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Comparative Histochemical Study of the proventriculus Between Bronze Fallow Cockatiel and White Eared Bulbul

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Abstract | The current study was designed to explore the chemical composition of the wall of proventriculus in white-eared bulbs and pasture chickens. For this study, 20 healthy adult birds (10 white-eared bulbuls and 10 bronze flocks) were purchased from the local market. All birds were slaughtered, and their stomachs were excised. Small fragments of tissue (6-8 mm) from the proventriculus were subjected to histochemical reactions of Haematoxylin, Eosin, and ABS pH 2.5, as well as Masson's trichrome stains. Sections of the proventriculus of these birds were collected. The mucosa of the proventriculus was folded, resembling the sulci of the intestine, and was covered by a simple epithelial column. The tunics of the submucosa were evidenced by many characteristics of well-developed, straight submucosal glands supported by the connective tissue capsules and extended from the base of the branched gland through the same lamina. In the white-eared bulbul, the Tunica Muscularis appeared to have been doubled. In contrast, the bronze-harvesting cockatiel seemed to have a three-layered structure, comprising an inner and outer longitudinal layer, as well as a middle circular layer. Serosa is composed of tissue that is fibrous, vessels, fatty tissue, and nerve bundles, all of which are composed of mesothelial cells. The histochemical reaction of the mucosal gland and the proventriculus gland is positive for both PAS and AB stains. This chemical reaction is used to differentiate the acidic and neutral composition of the mucopolysaccharides in the tissue. the proventriculus fold was taller in the bulbul from that of cockatiel with, characterized by a large number of goblet cells. The proventriculus gland is arranged in two rows in the cockatiel and one row in the bulbul.

Keywords | Gizzard, Proventriculus, birds

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INTRODUCTION

Typically, the digestive systems of birds are adapted to their diets and the origin of their species. In contrast to mammals (Alkafagy *et al*., 2022), birds have a more sensitive stomach, which is attributed to their diet (Langlois, 2003). The stomach's content of glandular tissue (proventriculus) is covered by a layer of glandular mucosa that produces juices similar to those found in Pepsodent. The rapid movement of bolus or food takes place in the stomach where food is reduced to the smallest possible particles that can be absorbed through the intestine for further digestion and absorption by the body (Saleem, 2012; Strack, 1999). The proventriculus is the glandular stomach of birds and is the site of the significant production of food-digesting enzymes that have been noted up to now (Bacha and Bacha, 2000). The proventriculus has four distinct components, each composed of four different tunics (Al-Khafaji and Al-Kafagy, 2024). A tunic is a special layer of tissue; each of the four tunics has its characteristics. The mucosa of the tunica is a simple columnar epithelium. In older ages, the height of the epithelium increases in thickness. With the thickness of the mucosa and submucosa, the latter is packed with proventricular glands (Al-Kafagy *et al.*, 2022; Ali *et al.*, 2024; Al-Sailawi *et al.*, 2024).

Proventriculus is categorized by different types and depending on the type of food ingested, it was split into four different clothing that are tunica or layers; the composition of the diet, the way they eat, and the quantity of food consumed affects the histology of their digestive system (Zaher *et al.*, 2012). The glandular vestibule has macroscopically large papillae covered with numerous minute folds. Individual glands are clustered into lobules, each with a common lumen located near the surface of the gland. Ultimately, the lumen forms a common duct that leads to the surface through the tips of the small papillae. The surface epithelium of the folds is simple columnar, and each fold has a core of lamina propria (Nasrin *et al.*, 2012).

