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Comparative age related histological observations on bursa of fabricius of Kadaknath and Narmada Nidhi birds

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ABSTRACT

Poultry meat is one of the most important sources of protein for human consumption worldwide. Chicken and turkeys are considered as healthy sources of protein because they have relatively low levels of saturated fat compared with red meat. Kadaknath is a native breed, found in the tribal dominated Jhabua district of Madhya Pradesh, India. But its meat is priced three times more than that of broiler chicken. Narmada Nidhi is an improved location specific breed of chicken developed by the College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India. This breed is a dual purpose coloured one, which is good for tribal and rural areas of the country for poultry farming. Bursa of fabricius, in Kadaknath and Narmada Nidhi breeds of birds, were studied and compared histologically. It was found to have wall made of four layers, tunica serosa, tunica muscularis, viz. tunica submucosa and tunica mucosa from outside to inside. In both the breeds, internal surface of the wall was thrown into folds known as plicae, lined by pseudo stratified epithelium except at the crypts where columnar epithelium was seen. Four types of epithelium in follicles and two types of epithelium, (FAE and IFE), attached to plicae of bursa of both the breeds were identified. Septae thickness in bursa of fabricius of Narmada Nidhi was found to be more. Similarily, cortex was more densely packed in bursa of Narmada Nidhi.

Keywords: Bursa of fabricius, Histology, Kadaknath, Narmada Nidhi, Native breed, Poultry meat

Poultry have existed for over 150 million years on earth, dating back to the original wild jungle fowl. Now the list of species under poultry include ducks, geese, turkeys, pigeons, chickens and so on. Chickens were domesticated in South East Asia at least 8000 years ago (Lawal and Hanotte 2021). Around the world, people consume poultry, ducks, geese, guinea fowl, pheasants, turkeys, and even more exotic birds, like ostriches. Kadaknath, which is locally known as Kalamasi, is a native breed of chicken found in Jhabua district of Madhya Pradesh, which is dominated by tribal people, though its meat is valued three times more than that of broiler chicken. Narmada Nidhi is an improved location specific breed of chicken developed by the College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh. This breed was developed by crossing Jabalpur Colour (coloured broiler) breed of chicken with Kadaknath (native). The final crossed breed has 75% inheritance of Jabalpur colour breed and 25% inheritance of Kadaknath breed. The lymphatic system plays a vital role in the defence mechanisms against all pathogens. In poultry, it consists of spleen, thymus and the bursa of fabricius, and is divided into two distinct morphologically and functionally different components (Cooper *et al.* 1966). The bursa of fabricius is a globular or spherical lymphoepithelial organ. The inner surface is thrown into folds which obscure the lumen. Its growth is correlated with the rapid body growth. It regresses and disappears at the time of sexual maturity. Perusal of literature on the topic revealed that no work has been done on comparative studies on histological characteristics of bursa of fabricius of Kadaknath and Narmada Nidhi breeds of poultry in India as well as abroad.

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MATERIALS AND METHODS

The present study was conducted in the Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Rewa (Madhya Pradesh). Seventy two unvaccinated chicks (0^{th} day old), thirty six each of Kadaknath and Narmada Nidhi breeds were reared separately at College Poultry Farm, Rewa, from 0^{th} day old to above 32^{nd} week of age. These birds were divided into six groups, viz. 0^{th} to 2^{nd} week, 2^{nd} to 4^{th} week, 4^{th} to 8^{th} week, 8^{th} to 16^{th} week, 16^{th} to 32^{nd} week and above 32^{nd} week, and each group contained six birds of both the breeds. After getting permission from the ethical committee of the parent institute, birds were sacrificed ethically at respective

age intervals and tissue samples from bursa of fabricius were taken from the same age groups of both the breeds of birds. Whole specimens and the tissue samples were then preserved in 10% Neutral Buffer Formalin. Tissue samples were fixed after proper labelling. After fixation, they were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968). The paraffin embedded tissue blocks were cut in sections. The sections of 4 to 5 µm thickness were obtained with the help of rotary microtome. These sections were floated in water bath (50°C) and mounted on albumin coated glass slides. They were dried at 37°C in hot air oven and preserved carefully for staining. Subsequently, they were subjected to routine histomorphological staining methods viz. Haematoxylin and Eosin, Fontana-Masson (melanin stain) and PAS–Alcian Blue.

