AATCC Review

25 October 2022: Received
28 January 2023: Revised
11 February 2023: Accepted
22 March 2023: Available Online

www.aatcc.peerjournals.net

Research Article Open Access

Histomorphometrical and Histochemical studies on the glandular stomach *[proventriculus]* of Poonchi bird of Union Territory of Jammu and Kashmir

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ABSTRACT

Histologically, proventriculus of Poonchi bird consisted of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Tunica mucosa was arranged in folds of variable height. Lamina epithelialis consisted of simple columnar epithelium. Tunica submucosa presented compound tubule-alveolar submucosal/proventricular glands which varied from round, oval, hexagonal or polymorphic lobules separated by connective tissue. Within each lobule, numerous glandular tubules and alveoli had radiating arrangement lined by cuboidal epithelium. Tunica muscularis consisted of three layers of smooth muscles. Tunica serosa consisted of loose connective tissue containing nerves and blood vessels. Thickness of tunica mucosa was 490.86 ± 43.47 µ. Tunica submucosa was 1641.16 ± 116.49 µ thick. Thickness of tunica muscularis was 427.99 ± 22.06 µ. The outer muscle layer was thickest (114.37 ± 9.81 µ). The thickness of tunica serosa was 83.68 ± 3.64 µ. Histochemically, the mucosal epithelial lining showed strong positive reaction to PAS-AB (pH 2.5). The glandular secretions were strongly positive to PAS. The epithelial lining of secretory ducts and secretory units of the compound tubule-alveolar glands showed strong reaction with both PAS stain and PAS-AB stain indicating the presence of both neutral and acid mucins. With PAS-AB stain, the cells towards the central cavity-stained reddish purple whereas towards bottoms, cells were bluish indicating acidic mucins. Submucosal glands and tunica muscularis layer showed strong reaction for basic proteins. Cells of sub-mucosal glands showed moderate to strong reaction for lipids in supra-nuclear zone.

Keywords: *Gland, Histomorphology, Histochemistry, Poonchi, Proventriculus*

INTRODUCTION

The Union Territory of J&K belongs to the greater Himalayan Mountain range which exerts significant influence on its agro-climatic conditions. Poonch has a humid subtropical climate which is much cooler than rest of India due to its moderately high elevation and northerly position. Indigenous poultry rearing provides sustainability to the local people. The local Poonchi bird weighs about 2.1-2.5 kg (cock) and 1.6-1.8 kg (hen). Females are combless whereas males presents a red coloured comb.

The digestive apparatus comprises the organs concerned with the mechanical reduction, chemical digestion and absorption of food. The most active part of digestive system of birds is a stomach which is formed of two parts, namely glandular portion (proventriculus) and the muscular stomach (gizzard) which is located caudal to the proventriculus [1]. Both parts are characterized by a great morphological and function variability, both between and within species [2]. The glandular stomach (proventriculus) is relatively small in graminivores but quite large and distensible in carnivores that ingest large food items [2]. The proventriculus secretes HCl and pepsin needed for protein digestion [3].

Literature is available on the histomorphology of proventriculus of red jungle fowl [4], Coot bird [5] mallard [6] Black-tailed Crake [7] uttara fowl [8], Egyptian mallard [9], Iraqi falcon [10], adult starling bird [11] domestic fowl and Kestrel [12] Japanese quail [12], guinea fowl [14] and ostrich [15]. Micrometrical studies had been done on the proventriculus of uttara fowl [16] Japanese quail [17] and ostrich [18]. Data is also available on the histochemistry of mallard [19], Black-tailed Crake [20] moorhen [21] turkey [22], Iraqi falcon [23] and guinea fowl [24]. But no data is available on the histomorphometry and histochemistry of proventriculus of local birds of Poonch district of UT of J&K. Therefore, the present study was undertaken to generate information on histomorphometry and histochemistry on the proventriculus of Poonchi bird as this bird can survive in cold weather by utilizing locally available feed. The study will further help to unveil the distribution and localization of various histochemical components.