MATERIALS AND METHODS

Twenty samples were employed that were divided into two subsystems, the first (10 samples from each bird) was used for histological evaluation, the second (20 samples from each bird) was reserved for future use (10 samples from each bird) was employed for histochemical analysis. Specimens of the proventriculus were flushed with tap water. The samples were fixed with water and then with 10% non-aqueous formalin. After hydration with increasing concentrations in the presence of methyl benzoate, samples of alcohol were then embedded in paraffin. The samples were sectioned with a microtome (4-5 µm thick) and then stained with hematoxylin and eosin to visualize general structural features. Additionally, the periodic acid-Schiff (PAS) method was employed to detect neutral polysaccharides, and alcian blue was used to identify acid polysaccharides (Al-Mahmood, 2020). Light microscopy was utilized to examine tissue components. Joe's micro-camera can take small images and, together with software, can measure the tiny size characteristics of the skin in terms of height, mucus thickness, and glandular stomach, as well as four layers of mucosa, submucosa, muscularis layer, and serosa (Bancroft and Gamble, 2008). Together, it evaluates the quality of images taken with a digital camera (Scope Image 9.0 – China) and appropriate image editing software (Bancroft *et al.*, 2019).

RESULTS

The tunica mucosa of the eared bulbul of the proventriculus (glandular stomach) was measured at about 301.696±2.97 µm, and the mucosa of the bronze fallow cockatiel was measured at (146.26±8.5). Tunica mucosa consisted of fingerlike projections (mucosal folds) that were longer than those in the bronze fowl cockatiel. The proventriculus was highly folded, lined with simple columnar epithelium, and measured about 15.148±0.1 µm. The crowded fold was known as plicae and were separated by grooves called sulci. While in the bronze fowl cockatiel, the lining epithelium was less than that in the eared bulbul, which measured about (13.22±0.93), the mucosal fold was separated by the sulci. The mucosa of the bronze fawn cockatiel was less folded than that of the eared bulbul. The lamina propria of both birds (white-eared bulbul and bronze fallow cockatiel) occupied the core of the mucosal fold and consisted of loose connective tissue. The blood vessels and lymphocyte aggregation were present in the lamina propria, along with connective tissue fibers of the mucosal fold. The superficial glands (simple tubular glands) are lined with simple cuboidal epithelium and are located in the lamina propria at the base of the mucosal folds. The muscularis mucosa is absent in both birds (Figures 1, 2 and Table 1).

The tunica submucosa of the eared bulbul was the thickest layer of the proventriculus wall that occupied the proventriculus gland, with the presence of connective tissue fibers that make the connective tissue septum or separators between the proventriculus glands. The blood vessels were present in large numbers in the tunica submucosa. The bronze fawn cockatiel has the same histological structure as that of the eared bulbul, but it is thinner. The tunica submucosa of bulbul and cockatiel was measured at about 922.70±2.05 µm and 911.15±6.83 µm, respectively (Figures 1, 2 and Table 1).

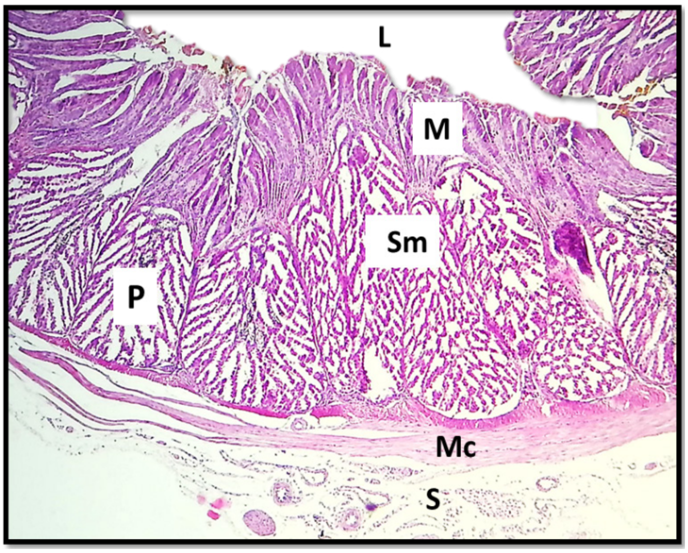

Figure 1: The cross section of the white-eared bulbul's proventriculus demonstrates: Mucosa (M), submucosa (Sm), muscularis (Mc), serosa (S), and lumen (L). HandE color. X40.

Table 1: The thickness (mucosa, submucosa, muscularis) and height of the vestibular epithelium of the two bird species.