RESULTS AND DISCUSSION

In bursa of fabricius of both the breeds of birds under study, the wall of the organ was made of four layers, viz. tunica serosa, tunica muscularis, tunica submucosa and tunica mucosa from outside to inside. Tunica serosa was thin and enclosed the organ in both the breeds. The connective tissue was observed to be filled with follicles of different sizes and shapes. The lining epithelium was made of pseudo stratified columnar epithelium in both the breeds at all age groups whereas in crypts, it was columnar epithelium. Four different types of epithelium were found in follicles in both the breeds in the present study. Type 1 cells were principal cells, they were pseudo stratified columnar type; Type 2 cells were placed basally; Type 3 cells were apical cells and Type 4 cells were goblet cells, which were scattered amongst the columnar cells. The epithelium covering the plicae were of two types, one was Follicle Associated Epithelium (FAE) and the other was, Interfollicular Epithelium (IFE). FAE was pseudo stratified ciliated columnar epithelium whereas IFE was simple columnar in crypts which was dissimilar to the observations of Karadeg Sari *et al.* (2015) who suggested two different types of epithelial cells on the plicae, follicle associated epithelium (FAE) identified as simple squamous and cuboidal in shape, which was associated with the lymphoid follicle and Interfollicular epithelium (IFE) which was identified as columnar in shape. However, the findings are similar to that of Davenport and Allen (1995) who reported that the epithelium had features of pseudo stratified columnar epithelium. IFE was observed to be covering the rest of the plical part. Columnar cells were darkly stained and these were placed between the follicles. FAE was observed to continue without any interruption or gaps in both the breeds (Fig. 1). It was found to be distended in early age groups in both the breeds. Lymphoid follicles completely filled each plicae in both the breeds. The follicles varied in shapes and sizes, being separated by interfollicular connective tissue (Fig. 4). Each follicle was composed of outer dark cortex and inner lighter medulla (Fig. 2). These findings were similar to those of Kanasiya *et al.* (2018) who suggested that the follicles in Kadaknath breed were divided into cortex and medulla. In early age (0^{th} to 2^{nd} week), the distinction between medulla and cortex was not clear in both the breeds. Gradual differentiation was visible at around 2^{nd} to 4^{th} week of age in both the breeds. The clear cut cortex and medulla distinction was visible in 4^{th} to 8^{th} week of age in the Kadaknath as well as Narmada Nidhi birds which was in accordance to the findings of Yadav *et al.* (2020). But this finding was different from that of Kanasiya *et al.* (2018), who suggested that there was no clear cut distinction between cortex and medulla. Medulla and cortex were divided by a corticomedullary junction. In the present study, a layer of epithelial cells resting on a clear and distinct basement membrane made the corticomedullary junction (Fig. 5). Cortex was densely packed in Narmada Nidhi breed as compared to Kadaknath breed birds at the age of 8^{th} to 16^{th} week. Medulla had loosely packed cells. The thickness of tunica mucosa increased till 8^{th} to 16^{th} week age beyond which it started diminishing in Narmada Nidhi birds while it was after 16^{th} to 32^{nd} week in Kadaknath birds. These findings were similar to those of Yadav *et al.* (2020). Large blood vessels were also seen in the connective tissue core. At 8^{th} to 16^{th} week age, follicles were well developed in Kadaknath breed birds with respect to that in Narmada Nidhi birds. Each follicle was composed of outer dark cortex and inner lighter medulla (Fig. 2). These findings were similar to those of Kanasiya *et al.* (2018) who suggested that the follicles in Kadaknath breed were divided into cortex and medulla. In early age (0^{th} to 2^{nd} week), the distinction between medulla and cortex was not clear in both the breeds. Gradual differentiation was visible at around 2^{nd} to 4^{th} week of age in both the breeds (Fig. 3). At the age of 4^{th} to 8^{th} week, the follicular size in Kadaknath birds was smaller and interfollicular septa was thin in Kadaknath breed birds as compared to that in Narmada Nidhi breed birds (Fig. 6). The present observations showed that there was early development of bursa of fabricius in Narmada Nidhi breeds as compared to that in Kadaknath breed birds. In the present study, a layer of epithelial cells resting on a basement membrane made the corticomedullary junction (Fig. 5). But corticomedullary epithelial layer was reported to be absent in quail (Nagy *et al.* 2004). Also, at 4^{th} to 8^{th} week age, corticomedullary junction and demarcation between cortex and medulla was not very clear in Kadaknath breed birds with respect to that in Narmada Nidhi birds which was dissmllar to the observations of Leena *et al.* (2012) in domestic fowl who reported that cortex and medulla were clearly differentiated by 4^{th} week of age. Tunica muscularis layer was found to be composed of longitudinal and circular smooth muscle fibres in both the breeds of birds under study (Fig. 7). The findings were in agreement with those of Yadav *et al*. (2020) who stated that the tunica muscularis layer was formed by circular and longitudinally arranged smooth muscle fibres in Kadaknath birds. Inner longitudinal layer penetrated the connective tissue core at some areas. Tunica muscularis was thin in Kadaknath birds

