

# Anatomical, Histometrical, And Histochemical Comparative Study Between Stomach Of Ring Necked Parakeet - *Psittacula krameri* (Scopoli, 1769) And Black Shouldered Kite - *Elanus caeruleus* (Desfontaines, 1789)

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## Abstract

The present study aims to investigate the anatomical, histometrical, and histochemical aspects of the stomach in two species of birds, *Psittacula krameri* (Ring-necked parakeet) and *Elanus caeruleus* (Black-shouldered kite). The stomach tissues in the two species were stained with Hematoxylin and Eosin for general histological study and Periodic Acid Schiff (PAS)-Alcian blue (AB) pH 2.5 for histochemical study. An automated analysis system was used for histometrical study. The stomach in two species of birds was divided into proventriculus and ventriculus (gizzard); in the parakeet, proventriculus appeared as fusiform shape and the isthmus separated the gizzard from proventriculus, while in the kite, the proventriculus was pear shape, and the isthmus was absent. In the parakeet, the gizzard was a spherical shape with a thick koilin layer, while the kite has pear shape gizzard with a thin koilin layer. The mucosa was possessed simple tubular glands which were larger and more in the kite; the submucosa was rich with compound tubular glands which were longer and wider in the kite. The histochemical results showed a positive reaction of epithelial cells and simple tubular glands with PAS-AB stains; the koilin and deep gastric glands showed a positive reaction with the PAS stain only.

**Keywords:** Histochemical, Histometrical, Kite, Parakeet, Stomach.

## Introduction

In birds, the process of digestion in the digestive system takes place by mechanical and chemical action to absorption the nutrients from their ingested food; the stomach structure in birds is various in shape, size, and weight according to the feeding nature (Hassouna, 2001). It is the most important part of the digestive system in birds; there are two parts of the stomach in birds, the glandular part, proventriculus, and the muscular part ventriculus or gizzard (Hristov, 2020).

The birds are divided into two types according to the nature of the diet, the first type was soft diet eating birds in which the main role of the gizzard is the storage of food; the stomach has a big single chamber such as kestrel and owl (Abumandour, 2013, 2014; Al-Saffar, and Al-Samawy, 2014). The second, hard diet eating birds in which the main role of the gizzard was the mechanical treatment of the food, the stomach has two

chambers with a thick wall of ventriculus such as turkey and sparrow (Hassouna, 2001).

The stomach of birds was divided into three types according to nature of diet as noted by (Hassouna, 2001); first type, soft eating birds as in kestrel and owl in which the main function of the gizzard was the storage of food. Second type, hard diet eating birds as in turkey and sparrow in which the The stomach of birds was divided into three types according to nature of diet as noted by (Hassouna, 2001); first type, soft eating birds as in kestrel and owl in which the main function of the gizzard was the storage of food. Second type, hard diet eating birds as in turkey and sparrow in which the The stomach of birds was divided into three types according to nature of diet as noted by (Hassouna, 2001); first type, soft eating birds as in kestrel and owl in which the main function of the gizzard was the storage of food. Second type, hard diet eating birds as in turkey and sparrow in which the first type, soft eating birds as in kestrel and owl in which the main function of the gizzard was the storage of food. Second type, hard diet eating birds as in turkey and sparrow in which the

The hydrochloric acid and pepsinogen are secreted by the proventriculus; the gizzard mixed the food with these materials due to the strong muscles and thick koilin layer of the gizzard wall (Svihus, 2014; Al-Juboory et al., 2016).

The current study investigated the anatomical, histometrical, and histochemical aspects of the stomach in the *Psittacula krameri* (Scopoli, 1769) and *Elanus caeruleus* (des Fontaines, 1789), and its relationship with the type of food consumed for both species.

## Materials And Methods

Twenty adult birds were used (10 Ring-necked Parakeets and 10 Black-shouldered Kite). The birds were brought from Suq Al-ghazl in Baghdad and checked for their health status. The birds were anesthetized by using chloroform. Tissue samples of the proventriculus and ventriculus were removed and immersed in formalin for 24h. After fixation, the specimens were dehydrated with alcohol (80, 90, 95, and 100%), then cleared with xylene, embedded in paraffin wax. The samples were sections to a thickness (5mm) by using a rotary microtome, and Stained routinely with Hematoxylin and Eosin stains for general histological study, also special stains of Periodic Acid Schiff (PAS)-Alcian blue (pH 2.5) were used for the histochemical study (Suvarna et al., 2019). Later, the sections were examined by an Olympus microscope. Images were taken by the digital microscopic camera for histometrical analysis. The automated computer-adopted image analysis system Image J<sup>®</sup> was used.

