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Histology, micrometry and histochemistry of proventriculus of Pigeon (*Columba livia domestica*)

Shailja Bansal, DM Bhayani and Omprakash Meel

Abstract
This study investigated the histological characteristics and histochemical properties of the proventriculus in pigeons. The luminal surface of the proventriculus exhibited papillae surrounded by folds, with plicae and sulci structures. The epithelium lining the folds consisted of columnar cells with round to oval nuclei. Proventricular glands were found in the deeper part of the lamina propria, organized in lobules opening through excretory ducts. The lamina muscularis, tunica submucosa, and tunica muscularis exhibited characteristic layers of connective tissue and smooth muscle fibers. The outermost layer, tunica serosa, consisted of loose connective tissue and blood vessels. The histological observations were consistent with previous studies in various bird species, although the location of proventricular glands differed in the pigeon proventriculus compared to turkeys. Micrometry measurements revealed the thickness of different layers in the proventriculus. Histochemical analysis indicated the presence of acidic and neutral mucosubstances in the epithelium, lamina propria, proventricular glands, and other layers. Elastic fibers were detected in blood vessels and the lamina propria.
In summary, this study provides a detailed histological and histochemical characterization of the pigeon proventriculus, expanding our understanding of its structural composition. These findings contribute to the existing knowledge base and serve as a reference for future research on avian digestive physiology and comparative anatomy.

Keywords: Proventriculus, histology, histochemistry, pigeon

Introduction
Histological studies have delved into the microscopic architecture of the proventriculus, providing insights into the cellular composition, organization of epithelial cells, and the presence of specialized structures such as proventricular glands. Histochemical analyses have further revealed the distribution of mucosubstances and other specific components within the proventriculus, shedding light on its secretory functions and physiological processes (Al-Saffar, and Al-Samawy, 2015, Mehra and Kumar, 2023) [1, 6]. Understanding the proventriculus of pigeons is not only important from an anatomical and histological perspective but also has significant implications for avian physiology, nutrition, and pathology. Investigations into the proventriculus can contribute to our knowledge of avian digestion, nutrient assimilation, and the development of effective dietary strategies for pigeon management and health.

Materials and Methods
The study was conducted on the proventriculus of pigeons. Pigeon carcasses were obtained from two sources: the kite festival organized by the Namo Namah Parivar Bird Camp -2020 in Ahmedabad, and the Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, K. U., Anand. All research work was performed in the Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Anand, Gujarat.

Sample Collection
A total of six pigeon carcasses were used for histological, and histochemical, and micrometrical studies.

Sample Processing, Dissection, and Preservation: The proventriculus and gizzard were dissected from the body after the pigeons were dissected out. The organs were washed with 0.5% phosphate-buffered saline solution and then fixed in 10% neutral-buffered formalin solution for 48 hours.
Lens, calibrated Vernier calipers, non-elastic thread, and a measuring tape.

Histological and Histochemical Study: The fixed tissues in 10% neutral buffered formalin were processed to create routine paraffin blocks and sections. The paraffin sections, with a thickness of 5-6 µm, were obtained using a microtome machine. The sections were then subjected to the following staining techniques:

1. Harris' hematoxylin and eosin stain for general architecture (Luna, 1968) [5].
2. Weigert's method for elastic fibers (Luna, 1968) [5].
3. Alcian blue method (pH 2.5) for muco-substances (Luna, 1968) [5].
4. PAS-Alcian blue method (pH 2.5) for acidic and neutral mucosubstances (Luna, 1968) [5].

Micrometrical Study
After preparing permanent histological slides, the thickness of the four layers: tunica mucosa (including the epithelium, lamina propria, and lamina muscularis), tunica submucosa, tunica muscularis, and tunica adventitia/serosaof the proventriculus and other organs of the digestive tube of pigeons were measured in microns (µm). The measurements were taken using a graduated 10X eyepiece and a 45X objective lens after calibration.