MATERIALS AND METHODS

Sample collection: Carcass of 06 Poonchi birds was obtained from Division of Animal Genetics and Breeding, F.V.Sc & A.H., SKUAST-Jammu. Immediately after collection, tissue samples of

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DOI: https://doi.org/10.58321/AATCCReview.2023.11.02.246
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proventriculus were fixed in 10% Neutral Buffered Formalin for 24 hours.

Sample processing for light microscopy: For histomorphology ,tissue samples were processed and sections of 5 µ thickness were obtained. The sections were subjected to various histological and histochemical methods as detailed hereunder.

1. Hematoxylin and Eosin stain for routine histomorphology
2. Periodic Acid Schiff's method for neutral mucin
3. PAS-AB (pH 2.5) for neutral and acid mucin
4. Bromphenol blue for basic proteins
5. Sudan Black B for lipids

Micrometrical parameters recorded: Different micrometrical parameters were recorded:

a. Thickness of Tunica mucosa (µ)
b. Thickness of Tunica submucosa (µ)
c. Thickness of Tunica muscularis (µ)
d. Thickness of inner, middle and outer muscle layers (µ)
e. Thickness of Tunica serosa (µ)
f. Maximum and minimum diameters of glandular lobule (µ)

Statistical analysis: The results were presented as Mean ± Standard Error. The data was subjected to standard statistical analysis [25].

RESULTS AND DISCUSSION

Proventriculus of Poonchi bird consisted of four tunics from inside to outside as tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Fig. 1). Similar findings were observed by [26] in guinea fowl. However, the proventriculus of Asiatic swiftest (Marshall and Folley 1965) and Black-tailed crake [27] had three layers namely tunica mucosa, tunica muscularis and tunica serosa.

Tunica mucosa

Tunica mucosa was thrown into folds (Fig. 2) of variable height as earlier reported [28] in Red jungle fowl in turkey. The mucosa was investigated regular intervals forming mucosal plicae as reported by [1] in Japanese quail, [28] in Vanraja breed of poultry and [29] in guinea fowl. The tunica mucosa consisted of three layers namely lamina epithelialis, lamina propria and lamina muscularis (Fig. 2). The lamina epithelialis consisted of simple columnar epithelium as also reported by [12] in [6] in Japanese quail, [9] in cattle egret, [12] in turkey and [16] in guinea fowl. In moorhen, the mucosa had branched folds lined by simple cuboidal epithelium [17]. The lamina propria occupied the space between the columnar cells of mucosal folds and consisted of fibroblast cells, collagen fibers, elastic fibers, infiltrated lymphocytes and blood vessels (Fig. 3). Similar findings were made by [13] in common moorhen (Gallinula chloropus). Lamina propria was separated from underlying submucosa by lamina muscularis mucosae which was formed by 1-2 layers of circularly arranged smooth muscle fibers along with connective tissue fibers. [12] in red jungle fowl reported that the lamina muscularis mucosae consisted of inner and outer layers of smooth muscle fibers.

Tunica submucosa

Tunica submucosa occupied most of the wall thickness of proventriculus. This layer presented numerous deep gastric glands known as submucosal or proventriculus glands (Fig. 1) similar to the findings of [5] in patridge and [4] in mallard. However, [5] included these proventricular glands in tunica mucosa. These glands were compound tubulo-alveolar glands similar to the findings of [12] in Japanese quail whereas [7-10] in mallard observed branched tubular glands. However, [13] reported that such glands were absent in the submucosa of proventriculus of chicken. The shape of these glands varied from round, oval, hexagonal or polymorphic lobules and was separated from each other by connective tissue sheath (Fig. 4). This was in agreement with the findings of [14] in Vanraja breed of poultry. Within each lobule, numerous glandular tubules had radiating arrangement (Fig. 5) and were lined by cuboidal epithelium. Numerous alveoli or tubules open together into a wide central cavity. The duct system and central lumen of the proventricular glands was lined with simple [15] columnar epithelium (Fig. 6). Similar findings were made by 1in coot birds.

