



Comparative histological and ultrastructural studies of thymus of indigenous and cross-bred broiler breeds of poultry of India

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India is a country where the weather is quite extreme and environment has lot of humidity. In such climatic conditions birds can be very prone to diseases and thus, poultry farmers are often at risk of heavy loss. Kadaknath is a very famed breed amongst the tribal group of Madhya Pradesh, a centrally located state of India. Another very sturdy broiler breed, known as Narmada Nidhi, was developed by College of Veterinary Science, Jabalpur in Madhya Pradesh, by crossing over Kadaknath breed and Coloured Broiler breed, under the ICAR scheme. The lymphoid tissue plays a salient role in the protective process countering pathogens. The defense system of poultry is made up of spleen, thymus and the bursa of fabricius and is categorized into two well-formed and operatively distinct components (Cooper *et al.* 1966).

Thymus in birds, is a twin organ in the neck constituting of segregated lobes of ovoid tissue. These are adjacent to vagus nerve and jugular vein and are quite active in hatchlings. Literature concerning age-dependent variations in avian thymus are scarce, fragmentary, and heterogeneous. The present study was aimed to compare structure of thymus between the aforementioned two Indian broiler breeds, which may guide us to a better acknowledgement of the anatomy of the organ in birds. This would in turn be greatly advantageous in the control and cure of different diseases.

The research was carried out in the Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Rewa, Madhya Pradesh for a period of about 10-12 months in 72 unvaccinated chicks (0 day old) chicks, 36 each of the two breeds under consideration. The chicks were raised separately at Rewa College Poultry Farm, till the age of above 32 weeks and then split into 6 groups and all groups had 6 birds each. The birds were

slaughtered ethically. The research study protocol was approved by the Institutional Ethical Committee for Animal Experimentation of College of Veterinary Science and Animal Husbandry, Rewa, Madhya Pradesh, following which the whole sample of thymus was taken and preserved for the investigation, after taking note of the position of the thymus *in situ*. The preserved tissue samples were fixed for 24 h in chilled 10% Neutral Buffered Formalin and then utilized for histomorphological studies as per the standard protocol (Singh and Sulochana 1997). Photography was done by employing Trinocular microscope fitted with camera.

Representative tissue samples from thymus were taken from the birds of same age group of both the breeds. Samples were then preserved in Karnowsky's fixative and were processed as per the standard protocol for electron microscopy in All India Institute of Medical Sciences (AIIMS), New Delhi. The desired photographs were taken after viewing in the electron microscope at AIIMS, New Delhi.

The results revealed that the thymic capsule was made of fibrous tissue, bordered by areolar connective tissue. The capsule was thin in case of younger birds of both the breeds, though, it slowly thickened with the progress of age because of growth in fibrous tissue. Fine septae were observed appearing from the capsule and pervading thymic lobes, finally segregating them into different sized lobules. The anatomy of thymic capsule was found to be alike to the observations of Kanasiya *et al.* (2018) who described that histologically thymus of Kadaknath was encapsulated by thin connective tissue capsule along with adipose tissue, finally segregating them into different sizes lobules. Similar findings were mentioned by Senapati *et al.* (2015) in quail, chicken and duck; Karmore *et al.* (2018) and Panwar *et al.* (2020) in Kadaknath birds, where they observed fine septae appearing from the capsule and pervading thymic lobes.

Cortex was noted to be peripherally located and darkly stained. It was formulated of crowded lymphocytes of medium, large and small size. Hassall's corpuscles in cortex were framed of reticulo-epithelial cells in both the breeds, under study. Lymphocytes were replaced by connective

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tissue accumulation following 18 weeks of period in Narmada Nidhi breed birds and beyond 24-32 weeks of age in birds of Kadaknath breed. Thymic cortex and medulla were distinctly marked in birds of Kadaknath breed at the age of 0-2 weeks. However, demarcation between the cortical and medullary regions was not easily recognizable at the age of 0-2 weeks in birds of Narmada Nidhi breed because of less discernible lobulation. Distinguishable lobules were clearly detectable in birds of Kadaknath breed and septae were noticed conveying blood capillaries and arteries.

