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

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Abstract | This study was conducted to investigate the histochemical structure of the duodenum in the White-eared Bulbul and Bronze Fallow Cockatiel. Twenty adult healthy birds, ten of each species (White Eared Bulbul and Bronze Fallow Cockatiel), were obtained from a local bird market in Hilla. Following anesthesia of the birds, their duodenum was dissected and collected. Small segments of tissue, 6-8 mm in length, from the duodenum were taken and subjected to preparation for histochemical study using stains such as hematoxylin and eosin, PAS, AB at pH 2.5, and Masson's trichrome. Results revealed a much thicker duodenal mucosa than that of the Bronze Fallow Cockatiel, which was denser in the White-eared Bulbul. It was comprised of numerous villi; those of the Bronze Fallow Cockatiel were smaller than those of the Eared Bulbul. Simple columnar epithelium cells have a villus-free surface. Brush border (microvilli) free surface columnar cells. Loose connective tissue layer crypts lieberkuhn/tunica muscularis. Brunner's glands are in the tunica submucosa. A two-layer thick inner circular and thin outer longitudinal layer of tunica muscularis, with the Auerbach plexus outer longitudinal layer. Serosa: This is a layer of thin, loose connective tissue that is covered by mesothelial cells. The duodenal gland exhibited a positive reaction to periodic acid-Schiff and Alcian blue stains, indicating the presence of secretions of both neutral and acidic mucopolysaccharide nature in both birds.

Keywords | Bird, Small intestine, Duodenum, Goblet cell, Histochemical

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INTRODUCTION

Cockatoos inhabit the whole of the Australian land, preferring the central parts to the coast. They mostly gather around fresh water bodies and prefer open spaces to forests (Pizzey and Knight, 1997; Recher, 2017). The body of the White Eared Bulbul is grey-brown with a jet-black head and a significant mark on its cheek. It has a lightly colored ring around its eye, whereas the vent is yellowish (Srivastava, 2012; Al-Jebori *et al.*, 2020). The duodenum's ascending and descending parts are occupied by the pancreas and are enclosed on all sides by the pancreaticoduodenal ligament (Al-Kafagy *et al.*, 2022; Mahmood *et al.*, 2022; Ali *et al.*, 2024; Al-Sailawi *et al.*, 2024).

The small intestine is composed of the mucosa, submucosa, muscularis mucosa, and serosa, respectively, from inside to outside (Kadhim *et al.*, 2014; Al-Khafaji and Al-Kafagy, 2024). The small intestine resembles the mammalian small intestine, which has a single-layered epithelial cell (absorptive, goblet, and enteroendocrine cells). Mucus-secreting goblet cells, which are situated at villi as well as crypts, reflect the interface between the gut and the flora. The structural appearance of villi forms a leaf-like structure

sticking into the gut wall it creating the spongy region and therefore is specific to the dimension of the small intestine (Casteleyn *et al*., 2010). The crypts of Leiberkuhn, simple tubular organs with a covering epithelium similar to that of the small intestine, are situated between the base of the villi and the layer of longitudinal smooth muscle fibers that comprise the muscularis mucosa (Nasser and Khaleel, 2021).

MATERIALS AND METHODS

This study examined the characteristics and differences in the macroscopic appearance and microscopic structure of the duodenum and jejunum of two bird species, based on their distinct diets. The Bronze Fallow Cockatiel (*Nymphicus hollandicus*) and the herbivorous or frugivorous bird, the White-eared Bulbul (*Pycnonotus leucotis*). Twenty adult healthy birds (10 Bronze Fallow Cockatiel and 10 white-eared bulbul) were purchased from bird owners in a local market (Spinning market in Baghdad) without sex differences and subsequently divided into two groups for histological and histochemical examinations.

Ten birds were used to conduct the histological examination. (Five birds of each species). The duodenum and jejunum were carefully removed and rinsed with saline to remove debris. They were then placed in an appropriate amount of fixative (at least ten times the volume of the tissue) as quickly as possible to avoid postmortem changes. (Gilbertson, 2012) Subsequently, individual animals of each species were cut open transversely with a sharp scalpel.

RESULTS

The histological study revealed that the duodenum of the white-eared bulbul is composed of four distinct tunicae: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. The tunica mucosa measured about (1358.15 ± 43.19µm) in thickness, which was thicker than that in the bronze fawn cockatiel. It showed epithelial mucosa, lamina propria, and crypt of Lieberkühn with the absence of muscularis mucosa. It is composed of numerous villi. The height of these villi was (1095.64±8.68 µm), which was significantly greater than the villi of the duodenum in the bronze fallow cockatiel (Figure 1, Table 1).