Thickness of tunica muscularis mean ± SE µm	Thickness of tunica submucosa mean ± SE µm	High of epithelium mean ± SE µm	Thickness of tunica mucosa mean ± SE µm	Bird
66.56±56±3.36	922.70±2.05	15.14±0.31	301.69±2.97	Bulbul
61.51±2.27	911.15±6.83	13.22±0.93	146.26±8.53	cockatiel

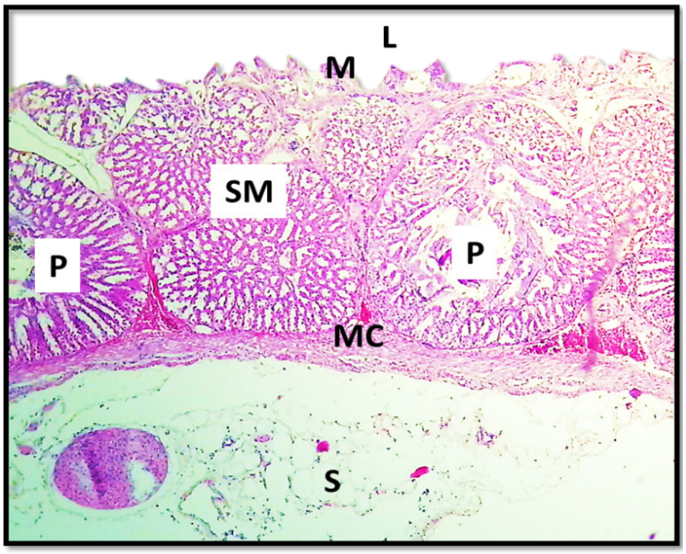

Figure 2: Cross-histological section of bronze fallow cockatiel proventriculus show: Mucosa (M), submucosa (Sm), muscularis (Mc), serosa (S), lumen (L), glandular stomach (P). HandE staining. X40.

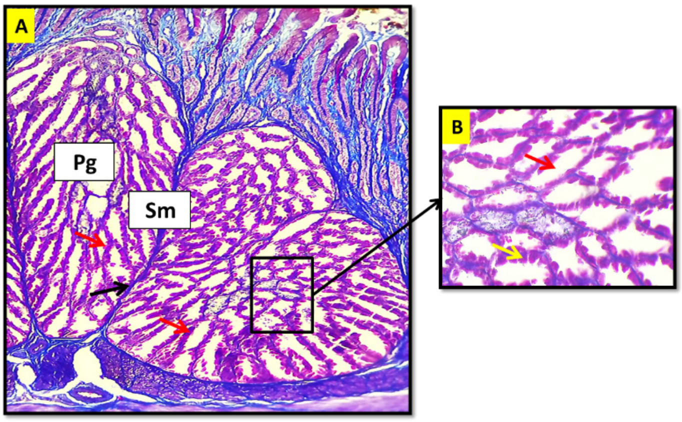

Figure 3: Cross-histological section of white eared bulbul proventriculus show: proventriculus gland (Pg), collagen bundles septa (black arrow), secretory unit (red arrow), simple cuboidal epithelium (yellow arrow).Trichromatic stain for masson.X100. B: enlarged section. X400.

The Proventriculus gland (tubular alveolar glands) in the white-eared bulbul was elliptical to circular in shape, grouped to form glandular lobules. The gland occupied the entire submucosal layer of the proventriculus wall, arranged in a single row with a central lumen. The proventriculus gland was covered with simple columnar epithelium. These glands were composed of numerous secretory units, each present in a separate lobule. Each unit opened into a duct, and this duct in turn opened into the central duct, which connected to the lumen. The proventriculus gland in the bronze fawn cockatiel has the same histological structure, except that it is arranged into two rows and appears elliptical, with a larger lumen than that in the eared bulbul.