In each thymic lobe, a lighter stained region containing less closely packed cells was identified as medulla. Lobulation was absent in the medullary region. This was conferring with the notings of Panwar *et al.* (2020) who referred that medulla was undivided by septae. It was also in accordance to the reportings of King and Mclelland (1984) who quoted thymic structure in aves to be homogenous as against to that in mammals which have lobules segregated by connective tissue. However, little or no lobulation was observed in certain lobes in a study conducted by Gulmez and Aslan (1999) in native geese. The cellular mass constituting medullary zone in current study, incorporated lymphoblasts, lymphocytes, mast cells, plasma cells, reticuloepithelial cells and also erythrocytes. In initial age groups, in birds of both the breeds under study, lymphoblasts dominated the medullary area. In the current investigation, medullary zone displayed the presence of macrophages eosinophils, basophils, heterophils and other lymphocytes such as plasma cells and mast cells in all age groups, in both the breeds of birds. The present investigation was in compliance to the opinion of Panwar *et al.* (2020) who remarked that thymocytes, reticuloepithelial cells, macrophages and myoid cells made the considerable part of parenchyma of thymus in different age groups of fowl. In both the breeds of birds under survey, results were in compliance with the reports of Kannan *et al.* (2018) in Nandanam chicken who illustrated the presence of granulocytes, mast cells and plasma cells which were few other cell types in all age groups.

In the current study, in all age groups, in both the breeds of birds, circular or oval, homogenous masses, entitled as Hassall's corpuscles containing lymphocytes and reticulo-epithelial cells were noted, arranged in central layers (Fig. 1). Core of Hassall's corpuscles was solid or cystic. Two types of Hassall's corpuscles were spotted: Type-I, that comprised of flattened reticulo-epithelial cells positioned in concentric manner and cornified mass was seen centrally in it and Type-II Hassall's corpuscles, in which hyaline mass was bordered by flattened reticulo-epithelial cells. Hassall's corpuscles had begun to develop at 0-2 weeks age in birds of Narmada Nidhi breed though, fully developed Hassall's corpuscles were seen in opulence at this age in Kadaknath breed birds. These corpuscles were observed in cortical region too in young age groups, till 8 weeks of age in birds of Narmada Nidhi breed. But they were smaller in size and were also less in number, with respect to that in

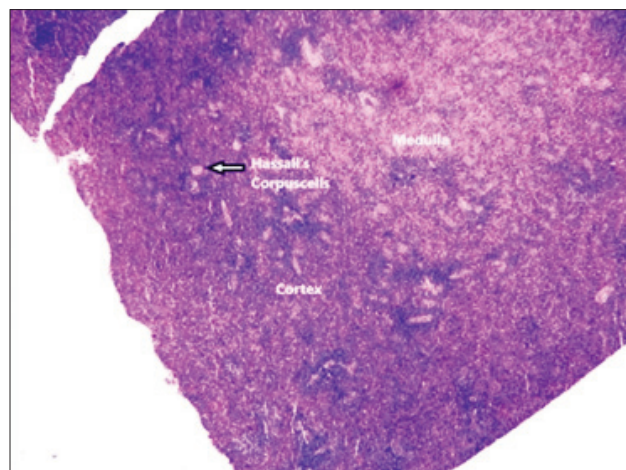


Fig. 1. Photomicrograph of thymus of 4-8 weeks old Narmada Nidhi bird showing Hassall's Corpuscles (white arrow) cortex and medulla. H&E (4×)

medullary zone. Though, their number increased in medulla with the increase in age of birds in Kadaknath breed as well as Narmada Nidhi breed. This was in accordance with the findings of Gulmez and Aslan (1999) who detailed that numerous Hassall's corpuscles were varying in shapes and sizes as found in medulla. Conversely, Muthukumaran *et al.* (2011) quoted that in turkey, three types of Hassall's corpuscles were observed.

