Comparative histochemical study of the major lymphoid organs of local hill fowl of Uttarakhand (Uttara Fowl) and Rhode Island Red (RIR)

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Abstract

The present study was conducted on the major lymphoid organs i.e. thymus, spleen and bursa of Fabricius of 24 birds of the local hill fowl of Uttarakhand (Uttara Fowl) and 24 birds of Rhode Island Red (RIR) divided into four age groups viz. day old, 1 month, 3 months and 6 months old birds with six birds in each age group. The capsule, trabeculae and Hassall’s corpuscles of thymus showed PAS positive and Alcian blue activities in both the birds. In case of the spleen of both Uttara fowl and RIR the capsule, blood vessels and some cells of white pulp showed moderate PAS positive activity. The apical border of surface epithelium of bursa of Fabricius showed PAS positive and Alcian blue activities in both the birds.

Keywords: Thymus, spleen, bursa of fabricius, PAS and alcian blue

Introduction

In the last four decades poultry production in India has taken a quantum leap, emerging from a traditional farming practice to commercial production system [15]. Uttarakhand have a total population of 26.01 lakh poultry and per capita availability (2015-16) of eggs and chicken in Uttarakhand is 37 and 1.15 kg/head respectively which is less than the national recommended level [11]. One of the indigenous dual purpose breed of bird found in Uttarakhand is local hill fowl or Uttara fowl which is generally reared under the backyard system in Kumaon division of Uttarakhand [7, 8]. The climatic condition of this division during winter is quite harsh, where environmental temperature occasionally goes down below freezing point. Meat of local hill fowl is very tasty and chewy, so it is very popular among the people of rural hilly areas. Due to low cholesterol level in the meat of local hill fowl it is considered appropriate for heart patients and obese people [13]. Another most popular dual purpose breed of poultry is Rhode Island Red (RIR) which is an American breed of domestic chicken. RIR originated from Rhode Island in New England and developed by crossing with red Malay Game, brown Leghorn and Asiatic native stock [3]. RIR is a good choice for the small flock owners because they can rear easily in marginal diets and poor housing condition. They have somewhat rectangular, relatively long bodies with flatten back and broad breast which make it a good meat producing birds. The major components of body defense mechanism are the innate and acquired or adaptive immunity. Innate immunity includes physical barrier like skin and mucous membrane, complement and cells like granulocytes, thrombocytes, macrophages and natural killer cells. On the other hand, acquired or adaptive immunity is mediated by immunocompetent cells viz., humoral response B-cells, cell mediated response T-cells and some other cells like phagocytic and adherent cells [5]. All these immunocompetent cells are located mostly in the lymphoid organs. With the advancement of bird age, the distinction between the primary and secondary lymphoid organs becomes less apparent [18]. Though some work on lymphoid system of broiler chicken [12], domestic chicken [10], Japanese quail [17], duck [20], turkey [1], Kadaknath [14] has been done, but still the comparative histochemical study on the thymus, spleen, bursa of Fabricius between Uttara fowl and RIR has not been explored. Keeping this fact in mind and viewing the increased popularity of both the birds, the present study was undertaken to develop a baseline data in this breed.
Materials and Methods
The present study was carried out on 24 birds of the local hill fowl of Uttarakhand (Uttara Fowl) and 24 birds of Rhode Island Red (RIR) procured from the Instructional Poultry Farm (IPF), Nagla, GBPUAT, Pantnagar. These birds (local hill fowl and RIR) were divided into four group viz. day old, 1 month, 3 months and 6 months old birds with six birds in each age group. The birds were sacrificed by cervical sub-luxation method. Feathers were removed manually and the organ of interest i.e. thymus glands, spleen and bursa of Fabricius were carefully dissected out. The representative tissue samples of around 4-5 mm were cut using BP blade and were fixed in 10% neutral buffered formalin. After overnight washing, dehydration (in graded alcohol), clearing (in xylene), impregnation and embedding (in paraffin wax) followed by mold preparation the tissue sections of 4-5 micron were cut using Leica rotary microtome (RM2125RT), Japan and stained by PAS technique- Carbohydrate [6] and Combined PAS-Alcian blue staining for acid and neutral mucins [2] for comparative histochemical studies. The stained tissue sections were examined under Nikon Microscope and photomicrography were performed with eclipse Ci-L/S microscope.