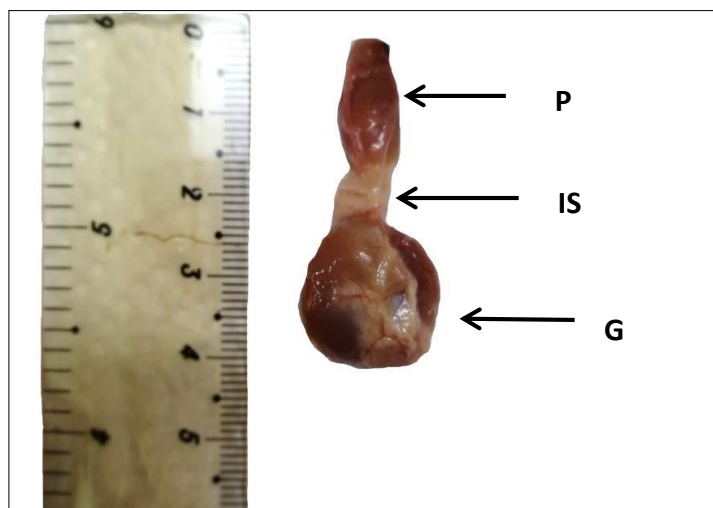
## Results

### Anatomical and Morphological description of stomach

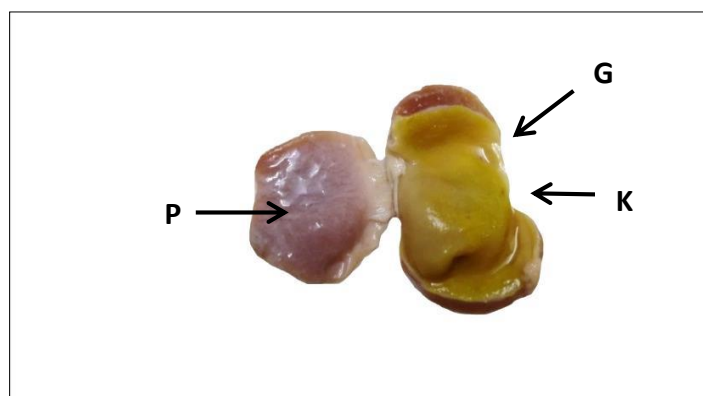
#### 1. Ring necked parakeet - *Psittacula krameri* (Scopoli, 1769)

The anatomical results showed that the stomach was situated in the left median part of the abdominal cavity. The stomach in the Ring-necked parakeet consists of two chambers, the glandular stomach or proventriculus, and the muscular stomach, ventriculus, or gizzard. The Proventriculus appeared as a thin-wall fusiform shape tube started at the narrow end of the thoracic esophagus in the abdominal cavity. The isthmus separated the proventriculus from the gizzard (Fig.1). The mean length of proventriculus was (1cm) and width (0.5cm).

The ventriculus or gizzard appeared in a Ring-necked parakeet as a disc or biconvex lens shape. It was a thick wall and with a smooth inner surface. Also, it was lined by thick keratinized tissues with yellowish-green in color called koilin (Fig. 2). The mean length of the ventriculus was (1.5 cm) and width (1.5cm).



**Figure 1.** Photographic pictures of the stomach in ring necked parakeet (G: gizzard, IS: isthmus, P: proventriculus).

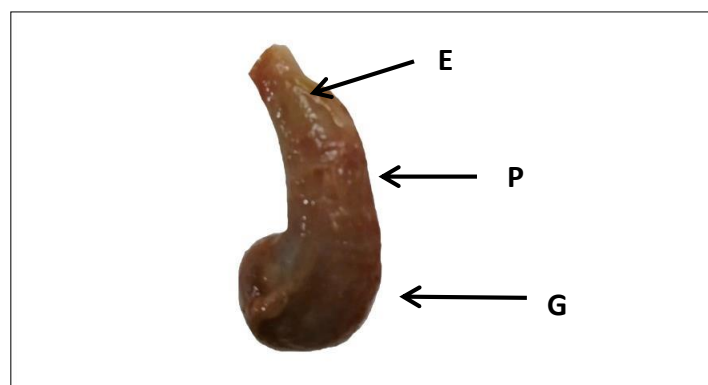


**Figure 2.** Photographic pictures of the stomach inner surface in ring necked parakeet (G: gizzard, K: koilin, P: proventriculus).

