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Histological, micrometrical and histochemical studies on gizzard of pigeon (*Columba livia domestica*)

Shailja Bansal, DM Bhayani and Omprakash Meel

Abstract
This study examined the histological features and histochemical properties of the gizzard in pigeons. The lateral wall of the gizzard displayed all four tunics, with an additional thick layer of koilin towards the luminal surface. The mucosa exhibited folds, followed by gastric pits formed by gastric glands. The lamina propria merged with the submucosa, lacking a distinct lamina muscularis. The tunica muscularis consisted of inner circular, middle oblique, and outer longitudinal layers of smooth muscle fibers. The tunica serosa formed the outermost layer. These findings were consistent with previous studies in broilers and *Elanus caeruleus*, but differed from observations in mallards and laughing doves. The central wall of the gizzard displayed koilin, a mucosa with folds, and gastric pits lined with cuboidal epithelium. The propria-submucosa exhibited dense connective tissue fibers, and hyaline cartilage was present in the middle portion. The tunica muscularis was absent, and the tunica serosa consisted of connective tissue fibers and mesothelial cells. Micrometry measurements revealed the thickness of various layers in the gizzard. Histochemical analysis showed the presence of neutral mucopolysaccharides and acidic mucosubstances in the epithelium, tunica muscularis, lamina propria-submucosa, and tunica serosa. Elastic fibers were observed in blood vessels, and collagen fiber bundles were interspersed within the tunica muscularis.
In summary, this study provides a comprehensive histological and histochemical characterization of the pigeon gizzard, highlighting the unique features of the lateral and central walls. These findings contribute to our understanding of avian digestive physiology and offer a basis for comparative anatomical studies.

Keywords: Gizzard, pigeon, micrometry, histology, histochemistry

Introduction
The literature was available on the histology of different birds Al-Saffar et al., (2015) [1] in mallard, Al-Kinany, (2019) [2] in Iraqi wild bird, Das et al., (2018) [3] in Kadaknath fowl and Hamdi et al., (2013) [4] in *Elanus caeruleus*.
The gizzard is a specialized muscular organ found in the avian digestive system. It plays a crucial role in the mechanical breakdown of food particles before they proceed to the intestines for further digestion and absorption. The gizzard of pigeons (*Columba livia domestica*) is of particular interest due to the unique anatomical and functional adaptations exhibited by this species.
Histological investigations shed light on the microscopic structure of the gizzard, revealing the arrangement and organization of its various layers and cell types. The histological analysis of the pigeon gizzard involves the examination of thin sections stained with specific dyes to highlight different tissue components. This examination enables the identification of the epithelium, lamina propria, tunica submucosa, tunica muscularis, and tunica serosa, providing insights into the cellular composition and organization of these layers.
Micrometry involves the precise measurement of the thickness of different layers within the gizzard. By quantifying the dimensions of the epithelium, lamina propria, tunica submucosa, tunica muscularis, and tunica serosa, micrometry allows for a more detailed characterization of the structural variations and adaptations within the gizzard. These measurements provide quantitative data that contribute to a better understanding of the functional aspects of the gizzard.
Histochemistry studies focus on the identification and localization of specific chemical substances within the gizzard tissues. Various staining techniques are employed to detect the presence of mucosubstances, such as acidic and neutral mucopolysaccharides, as well as elastic fibers. Histochemical analysis helps in identifying the distribution and composition of these substances within the different layers of the gizzard, providing insights into their functional roles and contributions to the overall digestive process.

Understanding the histology, micrometry, and histochemistry of the pigeon gizzard (*Columba livia domestica*) contributes to our knowledge of its structure-function relationships. This knowledge enhances our understanding of avian digestive physiology and provides a basis for comparative studies among different bird species. Furthermore, it has practical implications for avian health and nutrition, as abnormalities or variations in gizzard structure and function can impact digestive efficiency and overall health in pigeons and other avian species.

Materials and Methods

The present study focused on the gizzard of pigeons, specifically examining its histology, histochemistry, and micrometry. Pigeon carcasses were obtained from 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 conducted in the Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Anand, Gujarat.

A total of six pigeon carcasses were collected for the study, and used for histological, histochemical, and micrometrical studies. The carcasses were thoroughly defeathered to facilitate the dissection and improve access to the visceral organs. Dissection was performed by cutting through the abdominal wall and reflecting the thoracic region, while the cervical region was incised to expose the cervical, thoracic, and abdominal visceral organs. The gizzard was dissected from the body for histological, histochemical, and micrometrical studies. Prior to processing, the gizzard was washed with a phosphate-buffered saline solution and then fixed in 10% neutral buffered formalin solution for 48 hours.