Statistical Data Analysis: IBM SPSS Statistics 20 (Trial Version) was used for statistical data analysis. The biometrical and micrometrical data were presented as Mean±SE (Standard Error) values and coefficient of variation. An independent samples t-test was employed to determine significant differences between two groups, while One-Way Analysis of Variance (ANOVA) was used to determine significant differences among three groups.

Photography: Gross photographs of the organs were captured using a Sony Cyber-Shot DSC-HX400 Prosumer Camera (20.4 MP). Photomicrographs of the histological and histochemical slides were taken using Mag Vision.

Results and Discussion
Histology: The luminal surface of the proventriculus exhibited papillae surrounded by numerous folds. These folds displayed plicae towards the lumen, while their deeper portions formed depressions called sulci. The arrangement of these plicae and sulci was not well-defined (Figure 1). The folds were lined by a simple columnar epithelium consisting of columnar cells with round to oval nuclei arranged linearly towards the base of the cells (Figure 1). The lamina propria contained loose irregular connective tissue and fine blood capillaries. Proventricular glands were present in the deeper part of the lamina propria and were organized in lobules. The lobules varied in shape and size and were separated from adjacent ones by bundles of connective tissue fibers (Figure 1). The glandular lobules opened through excretory ducts, which were lined by a simple columnar epithelium (Figure 2, 3). The lobules of the glands were surrounded by connective tissue and blood capillaries. The lamina muscularis was a thin layer of smooth muscle fibers and was interspersed between the lobules in some areas (Figure 2). The tunica submucosa consisted of loose irregular connective tissue, fine blood capillaries, and small to medium-sized blood vessels. The tunica muscularis comprised an inner circular layer, which was thicker, and an outer longitudinal layer of smooth muscle fibers. The outer longitudinal layer of the tunica muscularis was not consistently observed in all areas. The outermost layer, tunica serosa, consisted of loose irregular connective tissue, a few mesothelial cells, and fine blood vessels.
These histological observations were consistent with previous studies conducted on broilers (Nasrin *et al*., 2012) [7], coot birds (Lafi and Ali, 2012) [4], Elanus caeruleus (Hamdi *et al*., 2013) [3], and mallards (Al-Saffar & Al-Samawy, 2015) [1]. However, Beheriy (2018) [2] reported the presence of proventricular glands in the lamina propria layer in turkeys, which differed from the present study where the proventricular glands were observed in the tunica submucosa layer of the pigeon proventriculus.

Micrometry: The mean ± SE values of the thickness of various layers were as follows: epithelium 18.50±1.35 µm, lamina propria 230±10.49 µm, lamina muscularis 67.83±3.40 µm, tunica submucosa 92±50 µm, tunica muscularis (circular) 123.95±5.95 µm, tunica muscularis (longitudinal) 43.17±2.81 µm, and tunica serosa 20±1.38 µm. In contrast, Das *et al*. (2017) reported the thickness of proventricular mucosa in different age groups of Kadaknath fowl, ranging from 323±24.722 µm to 712±46.13507 µm. Qureshi *et al*. (2017) reported thickness measurements in ducks, which significantly differ from the present study due to species differences.

Histochemistry: The epithelium exhibited a strong positive Alcianophilic reaction, indicating the presence of acidic mucosubstances as shown by PAS-AB and AB methods. Similar findings have been reported in Elanus Caeruleus (Hamdi *et al*., 2013) [3], Mallard (Saffar and Samawy, 2015), and Broilers (Mehra and Kumar, 2023) [6]. The lamina propria, proventricular glands, lamina muscularis, tunica submucosa, tunica muscularis, and tunica serosa showed a PAS-positive reaction, indicating the presence of neutral mucopolysaccharides. The glandular ducts also showed a PAS-positive reaction, with some areas displaying a weak Alcianophilic reaction (Figure 4, 5). These observations are consistent with findings in Elanus Caeruleus (Hamdi *et al*., 2013) [3], Mallard (Al-Saffar and Samawy, 2015) [1], and broilers (Mehra and Kumar, 2023) [6]. Weigert's method demonstrated the presence of elastic fibers in the blood vessels and lamina propria (Figure 6, 7).