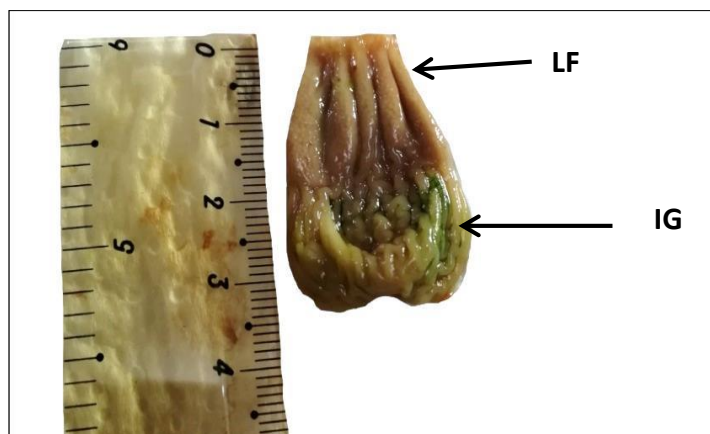
## 2. Black-winged Kite -*Elanus caeruleus* (desfontaines, 1789).

In the black winged kite, the proventriculus appeared as pear elongated shape, thick wall with five longitudinal muscular folds. It was a brown color. There was no isthmus separated proventriculus from the gizzard (Fig. 3). The proventriculus in Black-winged Kite appeared longer and wider than the proventriculus of Ring-necked parakeet. The mean length of proventriculus was (2 cm) and width (1 cm).

The gizzard in the black-winged kite was pear in shape with inner grooves on its surface; the mean length of the gizzard was (1.25 cm) and width (1.25 cm). Anatomical results showed that the koilin layer was very thin with poor yellowish green pigment inside the gizzard (Fig. 4).



**Figure 3.** Photographic pictures of the stomach in the black shouldered kite (E: esophagus, G: gizzard, P: proventriculus).



**Figure 4.** Photographic pictures of the inner surface to the stomach in the black shouldered kite (IG: gizzard inner grooves, LF: proventriculus longitudinal folds).

**The histological and histochemical description of stomach**

The wall of the stomach in two species of birds consisted of mucosa, submucosa, muscularis, and serosa.

**1. Ring-necked parakeet -*P. krameri*-**

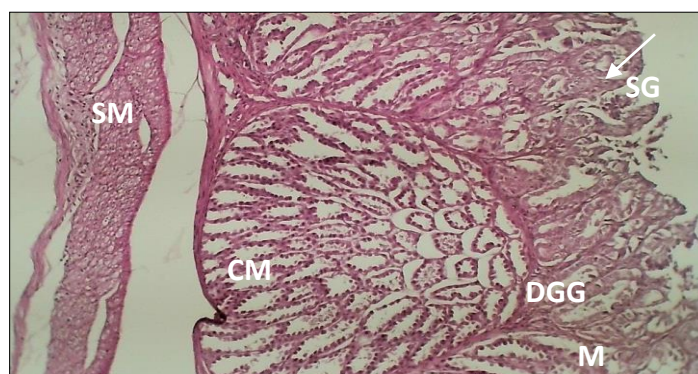
**A. Proventriculus**

The mucosa of the glandular stomach or proventriculus appeared as a finger-like shaped structure, the mucosal epithelium was lined with simple cuboidal epithelial cells with central oval nuclei. The epithelium of mucosa was rested on loose connective tissue represented by lamina propria with few simple tubular glands. No muscularis mucosa under the mucosa (Fig. 5). The mean thickness values of mucosa were (143.05±4.89µm) (Tab. 1).