Large euchromatic nucleus having compiled chromatin material and varying number of free polyribosomes were observed in large cells which were distinguished as lymphoblasts. Round nucleus was also seen in smaller lymphocytes containing multiple ribosomes and the chromatin material was seen congregated at the periphery. Macrophages and melanin pigment were notable (Figs. 2 and 3). Golgi complex and lysosomes were intermittently seen. Study revealed observations which were in harmony with the findings of Panwar *et al.* (2020) who discovered that smaller and medium lymphocytes were almost spherical in shape. In Nandanam chicken, Kannan *et al.*

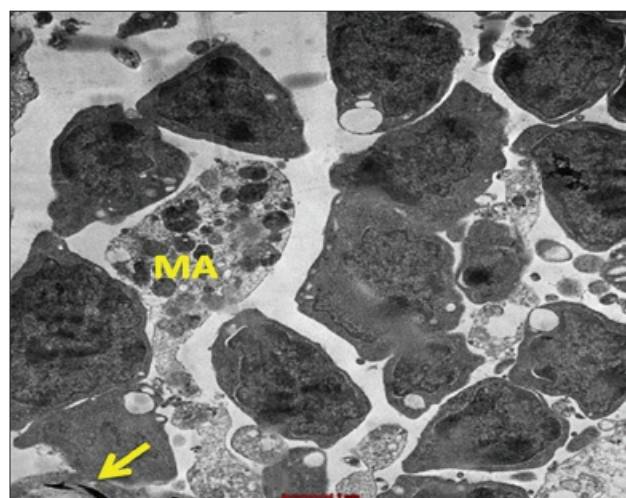


Fig. 2. Photomicrograph of thymus of 8-16 weeks old Narmada Nidhi bird showing macrophage (MA) and melanin pigment (arrow) (2600×).

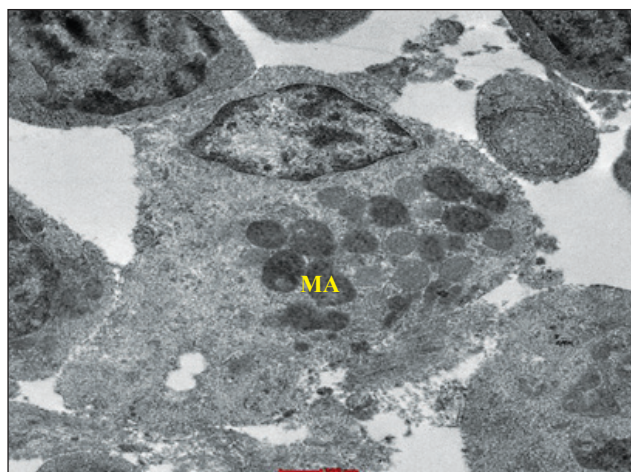


Fig. 3. Photomicrograph of thymus of 4-8 weeks old Kadaknath bird showing macrophage (MA) (5500 $\times$).

(2018) found that the larger lymphocytes possessed only one or two nuclei.

It was found that the two breeds of poultry exhibited resemblance in histology as well as ultrastructure of thymus. The parallelism in the results could be accredited to the fact that Narmada Nidhi has 25% genetic contribution of Kadaknath breed of poultry manifesting the alikeness in anatomy of the organ and the physiological hardiness of both the breeds under research.

SUMMARY

The present study was aimed to compare structure of thymus between the two poultry breeds, namely, Kadaknath and Narmada Nidhi. The aim was to understand the anatomical differences which could provide insights into how different breeds respond to diseases. The study involved histomorphological analysis of thymus tissue samples from 72 chicks (36 from each breed) that were divided into 6 groups with 6 birds in each group. The study results revealed that both poultry breeds exhibited similarities in the histology and ultrastructure of the

thymus. This similarity is likely due to the Narmada Nidhi breed having a 25% genetic contribution from the Kadaknath breed.

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