lymphoblasts, lymphocytes of various sizes (Fig.9) and all types of reticuloepithelial cells. Additionally, various types of Hassall's corpuscles were distributed in both cortex and medulla. This

observation aligns with the findings reported by Haseeb *et al.* (2014) in Aseel chicken and Jayachitra (2022) in turkey.

At 18 weeks of age, there was an increased thickness of the capsule and septa, signalling the onset of involutory changes. These changes were characterized by a decrease in the lymphoid population and a reduction in the size of lymphocytes, as in (Fig.4).

Degenerating reticuloepithelial cells were more numerous, and vacuole formation was evident in both the cortex and medulla. Hassall's corpuscles of different types with different sizes were noticed in both cortex and medulla (Fig.10). These findings are similar to those reported by Khenenou *et al.* (2012) and Kanasiya *et al.* (2018) in chicken.

At 32 weeks of age, the process of involution in the thymus was evident through several characteristics. There was a thickening of the capsule, attributed to an increased presence of collagen fibres. Additionally, signs of involution included the depopulation of cortical lymphocytes, invasion of connective tissue into the parenchyma, as illustrated in (Fig.5). Moreover, there was an increased number of Hassall's corpuscles and the presence of cysts, further indicating the features of an involuting thymus. These characteristics align with the findings reported by Bhattacharya and Binaykumar (1983) in chicken and Steinmann (1986) and Marinova (2005) in humans.

CONCLUSION

The present investigation established clear age-dependent histomorphological changes in the thymus of TANUVAS Aseel chickens. The early post-hatch thymus exhibited active lymphopoiesis, characterized by a prominent cortex, abundant lymphoblasts, well-developed reticuloepithelial cells, and Hassall's corpuscles. Progressive maturation with age was accompanied by increased connective tissue development and alterations in the cortical and medullary architecture. From 18 weeks onward, characteristic features of thymic involution, including lymphoid depletion, lymphocyte reduction, reticuloepithelial cell degeneration, vacuolation, connective tissue infiltration, and cyst formation, became evident. These changes were more pronounced at 32 weeks, indicating reduced thymic functional activity. The findings provide a useful morphological baseline for understanding thymic development, immune maturation, and age-associated involution in TANUVAS Aseel chickens.

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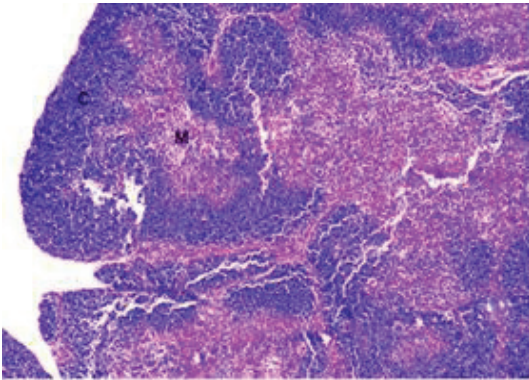


Fig.1. Photomicrograph of the thymus of day-old Aseel chicken showing the distribution of cortex (C) and medulla (M) H&EX100

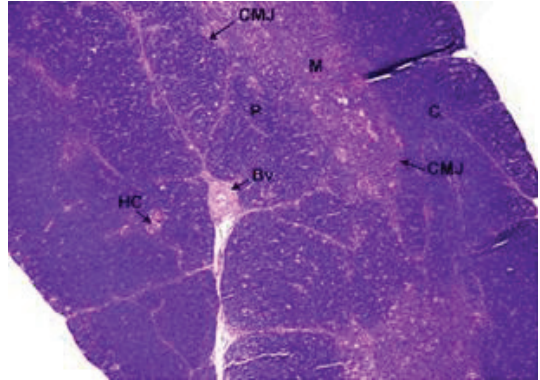


Fig.2. Photomicrograph of the thymus of four week-old Aseel chicken showing the parenchyma(P) Bv-Blood vessel C- Cortex M-Medulla CMJ – Cortico-medullary junction HC-Hassall's corpuscle H&E X100