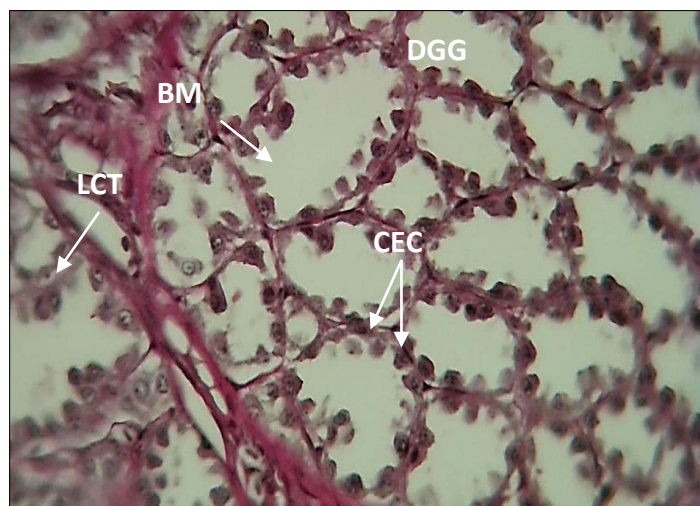
The submucosa was composed of loose connective tissue with a high supply of blood vessels and large oval to conical compound tubular glands called deep gastric glands filling most of the proventriculus wall and separating from each other by connective tissue. The histological examination revealed that the deep gastric glands consisted of numerous secreted units or tubes with different shapes and diameters and arranged with two rows, each one was lined with cuboidal epithelial cells with a central nucleus resting on a basement membrane (Fig. 6).

The results showed that the mean length values of the deep gastric glands were (373.21±37.67µm) and the width (280.80±24.52µm). The mean thickness values of submucosa were (724.43±22.179µm). The muscularis externa consisted of two layers, inner thick circular muscle fibers and outer thin longitudinal muscle (Fig. 5). Mean thickness values of the muscularis layer were (71.74±5.61µm). The outer layer was the serosa, which is composed of loose connective tissue containing blood and lymph vessels, and nerve ends (Fig. 5). The mean thickness values of serosa were (95.10±4.79µm).

The epithelium of the proventriculus showed a positive reaction with PAS- AB stains. The simple tubular glands appeared purple; in the compound tubular glands, the cells lining the ducts and central cavities showed a positive reaction with PAS- AB stains (Fig. 5).



**Figure 5.** Cross-section of the proventriculus wall in ring necked parakeet (CM: circular muscle, DGG: deep gastric glands, LM: longitudinal muscle, M: mucosa, S: serosa, SM: submucosa) (H&E) 100X.



**Figure 6.** Cross-section of the proventriculus deep gastric glands in ring necked parakeet (BM: basement membrane, CEC: cuboidal epithelial cells, DGG: deep gastric gland, LCT: loose connective tissue) (PAS-Alcian blue) 400X.

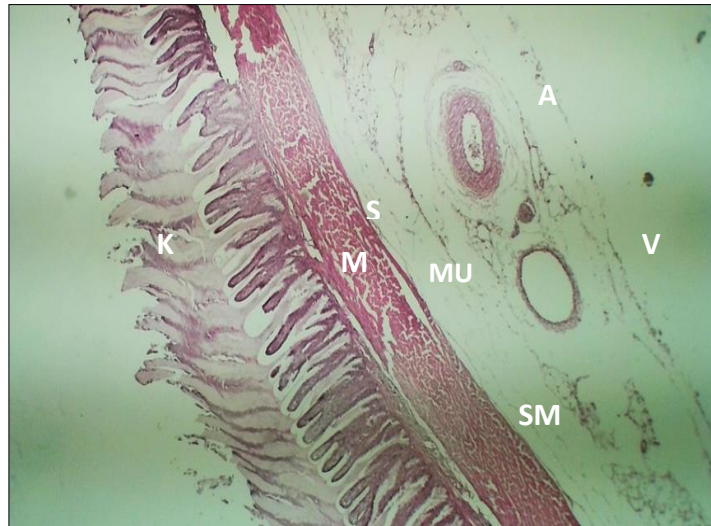
**Table 1.** Measurement of the Proventriculus layers in the ring necked parakeet *-P. krameri* and the black shouldered kite *-E. caeruleus*.

Proventriculus layers thickness	Ring necked parakeet (Mean $\mu\text{m} \pm \text{SE}$ )	Black shouldered Kite (Mean $\mu\text{m} \pm \text{SE}$ )
Glands length	373.21 $\pm$ 37.67	425.59 $\pm$ 18.88
Glands width	280.80 $\pm$ 24.52	293.90 $\pm$ 17.47
Mucosa	143.05 $\pm$ 4.89	171.22 $\pm$ 2.81
Submucosa	724.43 $\pm$ 22.179	819.09 $\pm$ 22.52
Musclaris externa	71.74 $\pm$ 5.61	247.18 $\pm$ 9.75
Serosa	95.10 $\pm$ 4.79	329.62 $\pm$ 14.26

### B. Ventriculus

The mucosa epithelium of the ventriculus was possessed thin folds, finger-like projection toward the lumen. The folds were lined with simple cuboidal epithelial cells with central oval nuclei (Fig.7, 9). The histological examination revealed that there was a layer of koilin in front of the epithelial folds, this layer was stained with eosin only and appeared with clear pink color (Fig. 7). The mean thickness values of koilin were (170.99 $\pm$ 6.10 $\mu\text{m}$ ). Lamina propria appeared as a connective tissue rich with lymph cells, blood vessels, and a few simple tubular glands, these glands were lined with simple cuboidal epithelial cells with a central spherical nucleus (Fig. 8).