The epithelial cells of the mucosa within the villi are lined by simple columnar epithelium made by columnar cells that have an oval nucleus at the basement membrane. The villi were taller than those in the mucosa of the bronze fowl cockatiel, which was measured at about (1095.64±8.68 µm). At the free surfaces of the columnar cells, the brush border (microvilli) showed that the epithelial mucosa was intermingling with a large number of goblet cells (Figure 2, Table 1).

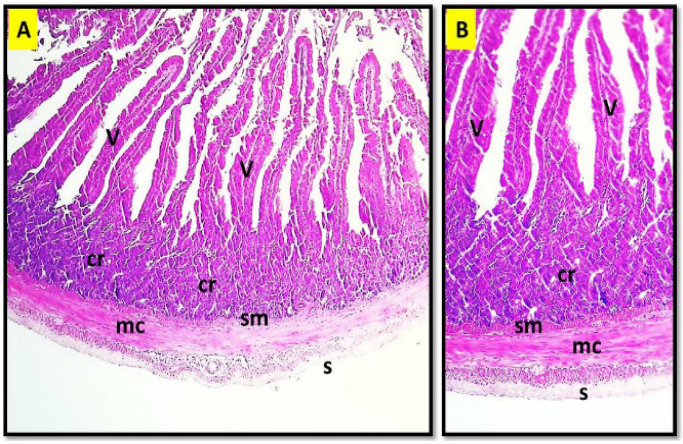

Figure 1: A: Histological sections of the duodenum in the bulbul demonstrate: villi (V), crypt (cr), the submucosa of the tunica (sm), the muscularis of the tunica (mc), and the serosa of the tunica (S). H&E color, x10. B: Exaggerated section x200.

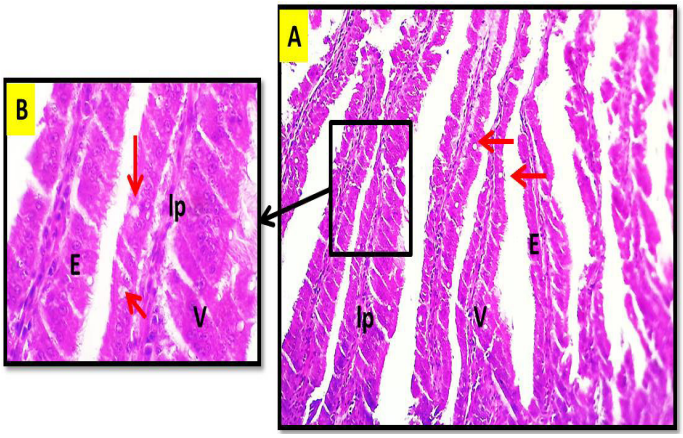

Figure 2: A: Histological sections of the duodenum of a bulbul's stomach: villi (V), laminae (lp), simple rectangular epithelium (E), and a Goblet cell (G). H & E staining. x100. B: enlarged section, X400.

Table 1: The thickness of the mucosa, submucosa, and muscularis, and the height of the epithelium, depth of crypt, and height of villi of the duodenum in both birds.

Bird	Thickness of mucosa	Thickness of submucosa	Thickness of muscularis	High of epithelium	Depth of crypt	High of villi
Bulbul	1358.15 ± 43.19	25.17±0.32	162.25±1.32	38.64±0.36	312.43±1.84	1095.64±8.68
Cockatiel	878.68±70	17.81±1.24	83.54±0.26	53.21±0.82	107.68±1.12	582.82±3.46