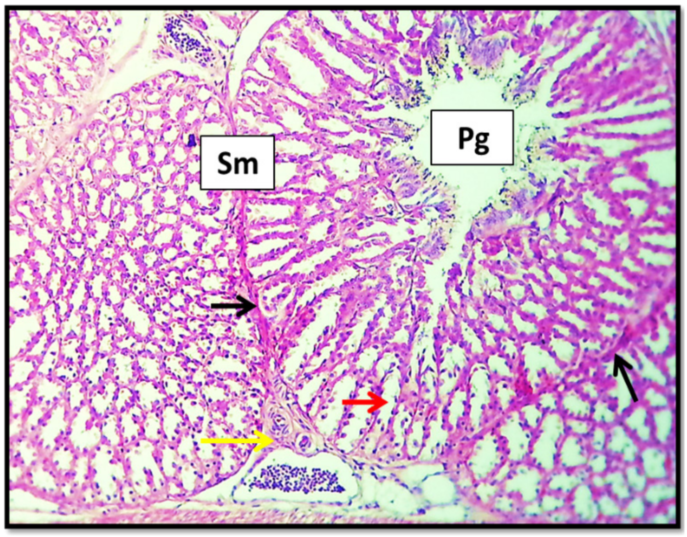

Figure 4: Cross-histological section of bronze fallow cockatiel proventriculus showing tunica submucosa (Sm), proventriculus gland (Pg), secretory unit (red arrow), collagen bundles (black arrow), blood supply) yellow arrow). HandE stain. X200.

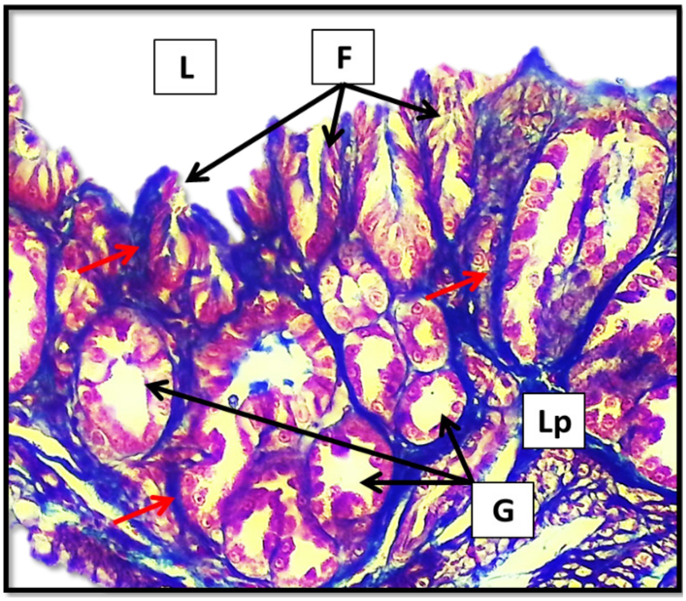

Figure 5: The cross section of bronze fallow cockatiel proventriculus show: lumen (L), mucosal fold (F), mucosal gland (G), lamina properia (Lp). Masson trichrom stain. X200.

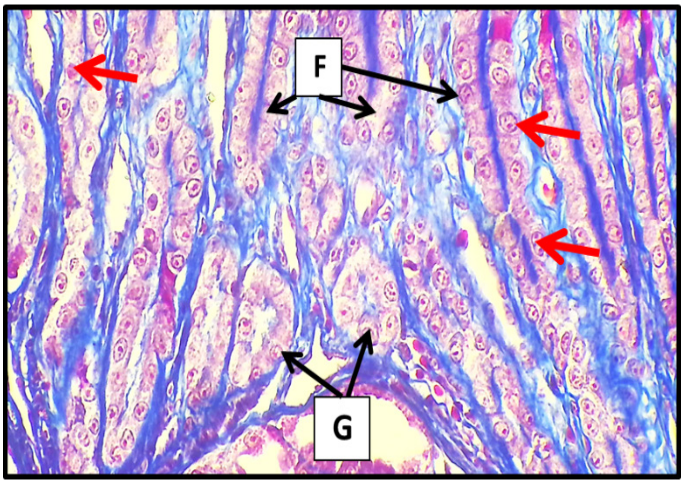

Figure 6: Cross-histological section of white eared bulbul proventriculus show: mucosal fold (F), simple columnar epithelium (red arrow), mucosal gland (G). Masson trichrom stain. X400.