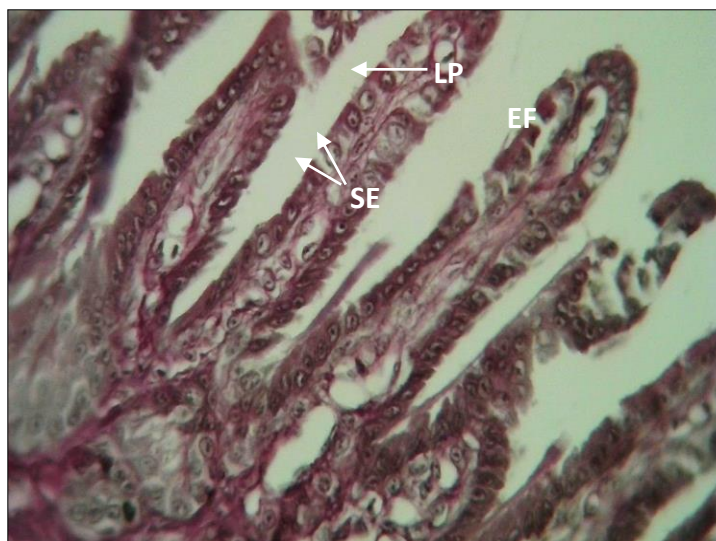
The mean thickness values of gizzard mucosa in the Ring-necked parakeets were (200.88 $\pm$ 4.29 $\mu\text{m}$ ). The submucosa appeared as dense connective tissue with blood vessels and collagenous fibers. The mean thickness values of submucosa were (59.40 $\pm$ 2.39 $\mu\text{m}$ ) (Tab. 2). The histological examination revealed that the muscularis externa consisted of a thick layer of inner circular muscle fibers and a thin layer of outer longitudinal muscle fiber (Fig. 8). The mean thickness values of muscularis were (1124.1 $\pm$ 18.54 $\mu\text{m}$ ). The outermost layer in the gizzard was the serosa that was composed of loose connective tissue lined with simple squamous epithelium rich with arterioles, veins, lymphoid vessels, and nerve ends (Fig. 7) The mean thickness values of serosa were (135.92 $\pm$ 4.65 $\mu\text{m}$ ). The surface covered the gizzard (koilin) showed a pink-colored positive reaction with PAS and a negative reaction with Alcian blue. The surface of mucosal epithelial cells that covered the tubular gland was stained strongly with PAS-AB stain (Fig. 9).



**Figure 7.** Cross-section of the ventriculus wall in ring necked parakeet (A: arteriole, K: koilin, M: mucosa, MU: muscularis, S: serosa, SM: submucosa, V: vein) (H&E) 40X.



**Figure 8.** Cross-section of the ventriculus wall in ring necked parakeet (M: mucosa, MU: muscularis, SM: submucosa, S: serosa) (PAS-Alcian blue) 100X.



**Figure 9.** Cross-section of the ventriculus wall in ring necked parakeet (EF: epithelium fold, GG: gastric gland, LP: lamina propria, SE: simple cuboidal epithelial cells) (PAS-Alcian blue) 400X.

**Table 2.** Measurement of the Ventriculus layers in the ring necked parakeet *-P. krameri* and the black shouldered kite *-E. caeruleus*.