Tunica muscularis

Tunica muscularis consisted of three layers of smooth muscles. Inner layer was arranged longitudinally whereas the middle and outer layers were arranged in circular manner (Fig. 7). Between middle and outer layer, nerves and ganglion cells were distributed (Fig. 8). This was similar to the findings of [15] in red-capped cardinal birds. [16] in chicken also reported the presence of muscle three layers, inner longitudinal, middle circular and outer longitudinal layer. [18-19] observed only one layer of smooth muscle fibre arranged in circular manner. [19] in duck and pigeon and [20] in moorhen reported the presence of two layers of smooth muscles, inner thick and longitudinally arranged and outer thin and circularly arranged. [21] in Japanese quail reported thick inner circular and thin outer longitudinal smooth muscle fibers. [22] in mallard reported inner thin longitudinal and outer thick circular layer.

Tunica serosa

Tunica serosa consisted of loose connective tissue containing nerves and blood vessels. These findings were similar to the findings of [23] in Japanese quail.

Micrometry

The thickness of tunica mucosa was 490.86 ± 43.47 µ. Tunica submucosa was 1641.16 ± 116.49 µ thick. The thickness of tunica muscularis was 427.99 ± 22.06 µ. The outer muscle layer was thickest (114.37 ± 9.81 µ) followed by inner layer (109.83 ± 11.27 µ) and least in middle layer (81.80 ± 7.74 µ). The thickness of tunica serosa was 83.68 ± 3.64 µ. The maximum and minimum diameter of the glandular lobules was recorded. The maximum diameter was 1829.39 ± 143.88 µ whereas the minimum diameter was 1231.24 ± 97.20 µ. In moorhen, the thickness of tunica submucosa was 143.12 ± 30.37 mm whereas tunica serosa was 2.50 mm thick [24-25] in Japanese quail recorded the thickness of different layers of proventriculus. The thickness of tunica mucosa was 553.42 µ, tunica submucosa was 2164.37 µ, tunica muscularis was 235.07 and tunica serosa was 22.69 µ. In ostrich, the thickness of tunica submucosa was 500.7 ± 47.2 µ, circular layer of tunica muscularis was 512.8 ± 59.6 µ, longitudinal layer of tunica muscularis was 517.8 ± 49.1 µ and tunica serosa was 145.21 ± 41.78 µ thick [25].

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Histochemistry

Histochemical studies of the proventriculus revealed that mucosal epithelial lining showed strong positive reaction to PAS-AB (pH 2.5) as deep magenta and blue staining (Fig. 9) indicating presence of neutral and acidic mucins. [26] mentioned that the presence of neutral and acid mucin acts as a barrier to protect the mucosal surface of proventriculus. [27] in quail and [28] in mallard reported that cells lining mucosal folds were strongly positive to PAS. The glandular secretion showed strong reaction to PAS (Fig. 10). Similar observation was made by [29] in mallard. Similar to findings of present study, neutral and acid mucins were detected in previous studies by [30] in guinea fowl and [31] in black-winged kite. The cells lining the secretory ducts and central cavities of the compound tubular glands showed strong reaction with both PAS stain and PAS-AB stain indicating the presence of both neutral and acid mucins (Fig. 10 and Fig. 11). With PAS-AB stain, the cells towards the central cavity stained reddish purple whereas towards bottoms, cells were bluish indicating acidic mucins (Fig. 11). Similar observations were made by Zhu (2015) in Black-tailed crake. [32] in mallard reported positive reaction of columnar cells to PAS-AB (pH 2.5) stain. Zhu (2015) in Black-tailed crake reported that the cells lining the epithelium in proventriculus appeared blue in color with PAS-AB (pH 2.5) stain indicating the presence of acidic mucin. Submucosal glands and tunica muscularis layer showed strong reaction for basic proteins (Fig. 12). Cells of sub-mucosal glands showed moderate to strong reaction for lipids in supra-nuclear zone (Fig. 13).