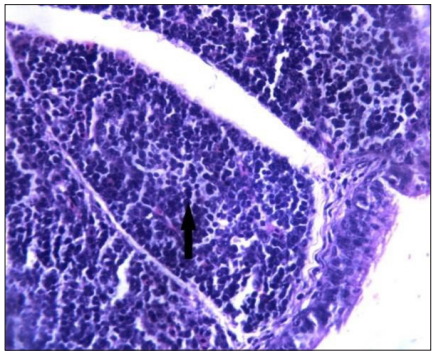

Fig. 1. Bursa of fabricius of 0 to 2 week old Kadaknath bird showing distended follicles and continuous epithelium. H&E (40×).

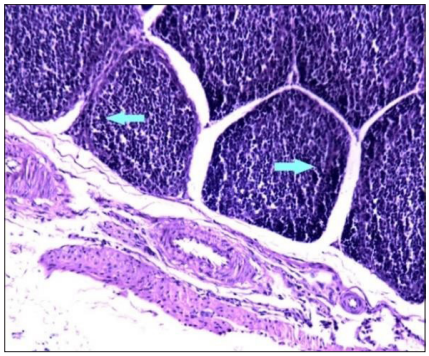

Fig. 2. Bursa of fabricius of 2 to 4 week old Narmada Nidhi bird showing differentiated cortex and medulla. H&E (20×).

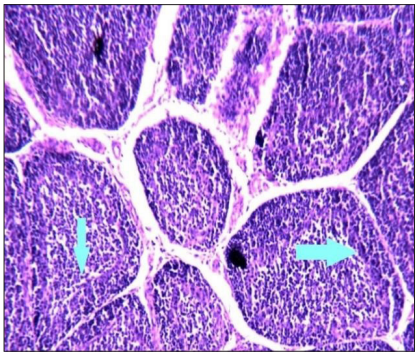

Fig. 3. Bursa of fabricius of 2 to 4 week old Kadaknath bird showing differentiated cortex and medulla. H&E (10×).

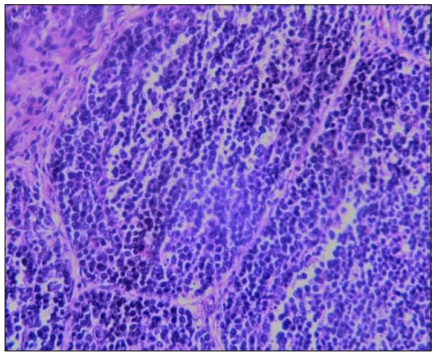

Fig. 4. Bursa of fabricius of 2 to 4 week old Kadaknath bird showing organized follicles. H&E (40×).