Villi in the duodenum of the white-eared bulbul appear. A thin layer of tissue that is easily coverable already covers the villi, whereas in the white-eared bulbul, it covers Lieberkühn crypts with connective tissue. The mean crypt depth for the white-eared bulbul is higher than that for the bronze ground dove; it measures about 312.43±1.84 µm, with no muscularis mucosa within the duodenal mucosa of the white-eared bulbul. The tunica submucosa of the white-eared bulbul duodenum measures about 25.17±0.32µm in thickness. It is a meager waste of interfascicular tissue that lies betwixt the crypts of Liberkühn and the tunica muscularis. Brunner's glands are located in the proper stratum of the duodenum of the white-eared bulbul. They also extend into the submucosa. This gland is structured as lobes separated by collagen septa and composed of cuboidal to columnar epithelial cells. Brunner's gland does not take up stain from alcian blue but takes up PAS stain, showing that this gland has a neutral secretion. The muscular wall of the duodenal loop of the bulbul observed was muscularis. It had relatively thick walls, approximately 162.25 ± 1.32 µm in thickness, consisting of two distinct layers. The inner layer was thick and composed of circular muscles, while the outer layer was thin and comprised longitudinal muscles, within which the Auerbach plexus was observed. This muscular wall is much thicker than that of the Fallow Bronze Cockatiel. The serous tunic was a thin, wasted layer of connective tissue, covered with mesothelial cells (Figures 3, 4 and Table 1).

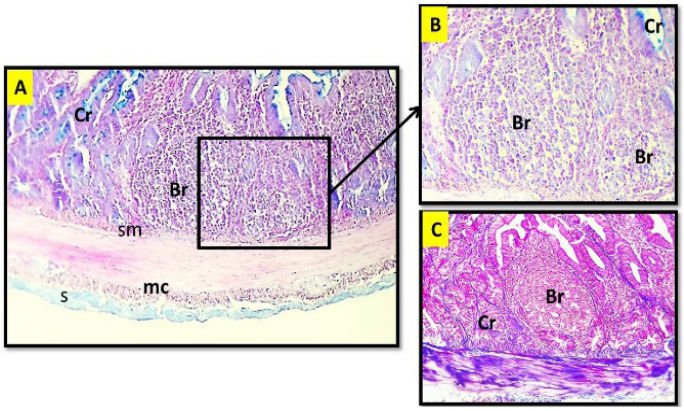

Figure 3: A: Histological sections of the duodenum in the bulbul animal exhibit: crypt (Cr), the submucosa of the tunica (sm), the muscularis of the tunica (mc), the serosa of the tunica (s), and the Brno's gland (Br). The Brunner's gland has a negative response to the alcian blue stain (random secretion). AB mark. X100. B: enlarged section. X400. C: The Trichrom Stain of Masson. x20.

The histological results indicated that the duodenal tunica mucosa in bronze fallow cockatiels measured approximately 878.68 ± 70 µm in thickness, characterized by crowded villi that appeared to be branched at their apical parts. The villi of the bronze fallow cockatiel were shorter than those of the white-eared bulbul, it measured about 582.82±3.46 µm in length. Villi were found to be lined with simple columnar epithelium bearing a brush border and to be characterized by goblet cells (Figure 5, Table 1). The proper lamina of the duodenum villi of the bronze fawn cockatiel is a thin, loose connective tissue layer. In contrast, the appropriate lamina under the villi is a thick layer of connective tissue that is occupied by crypts of Lieberkühn. The crypt depth mean in bronze fallow cockatiel was less in width than that in white-eared bulbul, which measured about (107.68±1.12 µm). The duodenum mucosa of the bronze fawn cockatiel appeared without muscularis mucosa (Figure 5, Table 1).

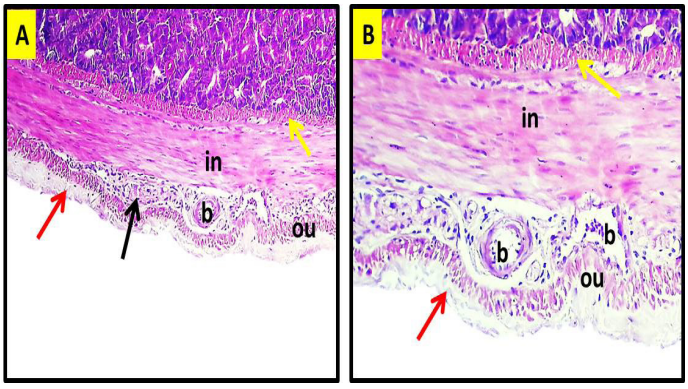

Figure 4: A: The histological composition of the duodenum of a bulbul is as follows: the submucosa layer (yellow arrow), the inner layer of the tunica muscularis (in), the outer layer of the tunica muscularis (ou), the blood vessels (b), the auerbach plexuses (black arrow), and the tunica serosa (red arrow). H&E staining.x100. B: enlarged section.x200.