The musculature of the eared bulbul consists of two layers of smooth muscle fibers, an inner circular layer and an outer longitudinal layer. The outer longitudinal layer is thicker than the inner circular layer. In the Bronze Eagle-crowned Cockatiel, this layer consists of three layers of smooth muscle fibers: The inner and outer circular layers and the middle layer of longitudinal smooth muscle fibers. The middle layer (longitudinal muscle fibers) was thicker than the inner and outer layers. The tunica muscularis of the eared bulbul and bronze fallow cockatiel was measured at about 166.56±3.36 µm and 61.51±2.2 µm, respectively (Figures 7, 8 and Table 1). The tunica serosa in both birds (the white-eared bulbul and the bronze fallow cockatiel) was composed of mesothelial cells, loose connective tissue, and adipose tissue. The tunica serosa was rich in blood supply, lymphatic vessels, and nerve fibers.

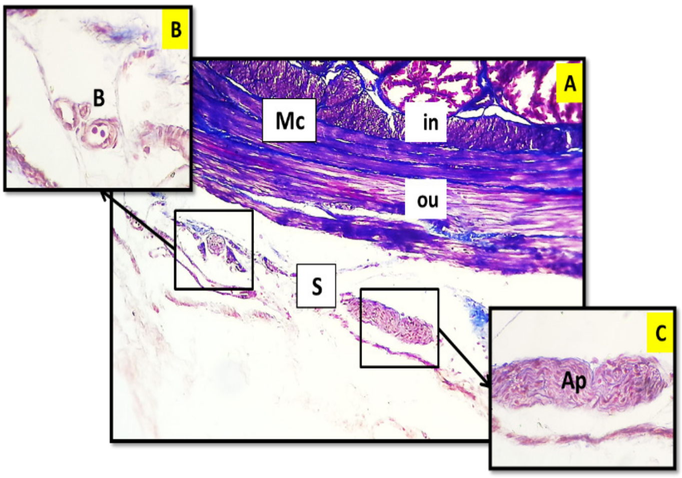

Figure 7: The cross section of the white-eared bulbul's proventriculus demonstrates: the muscularis layer (Mc), the serosa layer (S), the inner layer (in), the outer layer (ou), the blood supply (B), and the auerbach plexus (Ap). Masson trichrom color. X100. B and C: enlarged view.X400.

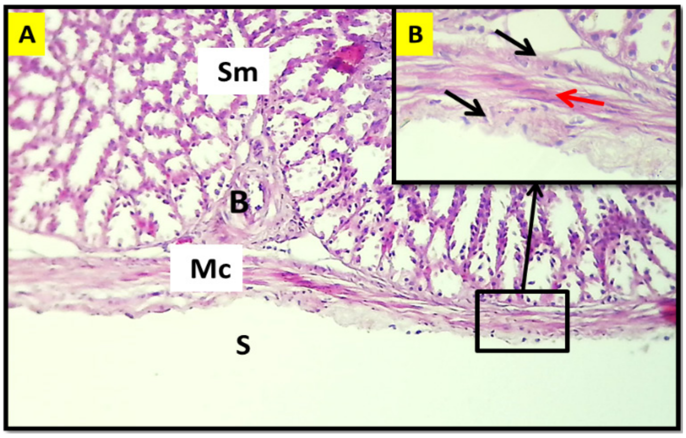

Figure 8: Cross-histological section of bronze fallow cockatiel proventriculus show the following composition: the submucosa (Sm), the muscularis (Mc), the serosa (S), the blood supply (B), the inner and outer muscular layers (black arrows), middle muscular layer (red arrow). HandE color.X100. B: enlarged section X400.

The histochemical study of the proventriculus gives the same reaction in both birds (white eared bulbul and bronze-fallow cockatiel). The mucosa exhibited a positive response to the alcian blue stain and a negative response to the periodic acid Schiff, reflecting acidic secretion in the mucosa. The mucosal gland and the proventriculus gland did not take alcian blue stain but reacted positively to periodic acid-Schiff, indicating that the secretion was neutral mucopolysaccharide.