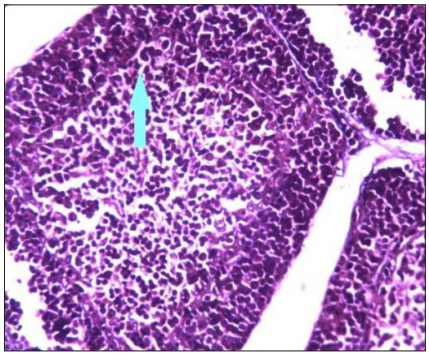

Fig. 5. Bursa of fabricius of 2 to 4 week old Narmada Nidhi bird showing organized follicles. H&E (40×).

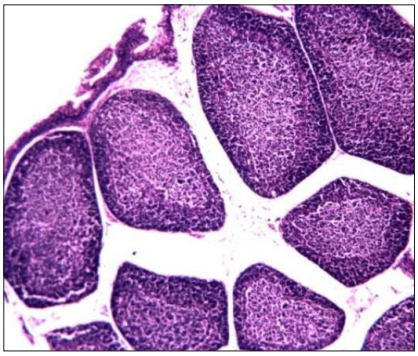

Fig. 6. Bursa of fabricius of 4 to 8 week old Kadaknath bird showing organized follicles. H&E (10×).

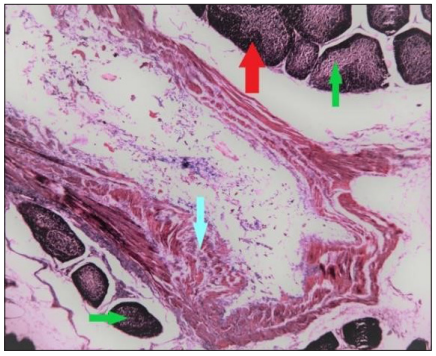

Fig. 7. Bursa of fabricius of 8 to 16 week old Narmada Nidhi bird showing thickened cortex (red arrow), regressing medulla (green arrow) and outer longitudinal and inner circular muscle fibres in tunica muscularis (blue arrow). H&E (4×).

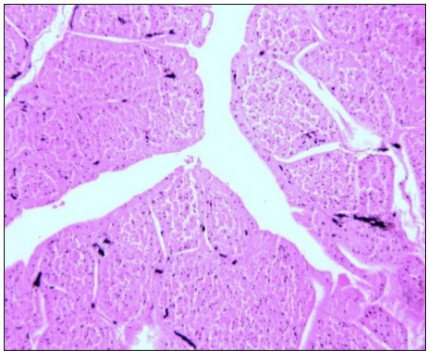

Fig. 8. Bursa of fabricius of 2-4 weeks old Kadaknath bird showing intense positive reactivity for Fontana Masson stain for melanin. (20×).

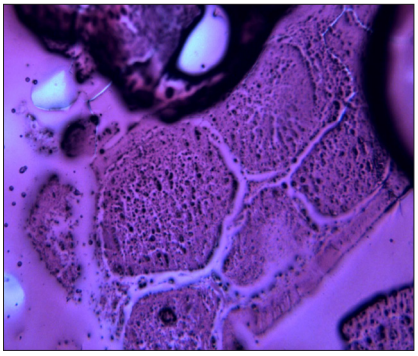

Fig. 9.. Bursa of fabricius of 2-4 weeks old Narmada Nidhi bird showing positive reactivity for PAS-Alcian Blue stain for mucopolysacarides. (20×).

as compared to that in Narmada Nidhi breeds at the age of 4th to 8th and 8th to 16th week age. It was found to be double layered in Narmada Nidhi birds at the age of 8th to 16th week (Fig. 7) while it was single layered in Kadaknath breed birds at the same age. At the age of 4th to 8th week, tunica muscularis was thick in Narmada Nidhi breeds bird and cortex was thin. Results suggested that Narmada Nidhi birds grow faster being a broiler breed. Connective tissue fibres formed the tunica serosa whose thickness increased initially with age but started to decrease after about 16th week in Kadaknath and Narmada Nidhi breeds. Tunica serosa was also found to be more in thickness at the age of 8th to 16th week in Narmada Nidhi birds with respect to that in Kadaknath birds.