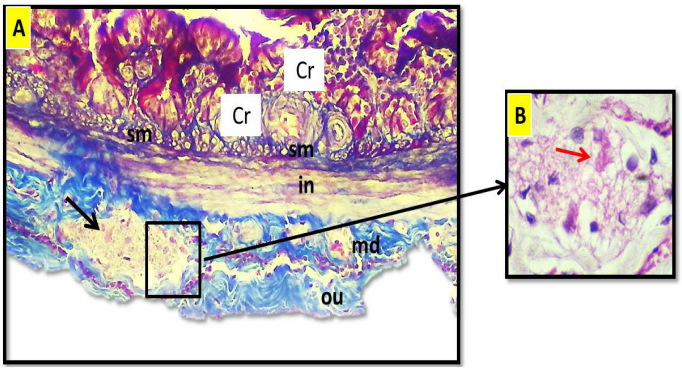

Figure 5: Histological section of duodenum in cockatiel shows: crypt of liberkhan (Cr), tunica submucosa (sm), inner muscular layer (in), middle muscular layer (md), outer muscular layer (ou), auerbach plexus (black arrow). Masson trichrom color. X200. B: enlarged section. X1000.

The tunica submucosa of the bronze fawn cockatiel duodenum was measured at about 17.81 µm in thickness. It comprises a layer of tissue that is loose and consists of the crypts of the mouth and the muscularis. Brunner glands are identical to those of the white-eared bulbul. The muscularis resembles that of the white-eared bulbul, featuring a thick inner circular muscle and a thin outer longitudinal smooth muscle fiber. It averages 83.54 µm in

thickness. Just behind the muscularis is the mesothelium, which is covered by a layer of loose connective tissue that forms the serosa (Figure 6, Table 1).

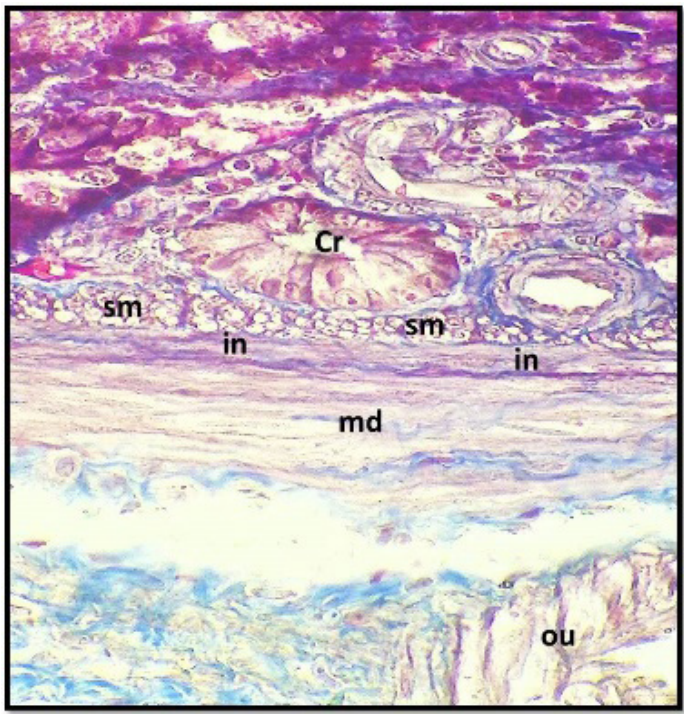

Figure 6: The histological composition of the duodenum in a Cockatiel is as follows: the crypt is located in the vicinity of Liberkhan (Cr), the tunica is sub-mucosa (sm), the inner layer is muscular (in), the middle layer is muscular (md), and the outer layer is muscular (ou). Masson trichrom stain. X400.

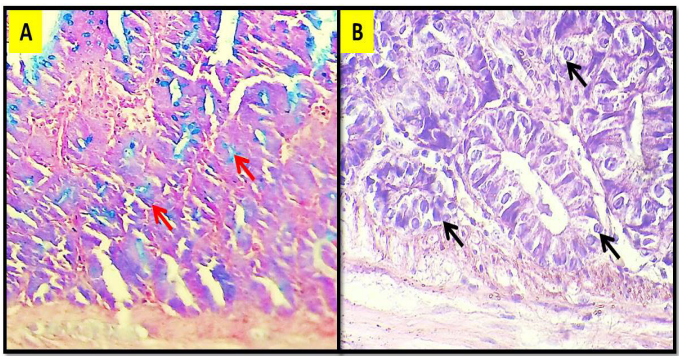

Figure 8: Histological section of duodenum in bulbul shows: the goblet cell in crypt was give positive reaction for AB stain (A, red arrow) and give positive reaction for PAS stain at villi (B, black arrow). (A: AB stain. X200, B: PAS stain. X400).