Histological and histochemical studies were conducted on the fixed tissues. Routine paraffin blocks were prepared, and sections of 5-6 µm thickness were obtained using a microtome machine. These sections were then subjected to staining techniques including Harris' hematoxylin and eosin stain for general architecture, Weigert's method for elastic fibers, Alcian blue method (pH 2.5) for muco-substances, and PAS-Alcian blue method (pH 2.5) for acidic and neutral mucosubstances (Luna, 1968) [6].

Micrometrical studies involved measuring the thickness of the gizzard's layers, including tunica mucosa (epithelium, lamina propria, and lamina muscularis), tunica submucosa, tunica muscularis, and tunica adventitia/serosa. These measurements were taken in microns (µm) using a graduated 10X eyepiece and a 45X objective lens after calibration. Statistical data analysis was performed using IBM SPSS Statistics 20 (Trial Version). The biometrical and micrometrical data were expressed as Mean ± SE values and coefficient of variation. An independent samples t-test was used to determine significant differences between two groups, while One-Way Analysis of Variance (ANOVA) was used to determine significant differences among three groups. Photomicrographs of the histological and histochemical slides were taken using Mag Vision.

Results and Discussion

Histology: The lateral wall of the gizzard in pigeons consisted of all four tunics, with an additional thick layer of koilin observed towards the luminal surface (Figure 1). The mucosa was lined by a simple columnar epithelium and formed folds. These folds were spaced widely apart and were followed by gastric pits in the deeper portion. The gastric pits were formed by gastric glands, which were lined by a simple cuboidal epithelium (Figure 1, 2). The lamina propria contained loose irregular connective tissue, merging with the submucosa to form the propria-submucosa due to the absence of a lamina muscularis. The deeper portion of the propria-submucosa was occupied by the gastric pits, which opened towards the free surface of the epithelium. The connective tissue in this region was denser and had a predominance of collagen bundles. Additionally, connective tissue, fine blood capillaries, and blood vessels were observed in this layer. This denser connective tissue was interspersed within the tunica muscularis (Figure 1, 2). The tunica muscularis mainly consisted of three layers, with the inner circular layer being thicker, a middle oblique layer, and an outer longitudinal layer of smooth muscle fibers. The outermost tunica serosa was present.

These observations align with findings in broilers (Nasrin et al., 2012) [7] and *Elanus caeruleus* (Hamdi et al., 2013) [4]. However, they differ from observations in mallards (Al-Saffar and Al-Samawy, 2015) [1] and laughing doves (Jamal, 2019) [5], where the lamina muscularis was present in the gizzard. In contrast, in the present study, the lamina muscularis was absent in the pigeon gizzard. The central (aponeurotic) wall of the gizzard exhibited an innermost layer of koilin, followed by a mucosa lined by a simple columnar epithelium forming folds. Similar to the lateral wall, the folds were widely spaced and followed by gastric pits formed by gastric glands lined with simple cuboidal epithelium. The propria-submucosa was significantly thicker and composed of dense regular connective tissue fibers. Hyaline cartilage was present in the middle portion of the propria-submucosa. The tunica muscularis was absent, and the tunica serosa consisted of connective tissue fibers and mesothelial cells,

Micrometry: The mean ± SE values of the thickness of koilin, epithelium, lamina propria, tunica submucosa, tunica muscularis (circular), tunica muscularis (longitudinal), and tunica serosa in the gizzard of pigeons were determined as 155.40±9.01 µm, 17.50±1.34 µm, 278.30±5.00 µm, 162.45±5.14 µm, 458.64±4.32 µm, 274.24±4.13 µm, and 15.78±5.23 µm, respectively. In contrast, Das et al. (2018) [3] reported different thickness values for koilin and tunica mucosa in birds of different ages. Similarly, Al-Kinany (2019) [2] reported different thickness values for tunica mucosa, tunica submucosa, and tunica muscularis in laughing doves. These variations in thickness can be attributed to species differences.

Histochemistry: In the lateral wall of the gizzard, the epithelium and tunica muscularis showed a strong PAS-positive reaction, indicating the presence of neutral mucopolysaccharides and acidic mucosubstances. The lamina propria-submucosa exhibited a strong PAS-positive reaction, indicating the presence of neutral mucopolysaccharides. The tunica serosa also showed a strong PAS-positive reaction (Figure 4). The epithelium and tunica muscularis also displayed an Alcianophilic reaction in AB staining. Elastic fibers were observed in the blood vessels, and bundles of collagen fibers were interspersed within the tunica muscularis (Figure 5).