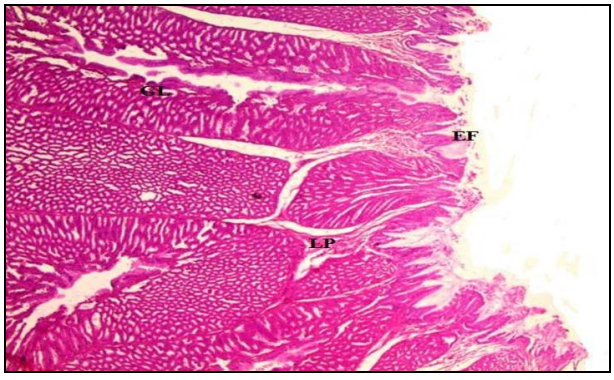

Fig 1: Photomicrograph of proventriculus showing epithelium fold (EF), laminapropria (LP) and proventricular gland (GL). Haematoxylin & Eosin stain,100X.

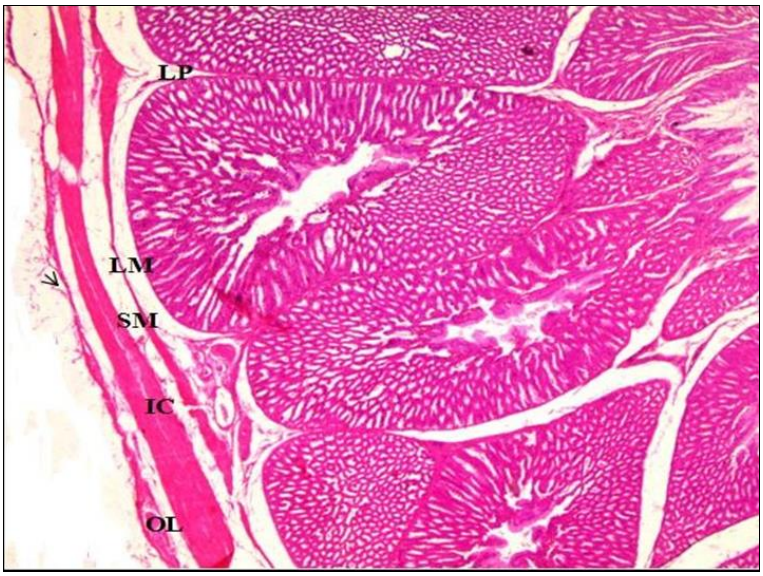

Fig 2: Photomicrograph of proventriculus showing laminapropria (LP), laminamuscularis (LM), tunicasubmucosa (SM), inner circular of tunicamuscularis (IC), outer longitudinal (OL) layer of tunica muscularisand tunica serosa (Black arrow). Haematoxylin & Eosinstain, 100X.

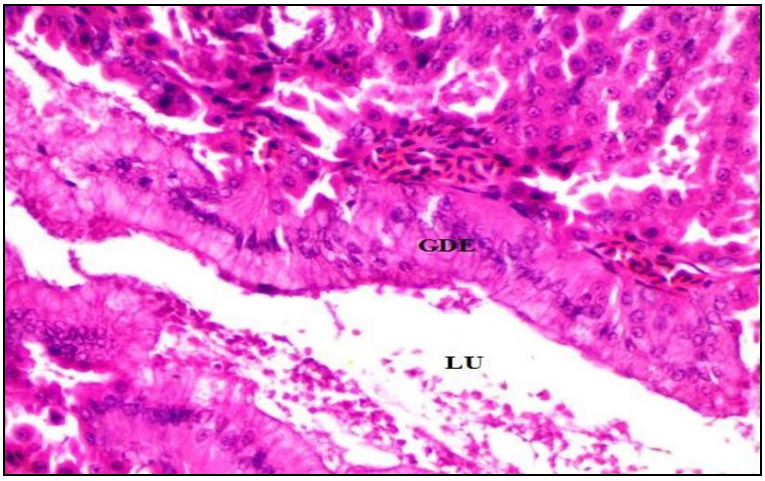

Fig 3: Photomicrograph of proventricular gl and showing simple columnar epithelium (GDE)and lumen of glandular duct (LU). Haematoxylin &Eosinstain, 400X.