Results of the histochemical examination of two duodenal sections of the small intestine of the white-eared bulbul and bronze-crowned cockatiel showed that the villus epithelium and mucus-secreting goblet cells in the epithelial crypts reacted distinctly to the stains of Alcian blue and periodic acid. The duodenal villi of the white-eared bulbul are found to be strongly positive for AB stain and moderately positive for PAS stain. However, the villi of bronze-crowned cockatiels are moderately reactive to AB stain and strongly reactive to PAS stain, which indicates that acidic mucopolysaccharides in white-eared bulbul are more than in bronze-crowned cockatiels. The crypts of the duodenum in the white-eared bulbul give a negative reaction for AB staining and a positive response for PAS, indicating neutral sites of mucopolysaccharides. Secretions from crypts of bronze fallow cockatiels were identical to those of bulbul with a weak AB staining reaction (Figures 7, 8, 9).

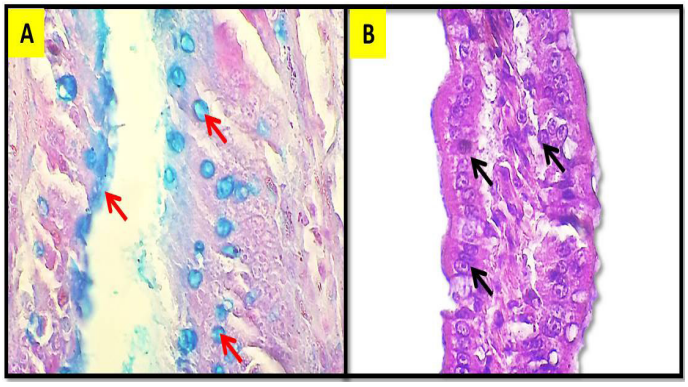

Figure 7: Histological section of duodenum in bulbul shows: the goblet cell in villi was give positive reaction for AB stain (A, red arrow) and give positive reaction for PAS stain at villi (B, black arrow). (A: AB stain, B: PAS stain. X400).

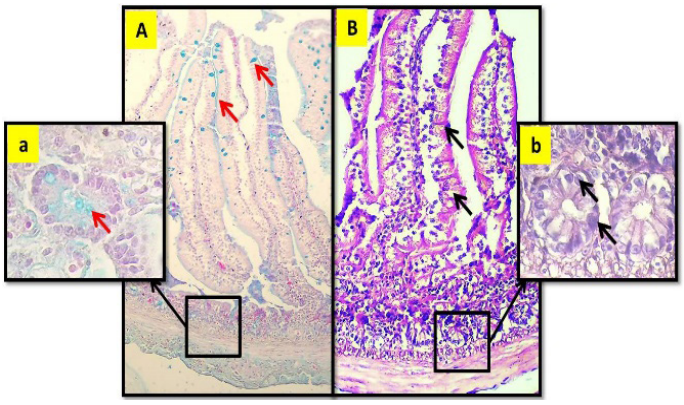

Figure 9: Histological section of duodenum in cockatiel shows: The goblet cell in villi and crypt was give positive reaction for AB stain (A, red arrow) and give positive reaction for PAS stain at villi (B, black arrow). (A: AB stain.x100, B: PAS stain. X100). (a and b: magnified section. X400).

DISCUSSION

The results of the present study are nearly identical to those of a previous study, which reported that the villi of the small intestinal mucosa in the duodenum contained the most significant number of cells, followed by the jejunum and the ileum. They are somewhat variable in size,

and are elongated. These villi are surrounded by simple columnar cells, a functionally absorptive cell type with an overnuclear-oval mechanism, where the basal part is either flat or appears to be provided with microvilli borders. Columnar cells are functionally accompanied by goblet cells, the cytoplasm of which stains lightly, and the number of which increases towards the ileum. Lie within loose connective tissue that is supplied with blood vessels and nerves, and well-developed lymphoid tissue.