In the central wall of the gizzard, the epithelium showed an Alcianophilic reaction in PAS-AB staining. The lamina

propria-submucosa and tunica serosa exhibited a strong PAS-positive reaction, indicating the presence of neutral mucosubstances. The cartilage displayed a strongly PAS-positive reaction along with a weak Alcianophilic reaction, indicating the presence of neutral mucopolysaccharides and traces of acidic mucosubstances in PAS-AB staining (Figure 4, 6). Similarly, AB staining also revealed an Alcianophilic reaction in the epithelium, and the cartilage showed traces of acidic mucosubstances (Figure 6).

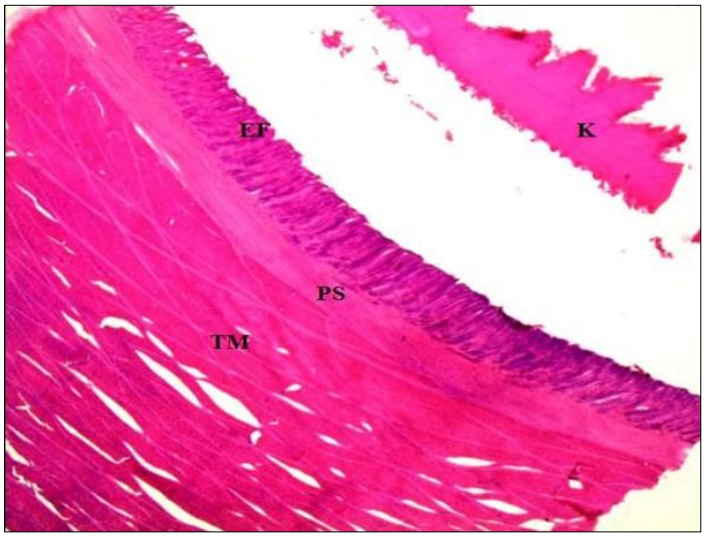

Fig 1: Photomicrograph of lateral wall of gizzard showing koilin (K), epitheliumfolds (EF), propria-submucosa (PS) and tunica muscularis (TM). Haematoxylin & Eosinstain, 40X.

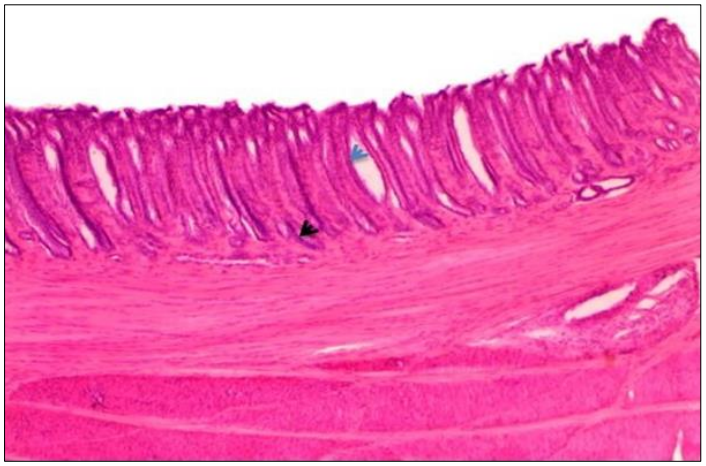

Fig 2: Photomicrograph of lateral wall of gizzard showing epithelium (Blue Arrow) and gastric pit (Black arrow). Haematoxylin & Eosinstain, 100X.

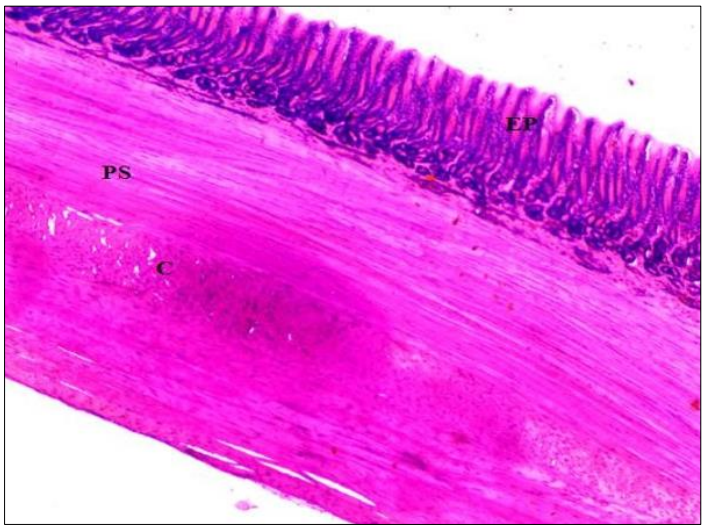

Fig 3: Photomicrograph of central wall of gizzard showing Epithelium folds (EP), Propria-Submucosa (PS) and Cartilage (C). Haematoxylin & Eosinstain,100X