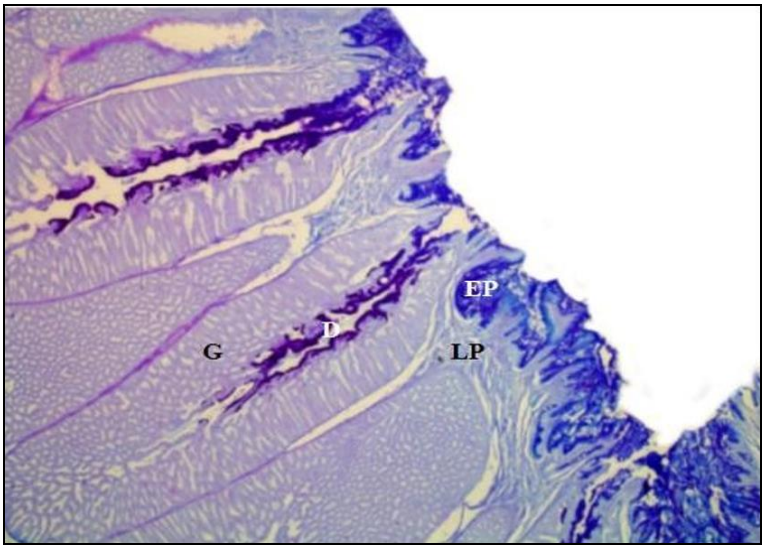

Fig 4: Photomicrograph of proventriculus showing neutral mucopolysaccharides in proventricular glands (G), glandular duct (D) and acidic mucosubstances in epithelium (EP),laminapropria (LP). PAS-ABstain,100X.

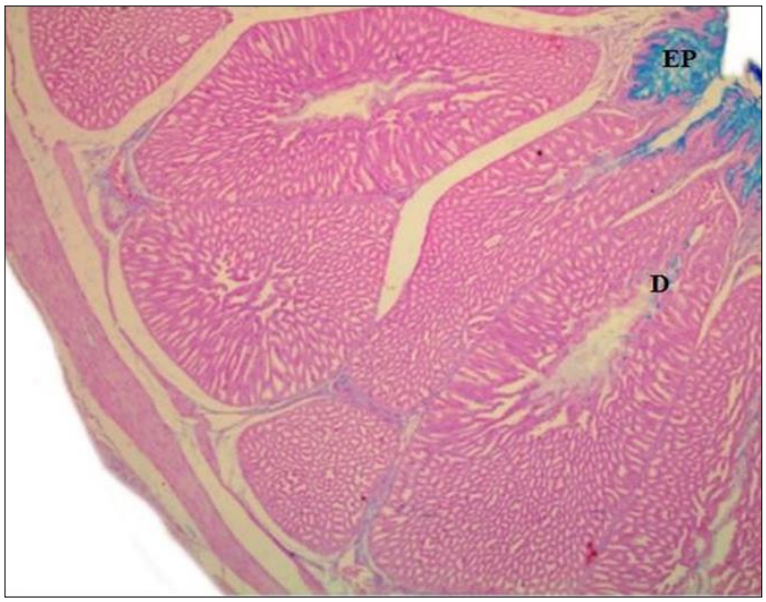

Fig 5: Photomicrograph of proventriculus showing acidic mucosubstances in the epithelium (EP) and glandular duct (D). ABstain,100X.

Fig 6: Photomicrograph of proventriculus showing blood vessel (BV) in between proventricular glands.Weigert'sstain,400X.

Fig 7: Photomicrograph of proventriculus showing blood vessels in tunica serosa. (TS) Weigert'sstain,100X.

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