Table 1. Showing different micrometrical parameters of proventriculus of Poonchi bird

Parameter (µ)	Values
Thickness of tunica mucosa	490.86 ± 43.47
Thickness of tunica submucosa	1641.16 ± 116.49
Thickness of tunica muscularis	427.99 ± 22.06
Thickness of inner layer of tunica muscularis	109.83 ± 11.27
Thickness of middle layer of tunica muscularis	81.80 ± 7.74
Thickness of outer layer of tunica muscularis	114.37 ± 9.81
Thickness of tunica serosa	83.68 ± 3.64
Maximum diameter of glandular lobule	1829.39 ± 143.88
Minimum diameter of glandular lobule	1231.24 ± 97.20

FIGURES

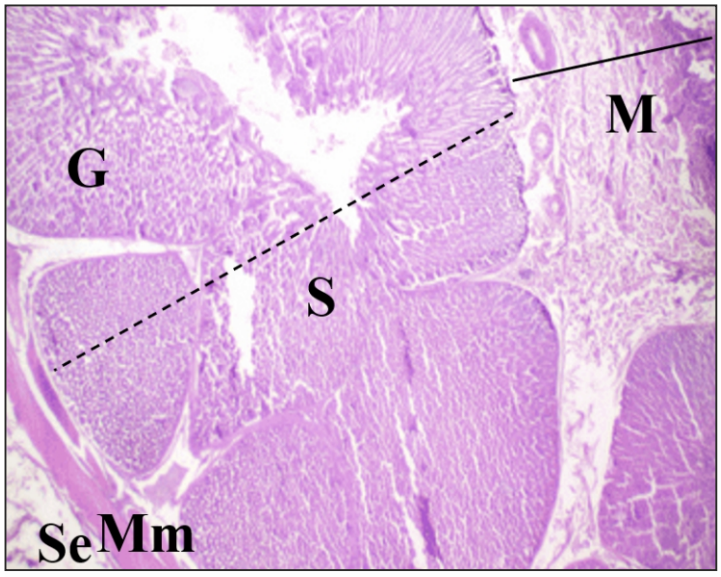

Fig. 1 Photomicrograph of proventriculus of Poonchi bird showing different layers, tunica mucosa (M), submucosa (S) containing proventricular/submucosal glands (G), muscular layer (Mm) and serosal layer (Se). H&E stain 40x

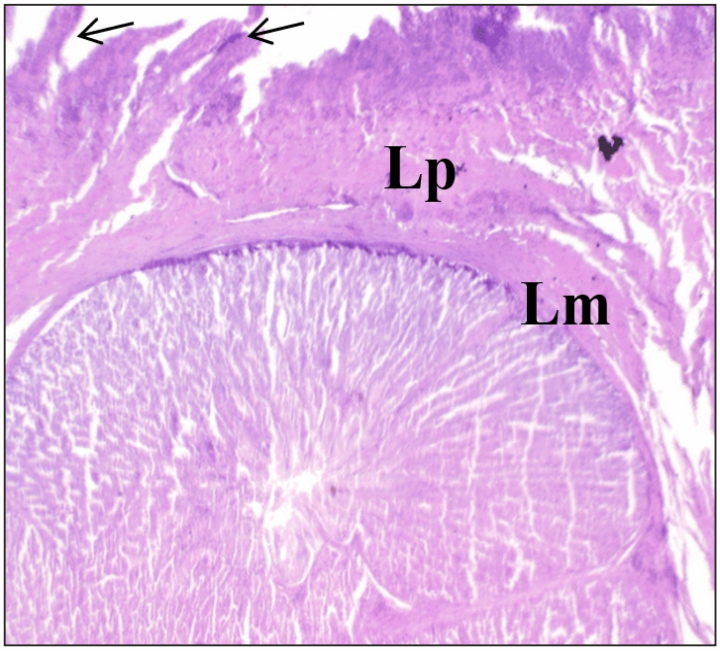

Fig. 2 Photomicrograph of proventriculus of Poonchi bird showing mucosal folds (arrow), lamina propria (Lp) and lamina muscularis mucosae (Lm). H&E stain, 400x