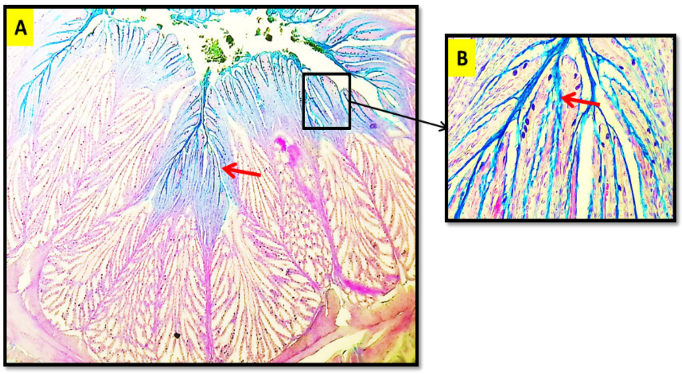

Figure 9: A: Cross histological section of white eared bulbul proventriculus show that the mucosa gland and mucosal surface give positive reaction for alcian blue stain. (acidic mucopolysaccharid) this reaction appear as blue in color and the proventriculus gland give negative response to the alcian blue stain. AB. Stain. X40. B: Magnified section. X400.

DISCUSSION

The glandular vestibule lacks papillae and ducts that lie directly on the mucosal surface. It has four basic membranes that are associated with tubular structures: The mucosal lamina, submucosa, muscularis, and serosa.

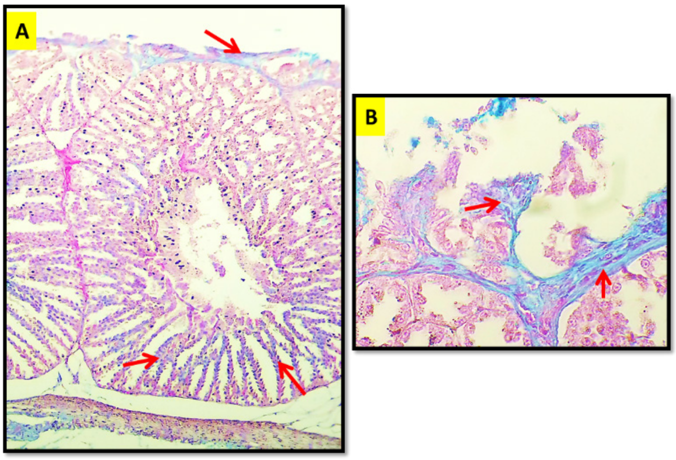

Figure 10: A: Cross histological section of bronze fallow cockatiel proventriculus show that the mucosa gland and mucosal surface and the proventriculus gland both have a positive response to alcian blue staining (acidic mucopolysaccharid) this reaction appear as blue in color (red arrow). AB stain. X100. B: Magnified section. X400.

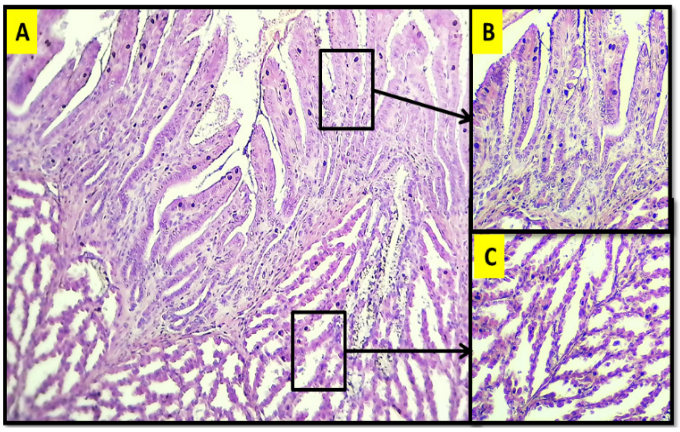

Figure 11: A: Cross histological section of white eared bulbul proventriculus show that the mucosa gland and mucosal surface and the proventriculus gland give positive reaction for periodic acid shif (neutral mucopolysaccharid) this reaction appear as magenta in color. PAS stain. X100. B and C: Magnified section. X200.