It is reported in this study that the results on the muscularis mucosa of the intestine of two bird species directly contrast the results of Dawood (2013) on the muscularis mucosa of the intestine of male and female domestic ducks. The muscularis mucosa is reported to have good development in the intestinal wall of ostriches. It is composed of longitudinally and circularly arranged smooth muscle bundles, as seen in macaws, such as the blue and gold macaw. This forms a separating layer between the submucosa and the lamina propria, connecting the two connective tissues. The absence of muscularis mucosa in the present results, where it is reported to be completely absent, is in agreement with the results of (Igwebuike and Eze, 2010; Alkafagy *et al*., 2019) that there is no such thing as muscularis mucosa since there has been reported to be continuous attachment between the connective tissue layer of the submucosa and the lamina propria.

In this study, the structure of the small intestine of birds belonging to the species Columba liva and Buteo buteo vulpinus (Al-Ghakany, 2013) was revealed to have well-organized submucosal groups of glands. This does not support the present study's results because the submucosal layer is only a thin development of connective tissue; therefore, it lacks submucosal glands or Brunner glands. Large numbers of well-developed lymph nodes in the lamina propria of the mucosa have been found to undermine the framework of the muscularis mucosa and extend into the submucosal layer (Bezuidenhout and Van Aswegen, 1990); this finding does not align with the results of the present study. In this study, the muscularis mucosa appeared double layered, parallel to the muscularis mucosa reported in references on African ravens and Japanese quail; the muscularis mucosa of references on peacocks and ducks comprises two smooth muscle fiber layers, arranged circularly in the interior and longitudinally on the exterior, with a layer of fluffy connective tissue between the muscularis mucosa.

The results of the present study on the serosa layer were, in most cases, similar to the histological findings of other studies on the intestinal tract of birds such as chicken and quail. These two surface layers exhibited very slow metabolic activity in the white-eared bulbul. The height of the duodenal villi of white-eared bulbul and cicadas differs and may be due to the different diets and related habits of these birds. Earlier workers reported that an increase in the height of the villi increases the intestinal surface area, thus accelerating food intake. This description is suitable and applicable to cockatiels, as their habit is to fly long distances, which requires high energy and necessitates the elimination of a high digestive load, potentially impairing their ability to fly or hunt. Factors resulting in a greater absorptive surface within the duodenal mucosal layer of falcons, changes are evidenced premised on the one hand by the impact of foods high in energy and low in fiber and easily digestible, and on the other hand by the hunting dependence of the birds and high metabolic nutrition due to their enormous body weight and long flight distance. This is supported by Alhaaik and Mahmood (2024), who state that changes in the absorptive unit can alter absorptive capacity. Also found was the fact that the morphology of the absorptive cell in pigeons indicated the absorptive capacity.

The duodenal high goblet cell percentage of the white-eared bulbul reflects a nutritive system that is high in fiber food; food that would be difficult to digest without goblet cells. The mucus that goblet cells secrete is very significant in the lubrication of the alimentary canal as well as in the enhancement of the process of digestion. If at all, there is a low goblet cell percentage found in the white-eared bulbul's duodenum, then it could be well attributed to the Brunner glands because for this specific species, it relies more on these special glands for mucus secretion; mucus that has a direct relationship with high fiber content in its diet. In their study, rock pigeons were attributed to a high number of goblet cells in their duodenal fiber diet content.

These findings are identical to those obtained in the small intestine of the Persian squirrel (Tootian *et al*., 2013) but differ from those found in the small intestine of the indigenous duck (Dawood, 2013). Goblet cells are known to secrete mucus molecules that are positive for the PAS reaction (red) at pH levels of 1.0 and 2.5, and also positive for the AB reaction (blue). This implies that there are neutral, acidic-COOH, and acidic-SO3H mucus in goblet cells. Located toward the anal end of the gut, goblet cells multiply in large numbers. They secrete mucus used for lubrication and, at the same time, offering protection to the mucosa from the clogging action of feces (Ghosh *et al*., 2011; Ikpegbu *et al*., 2014).

CONCLUSIONS

This study shows that the duodenum villi in bulbul were taller than those in cockatiel, with a large number of goblet cells, and the Paneth cells extended from the tunica submucosa toward the mucosa. The secretion of both birds was an acidic mucopolysaccharide with a weak neutral pH.

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NOVELTY STATEMENT

This study was investigated the histochemical structure of the duodenum in the White-eared Bulbul and Bronze Fallow Cockatiel.

AUTHOR'S CONTRIBUTION

Mustafa Fahem: Conceptualization, methodology, validation, writing original draft, writing review & editing. Siraj Moner Al-Kafagy: Conceptualization, methodology, review & editing, supervisor.

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Conflict of interest
The authors have declared no conflict of interest.

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