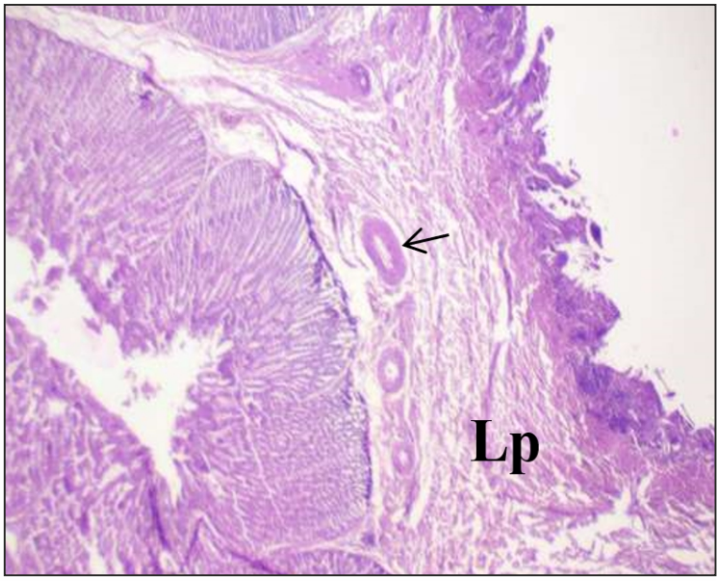

Fig. 3 Photomicrograph of proventriculus of Poonchi bird showing presence of blood vessels (arrow) in lamina propria (Lp). H&E stain, 40x

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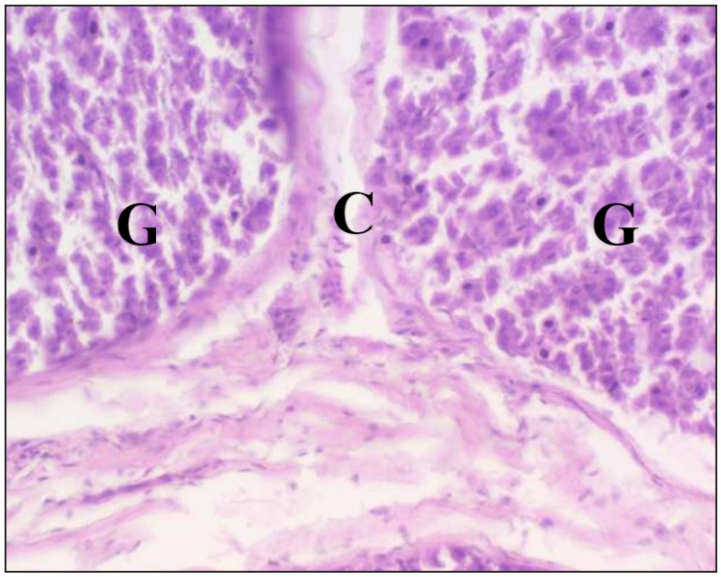

Fig. 4 Photomicrograph of proventriculus of Poonchi bird showing two adjacent glandular lobule (G) separated by connective tissue sheath (C). H&E stain, 400x

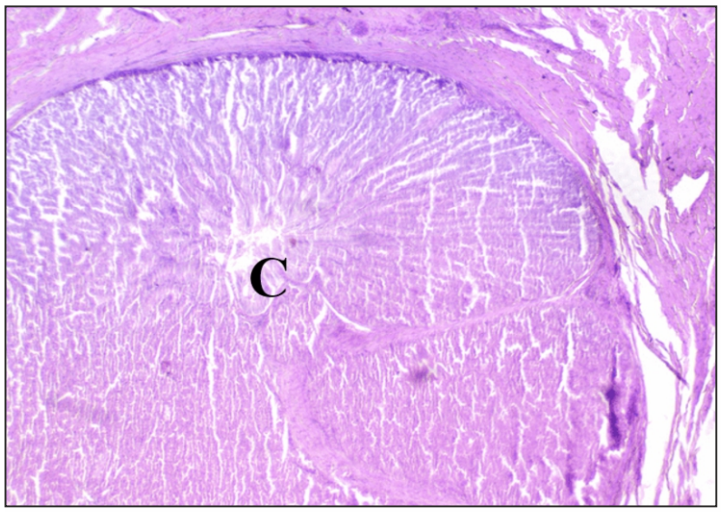

Fig. 5 Photomicrograph of proventriculus of Poonchi bird showing radiating arrangement of glandular tubules and central cavity (C). H&E stain, 100x