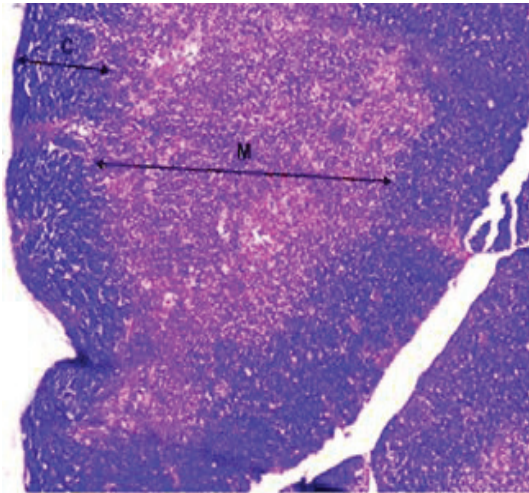


Fig.3. Photomicrograph of the thymus of eight week-old Aseel chicken showing the distribution of cortex(C) and medulla (M) H&E X 100

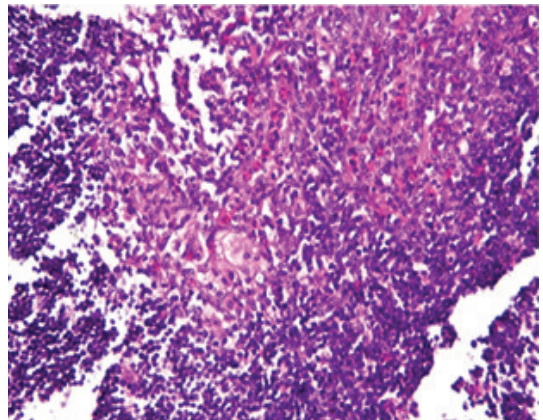


Fig.4. Photomicrograph of the thymus of eighteen week-old Aseel chicken showing onset of involutory changes H&E X 400

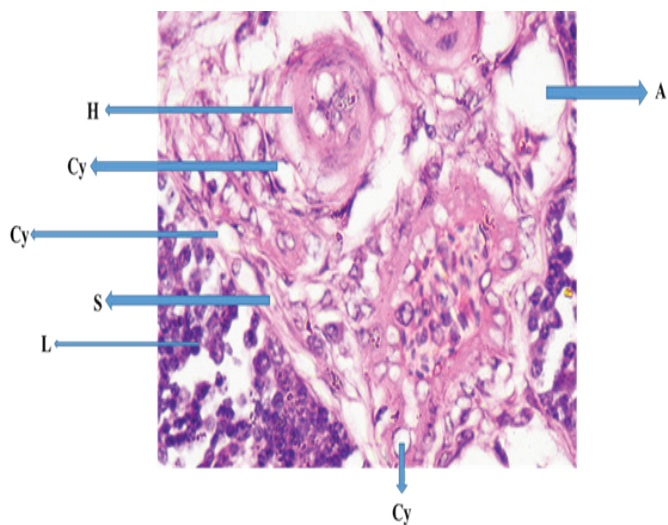


Fig.5. Photomicrograph of the thymus of Thirty two week-old Aseel chicken showing the marked involutory changes. H-Hassall's corpuscle Cy-Cyst S- Septal Fibres A-Adipose tissue L-Lymphocytes H&E X 1000

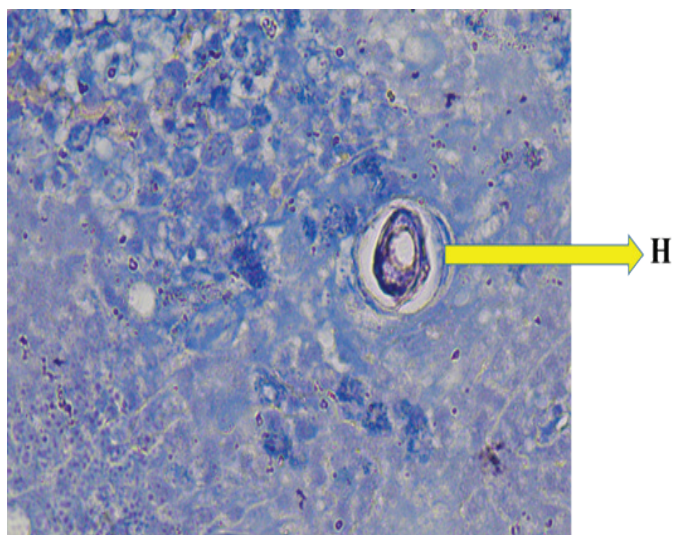


Fig.6. Photomicrograph of the thymus of Day-old Aseel chicken showing the Hassall's corpuscle (H) in the cortex PTAH X 1000