These layers are present in the glandular vestibule of various birds, including domestic pigeons (Bancroft *et al*., 2019), Japanese quail (Batah *et al*., 2012) and coots (Jassem *et al*., 2016). Recent studies have shown that the lining of the proventricular mucosa is filled with goblet cells. This contradicts the unexpected results of Eman's study (Das *et al*., 2017). Examination of the groin of *Gallinula chloropus* revealed that the fibers are located within the folds and have simple channels through which the lubricant can escape (Rossi *et al*., 2005). The lymphoid tissue in the gastric mucosa of Kadaknath chickens is diffuse or nodular. The delicate, smooth muscle fibers of the muscularis mucosa are evenly distributed throughout the glandular bundles. The submucosal layer comprises numerous glands within the submucosal tissue; a layer of cuboidal cells that project outward in a straight line (Taher *et al*., 2020) lines these glands. Studies have indicated that a fibrous sheath surrounds the red partridge (*Rhynchotus rufescens*) glands. Results observed are in corroboration with our earlier findings reported by Rossi *et al*. (2005). Al-Jebori *et al*. (2020) discussed that the muscular tissue in the partridge has an internal layer of muscle and an exterior layer of fat. In the domestic duck, the muscular tissue comprises inner longitudinal muscle fibers and outer circular muscle fibers, as well as proximal and distal parts of the musculature, with an additional layer located in the anterior part (Al-Kafagy *et al*., 2022). Loose connective tissue forms the serosa, which includes a large number of blood vessels, neurons, and adipocytes. It is topped with a layer of mesothelial cells, which harbor some cells, as found by Hussein *et al*. and their coworkers (Hussein *et al*., 2020). The serosa consists of collagen fibers, in addition to a layer of rounded cells. Histochemically, the apical surface of the folds is predominantly PAS-positive, with AB at 2.5 in the majority of cells, indicating the presence of both neural and acidic mucus. The cells lining the lumen of the submucosal glands of the two apical glands are PAS-positive, indicating the presence of mucus within them, with AB at 2.5, which tends to suggest that the mucus is a mixture of neutral and acidic components. Results thus far are in relatively good agreement with those of Taher *et al*. (2020) and the birds, respectively, and contradict the results of Udoumoh and Ikejiobi (2017), who found that the complex tubular glands in the vestibule of the African crow gland were negatively reactive to PAS.

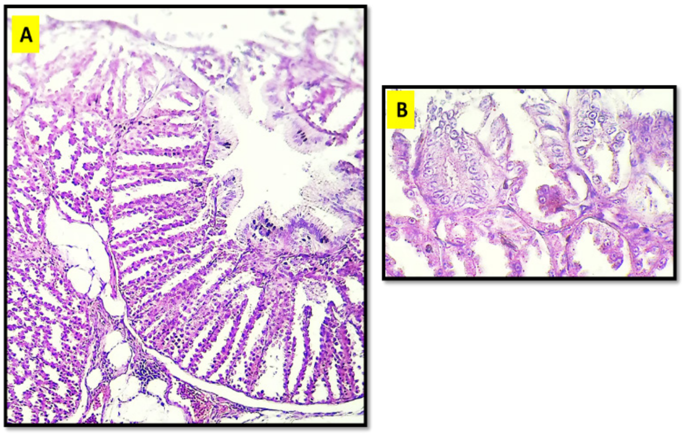

Figure 12: A: Cross-histological section of bronze fallow cockatiel proventriculus show that the mucosa gland and mucosal surface and the proventriculus gland give positive reaction (neutral mucopolysaccharid) this reaction appear as magenta in color. PAS stain. X100. B: Magnified section. X400.

CONCLUSIONS

This study reveals that the proventriculus fold in the bulbul is taller than in the cockatiel, characterized by a large number of goblet cells. The proventriculus gland is arranged in two rows in the cockatiel and one row in the bulbul. The secretion of both birds was acidic and neutral mucopolysaccharides.

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NOVELTY STATEMENT

The study examined the impact of dietary changes on the histological structure and secretory function of the Gizzard and Proventriculus.

AUTHOR'S CONTRIBUTION

Mohamed Ibrahim: Conceptualization, methodology, validation, writing original draft, writing review and editing. Siraj Moner Al-kafagy: Conceptualization, methodology, review and editing, supervisor.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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