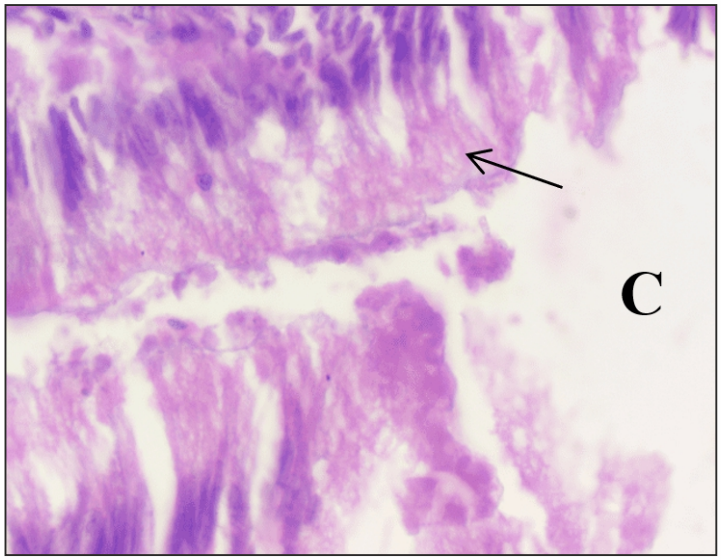

Fig. 6 Photomicrograph of proventriculus of Poonchi bird showing simple columnar epithelium (arrow) around the central cavity (C). H&E stain, 1000x

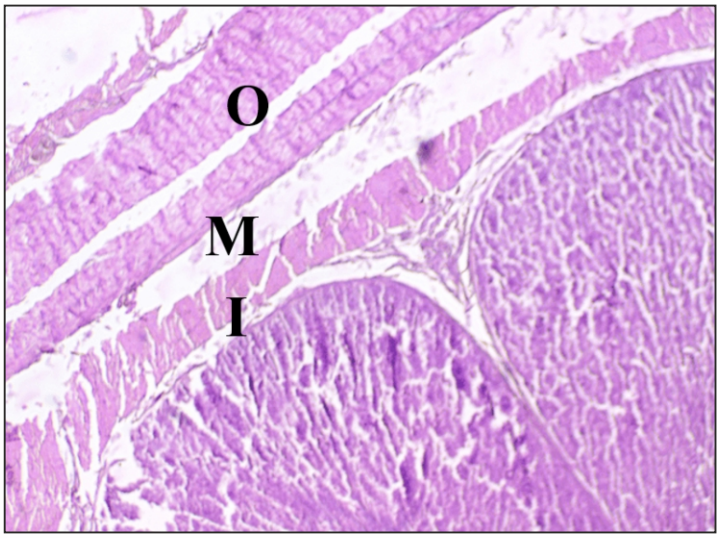

Fig. 7 Photomicrograph of proventriculus of Poonchi bird showing inner (I), middle (M) and outer (O) layer of tunica muscularis. H&E stain, 100x

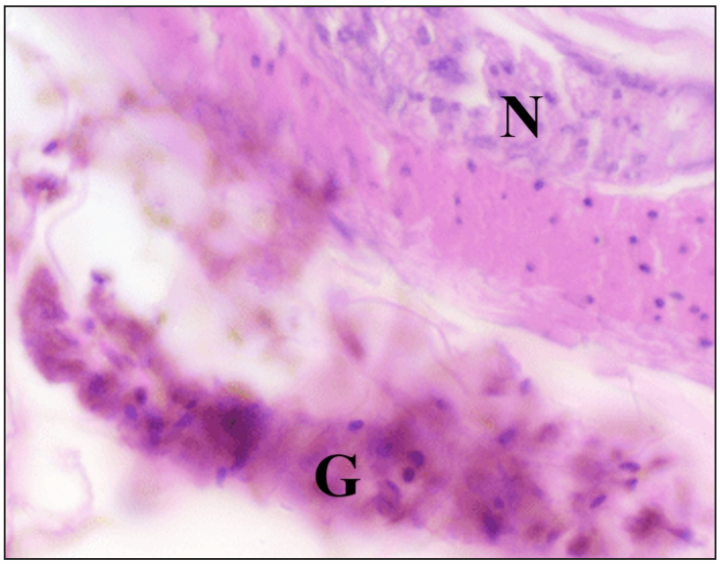

Fig. 8 Photomicrograph of proventriculus of Poonchi bird showing presence of nerve fibers (N) between the layers of tunica muscularis and ganglion cells (G). H&E stain, 1000x