Optimum growth of the organ was observed the age of 8th week in Kadaknath and Narmada Nidhi breeds. In contrast to the observations of Franchini and Ottaviani (1999) who reported that bursal regression started from

2nd month-old in White Leghorn chicken. In the present study, conducted in Kadaknath breed as well as Narmada Nidhi breed, bursa of fabricius started regressing after 18th week of age while in Kadaknath breed, it started regressing after 24th week of age onwards. This was similar to the results reported by Yadav *et al.* (2020) who stated that the bursa of fabricius started regressing at the age of 28th week post hatch in Kadaknath breed of poultry. Milicevic *et al.* (1986) also noted that the involution of bursa started at 24th week of age. It could be concluded that the timing of bursal regression probably depended on the breed of chicken, based on the environmental situation, and also determined genetically (Bellamy and Mohamed 1982). Elongated flask shaped follicles were observed in the plicae. The lymphocyte population was replaced by fibroblasts and adipose tissue. During the involutory phase, degenerating lymphocytes were found. Cysts were also seen in the medulla of Kadaknath as well as Narmada Nidhi breed birds at this stage. Lymphocytes aggregates or free lymphocytes were observed in the lumen in early involutary phase. Plical epithelium was found to be vacuolated and detached. The height of the epithelium of plicae also decreased as the involutory phase progressed. In both the breeds of birds under study, medullary region showed positive reaction for PAS–Alcian blue test (Fig. 9). Strong positivity was also seen in FAE and IFE cells for PAS–Alcian blue, especially in 8th-16th week age group. This may be attributed to increase in amount of deposition of polysaccharides in these regions with the advancement of age. Though tunica submucosa, tunica muscularis and tunica serosa showed weak positive results in all age groups in both the breeds of birds. Melanin pigment deposition was seen only in Kadaknath breeds in all age groups under study (Fig. 8). Fontana Masson staining revealed melanin presence in connective tissue in Kadaknath birds in all age groups but not in Narmada Nidhi bird. These findings were similar to the observations of Kanasiya *et al.* (2018) and Yadav *et al.* (2020). Melanocytes have been proven to produce key chemokines and cytokines (Gasque and Jaffar Bandjee 2015).

In both the breeds, the wall of the bursa was made of four layers, namely tunica serosa, tunica muscularis, tunica submucosa and tunica mucosa from outside to inside. Tunica mucosa was found to be thrown in folds (plicae) which were composed of connective tissue core (lamina propria) and the lining epithelium. The thickness of tunica mucosa increased till 8th to 16th week age in Narmada Nidhi birds beyond which it started diminishing while it was after 16th to 32nd week in Kadaknath birds. The lining epithelium was made of pseudo stratified columnar epithelium in both the breeds at all age groups whereas in crypts, it was columnar epithelium in both the breeds of birds under study. Number of goblet cells was higher in Narmada Nidhi birds in 8th to 16th week of age whereas their number was higher in Kadaknath birds in 16th to 32nd week of age. The epithelium covering the plicae were of two types, one was Follicle Associated Epithelium (FAE) and the other was, Interfollicular Epithelium (IFE). Tunica muscularis was thin in Kadaknath birds as compared to that in Narmada Nidhi breeds at the age of 4th to 8th age. It was found to be double layered in Narmada Nidhi birds at the age of 8th to 16th week while it was single layered in Kadaknath breed birds at the same age. Plasma cells, large, medium and small lymphocytes, macrophages and RBCs. Optimum growth of the organ was observed at the age of 8 week in Kadaknath and Narmada Nidhi breeds. In Kadaknath breed as well as Narmada Nidhi breed, bursa of fabricius started regressing after 16th to 32nd week of age.

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