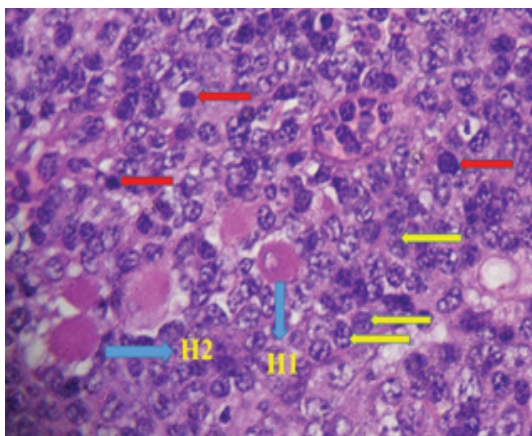


Fig.7. Photomicrograph of the thymus of Four week-old Aseel chicken showing the distribution of lymphoblasts(Yellow arrow) and lymphocytes(Red arrow) in the medullary region. H1- Type 1 Hassall's corpuscle H2- Type 2 Hassall's corpuscle H&E X 400

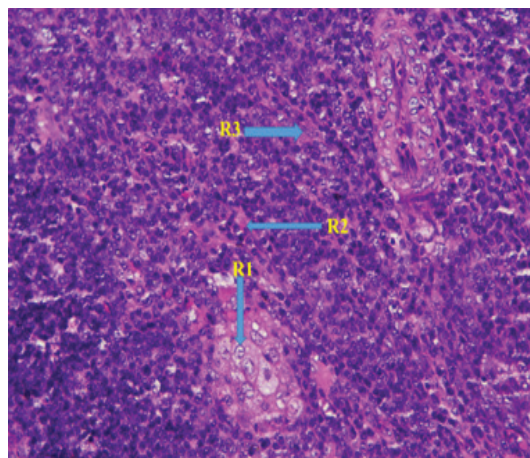


Fig.8. Photomicrograph of the thymus of Four week-old Aseel chicken showing the three types of Reticuloepithelial cells in the medullary region Type I-R1 , Type II- R2 and TypeIII-R3 H&E X 400

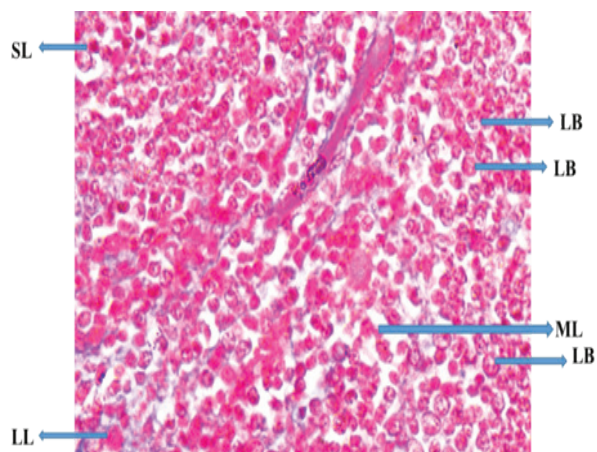


Fig.9. Photomicrograph of the thymus of Eight week-old Aseel chicken showing the distribution of lymphoblasts (LB) and lymphocytes of various sizes Small lymphocytes (SL) , Medium sized lymphocytes (ML), Large Lymphocytes (LL)in the medullary region. MTC X 400

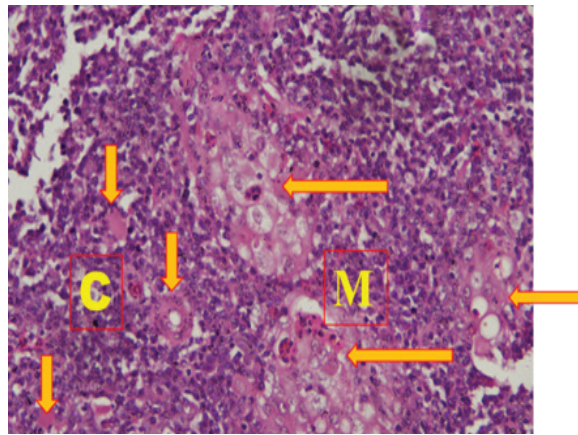


Fig. 10 Photomicrograph of the thymus of Eighteen week-old Aseel chicken showing the Hassall's corpuscles (Marked in arrows) in the cortex (C) and Medulla (M) H&E X 400

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