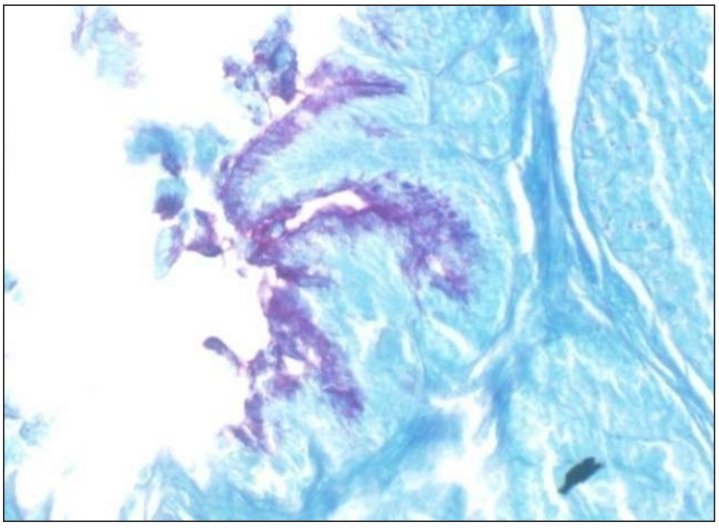

Fig. 9 Photomicrograph of proventriculus of Poonchi bird showing positive reaction of mucosal folds to PAS-AB (pH 2.5) stain. 400x

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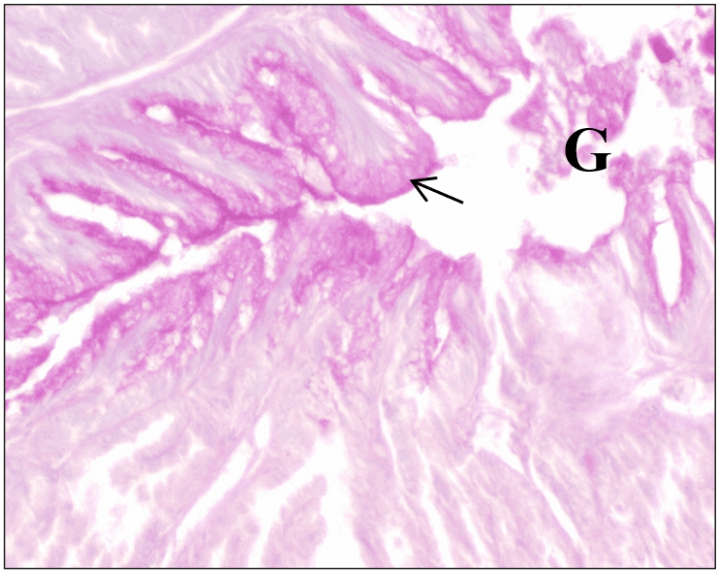

Fig. 10 Photomicrograph of proventriculus of Poonchi bird showing positive reaction of glandular secretion (G) and ducts (arrow) to PAS stain. 400x