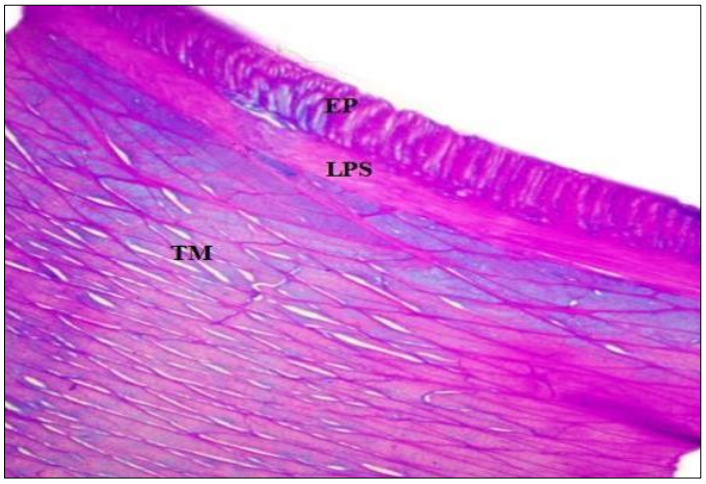

Fig 4: Photomicrograph of lateral wall of gizzard showing neutral mucopolysaccharides in Epithelium (EP), Lamina Propria Submucosa (LPS), Tunica Muscularis (TM)and traces of acidic mucosubstances in Epithelium (EP), Tunica Muscularis (TM). PAS-A Bstain, 40X.

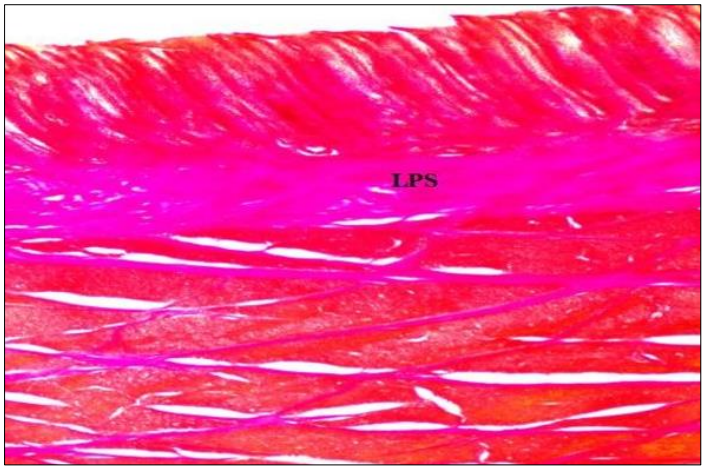

Fig 5: Photomicrograph of lateral wall of gizzard showing predominance of collagen fibers in Lamina Propria Submucosa (LPS) that inter spersed in tunica muscularis. Weigert'sstain,100X.

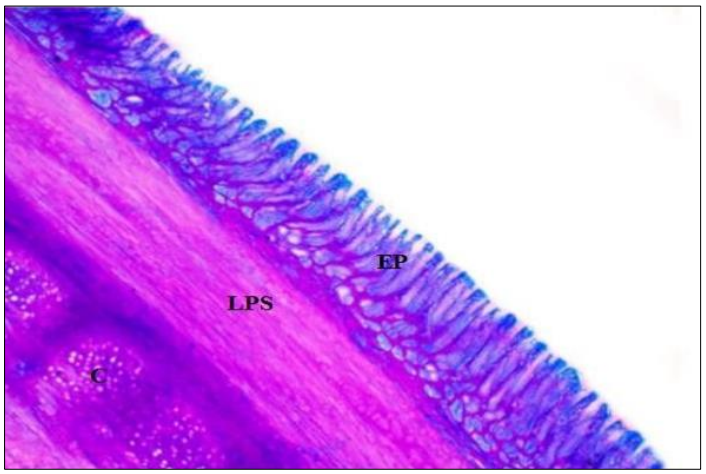

Fig 6: Photomicrograph of central wall of gizzard showing neutral mucopolysaccharides in Lamina Propria Submucosa (LPS), Cartilage(C) and acidic mucosubstances in Epithelium (EP), Cartilages (C). PAS-ABstain,100X.

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