Results and Discussion
The capsule, trabeculae (Fig. 1) and Hassall’s corpuscles of thymus were moderately PAS positive at 0 day, 1 month and 6 months of age in both Local hill fowl and RIR. There was intense PAS positive reaction in the cortical trabeculae at few places at the 3 months of age in both Local hill fowl and RIR. Mild Alcian blue activity was observed in the capsule and trabeculae of thymus in 0 day and 1 month of age in both Local hill fowl and RIR. Moderate and intense activities for Alcian blue was shown by capsule, trabeculae (Fig. 2) and Hassall’s corpuscles (Fig. 3 and Fig. 4) at 3 months and 6 months of age respectively in both Local hill fowl and RIR. The present findings were similar to the observations of Bhattacharya [4] in chicken and Gulmez and Aslan [6] in native geese. The capsule (Fig. 8), tunics of blood vessels (Fig. 5) and connective tissue present in the white pulp of spleen showed moderate PAS positive activity in all the age groups under study in both Local hill fowl and RIR. The present findings were similar to the observations of Singh [19] in keets. Similarly, the capsule (Fig. 8), intercellular region of white pulp (Fig. 7) and tunics of blood vessels (Fig. 6) of spleen showed mild to moderate activity for Alcian blue in 3 months of age group in both Local hill fowl and RIR. While [19] reported that there was no Alcian blue positive reaction in the spleen of keets. The apical border (Fig. 10) of both follicle associated epithelium and inter follicular epithelium, medulla of bursa follicle, tunics of blood vessels, tunica muscularis, tunica serosa showed moderate PAS reaction in 0 day and 1 month of age in both Local hill fowl and RIR. The centre of plica showed intense PAS positive reaction in 1 month of age in both the birds (Fig. 9). The intense PAS positive activity was observed at the apical border of epithelium and moderate activities were found at the sub-epithelial region, cortico-medullary junction, tunica muscularis, tunica serosa and all the layers of blood vessels in 3 months of age in both Local hill fowl and RIR and 6 months of age in RIR. The tunics of blood vessels showed intense PAS positive activity in 3 months of age in both the birds. In bursa of Fabricius the apical border of follicle associated epithelium and inter follicular epithelium showed moderate Alcian blue activity in 0 day of age in both Local hill fowl and RIR, which gradually decreased in subsequent age groups where mild to moderate activity was observed in 1 month and 3 months of age. No Alcian blue activity was observed in the apical border of follicle associated epithelium and inter follicular epithelium in 6 months of age in RIR (Fig. 11). The tunica muscularis, tunica serosa, inter follicular connective tissue, tunica intima and tunica media of blood vessel showed moderate Alcian blue activities in 0 day, 1 month and 3 months of age (Fig. 12) in both the birds. Intense activity was seen in the goblet cells of lining surface epithelium (Fig. 11). The present findings were similar to the observations of Gulmez and Aslan [6] in native geese and Jayachitra et al. [19] in turkeys.

Fig 1: Photomicrograph showing intense PAS positive reaction in the trabeculae (yellow arrow) of the thymus of 3 months old local hill fowl (PAS×400).

Fig 2: Photomicrograph showing intense Alcian blue activity in the trabeculae (yellow arrow) of the thymus of 6 months old RIR (Alcian blue×400).

Fig 3: Photomicrograph showing moderate Alcian blue activity in the Hassall’s corpuscle (yellow arrow) of the thymus of 3 months old RIR (Alcian blue×400).
Fig 4: Photomicrograph showing moderate activity of PAS (white arrow) and intense activity of Alcian blue (yellow arrow) in the Hassall’s corpuscle of the thymus of 6 months old local hill fowl (AB-PAS×400).

Fig 5: Photomicrograph of spleen showing moderate PAS positive activity in the tunics of blood vessels (a, b and c) and White pulp (d) in 3 months RIR (PAS, X400).

Fig 6: Photomicrograph of spleen showing mild Alcian blue activity in the tunics of central artery (red arrow) in 3 months RIR (Alcian blue, X400).

Fig 7: Photomicrograph of spleen showing moderate Alcian blue activity in the cells of white pulp (red arrows) in 3 months local hill fowl (Alcian blue, X1000).

Fig 8: Photomicrograph of spleen showing moderate PAS positive (a) and mild Alcian blue activity (b) in the Capsule of 3 months local hill fowl (AB-PAS, X400).

Fig 9: Photomicrograph of bursa of Fabricius showing moderate PAS positive activity in the centre of plica (a) and trabeculae (b) in 3 months RIR (PAS, ×100).

Fig 10: Photomicrograph of bursa of Fabricius showing moderate PAS positive activity in the apical border of surface epithelium in 1 month old local hill fowl (PAS, ×400).

Fig 11: Photomicrograph of bursa of Fabricius showing intense Alcian blue activity in the goblet cells (a) and epithelial cyst (b), but no activity in apical border of surface epithelium in 6 months RIR (Alcian blue stain, ×100).
Fig 12: Photomicrograph of bursa of Fabricius showing intense Alcian blue activity in the goblet cells and epithelial cyst (red arrows), moderate activity in the apical border of surface epithelium (green arrow) and moderate PAS positive activity in the connective tissue of centre of plicae and trabeculae (yellow arrows) in 3 months old RIR (AB-PAS,×100).

Conclusion
There was intense PAS positive reaction in the cortical trabeculae of thymus at the 3 months of age in both the birds. Mild Alcian blue activity was observed in the capsule of thymus in 0 day and 1 month of age but there are moderate and intense activities for Alcian blue was shown by capsule, trabeculae and Hassall’s corpuscles at 3 months and 6 months of age respectively in Uttara fowl and RIR. Histochemical studies of spleen revealed that the capsule, tunics of blood vessels and white pulp of spleen showed moderate PAS positive activity in all the age groups while these parts showed mild to moderate Alcian blue activities in 3 months of age groups. Histochemical studies of bursa of Fabricius revealed that the apical border of epithelium, tunic of blood vessels, tunica muscularis, tunica serosa showed moderate PAS reaction in 0 day and 1 month of age in both Local hill fowl and RIR. The apical border of follicle associated epithelium and inter follicular epithelium showed intense Alcian blue activity in 0 day of age in both the birds which gradually decreased in subsequent age groups. Intense activity was observed in the goblet cells. The present study revealed that there is no such major histochemical difference in the major lymphoid organs of Uttara fowl and RIR in each age group.

References