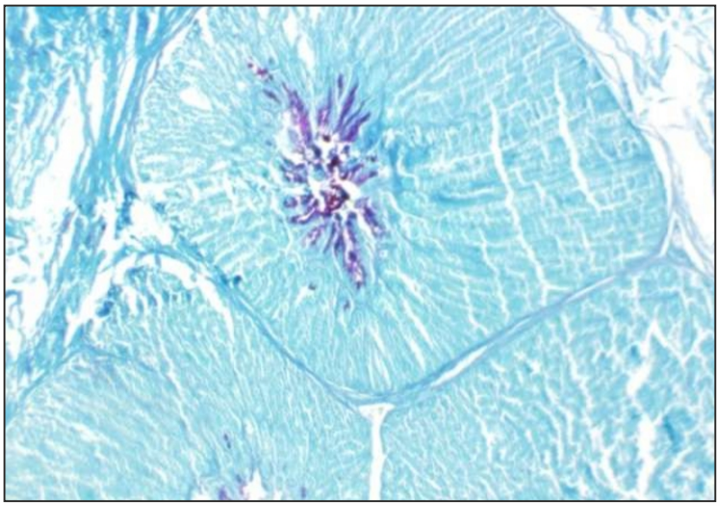

Fig. 11 Photomicrograph of proventriculus of Poonchi bird showing reaction of glandular lobule to PAS-AB (pH 2.5) stain. 100x

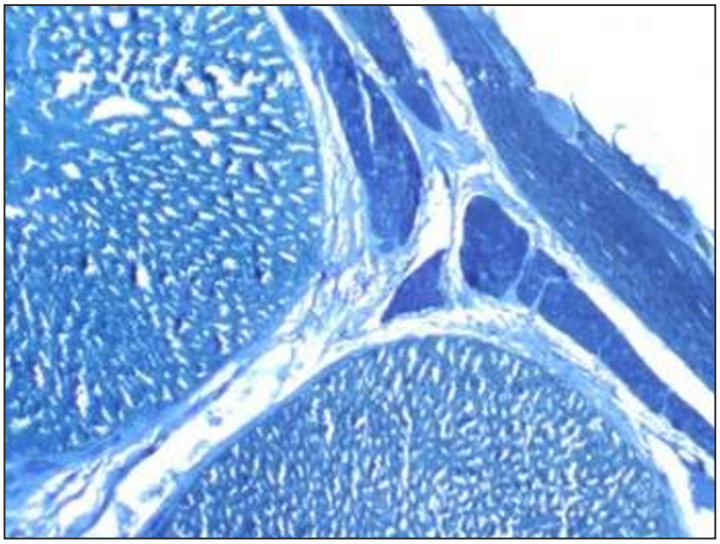

Fig. 12 Photomicrograph of proventriculus of Poonchi bird showing strong reaction of glandular lobule (G) and tunica muscularis (M) to basic proteins. Bromphenol Blue, 100x

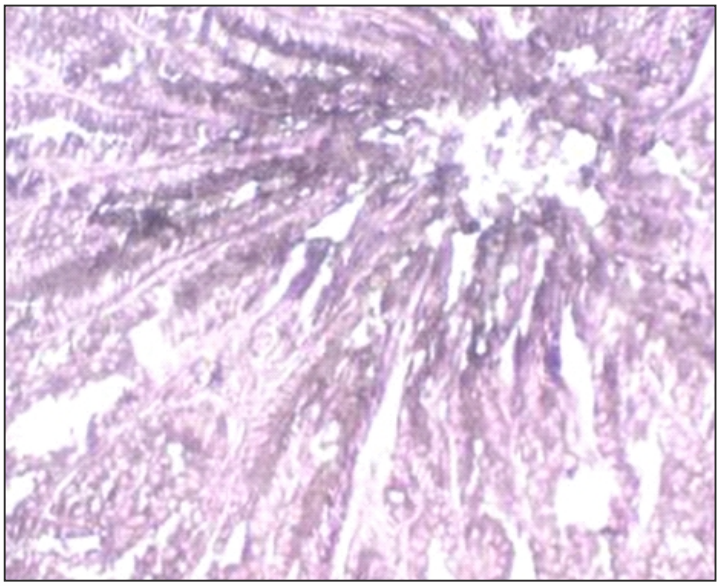

Fig. 13 Photomicrograph of proventriculus of Poonchi bird showing moderate to strong reaction of cells of glandular lobule (G) to lipids. Sudan Black B, 400x

CONCLUSION

The histological and histochemical studies were carried out on the proventriculus of Poonchi bird. The present study demonstrated that the histological architecture of proventriculus of Poonchi bird and the distribution of its mucins resembled with those of other avian species. Some of the variations may be associated with its diet and nutritious behavior.

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