Ventriculus layers thickness	Ring-necked parakeet (Mean $\mu\text{m} \pm \text{SE}$ )	Black-shouldered Kite) (Mean $\mu\text{m} \pm \text{SE}$ )
koilin	170.99 $\pm$ 6.10	Very thin
Mucosa	200.88 $\pm$ 4.29	289.3 $\pm$ 13.75
Submucosa	59.40 $\pm$ 2.39	61.46 $\pm$ 3.00
Musclaris externa	1124.1 $\pm$ 18.54	279.2 $\pm$ 7.20
Serosa	135.92 $\pm$ 4.65	181.74 $\pm$ 6.26

**2. Black winged kite *-E. caeruleus***

**A. Proventriculus**

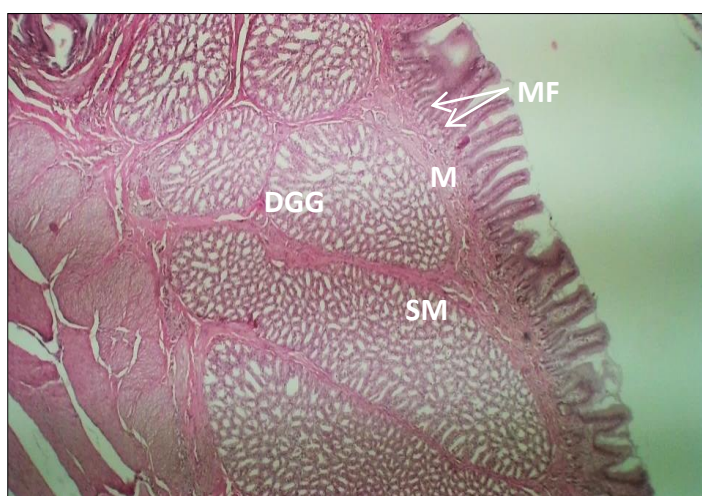
The mucosa showed numerous fingers-like shape folds called mucosal folds with flat apexes and their length measured (171.22 $\pm$ 2.81 $\mu\text{m}$ ) The mucosal epithelium was lined with simple columnar epithelium with clear cytoplasm cells and an oval dark nucleus (Fig.10, 12).

The lamina propria extended inside folds and contained blood vessels, lymphocytes, and simple tubular glands in the base of the folds which were lined by low columnar cells with spherical nuclei. The muscularis mucosae composed of smooth muscle arranged longitudinally in a thin layer extends inside the folds. These muscles surrounded the surface gastric glands and the deep proventriculus glands located under the muscularis of mucosa (Fig.10).

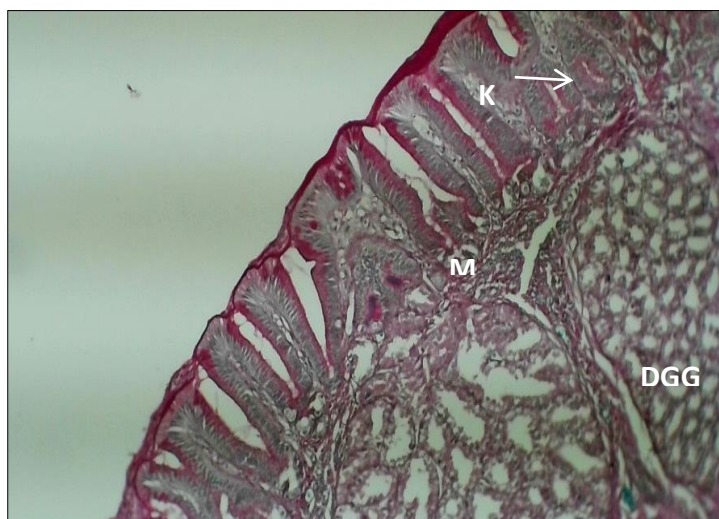
The thickness values of submucosa were (819.09 $\pm$ 22.52 $\mu\text{m}$ ). The most space of the proventriculus wall consisted of dense connective tissue rich in blood vessels and filled with deep gastric glands. Each gland was surrounded by a thin connective tissue, chief cells, blood vessels, and smooth muscles, each gland consisted of several secretory tubules that empty their secretions in collecting ducts (Fig.11). The mean length values of these glands were (425.59 $\pm$ 18.88 $\mu\text{m}$ ) and the width (293.90 $\pm$ 17.47 $\mu\text{m}$ ) (Tab. 1).

The muscularis externa consisted of two layers of smooth muscle each oriented in a different plane: an inner circular layer and an outer longitudinal layer, the muscle layers separated by the connective tissue containing blood vessels, and nerves (Fig.10). The mean thickness values of muscularis externa were (247.18 $\pm$ 9.75 $\mu\text{m}$ ) The serosa consisted of connective tissue with blood vessels and lymphocytes and was covered by simple squamous cells or mesothelium, the mean thickness of serosa was (329.62 $\pm$ 14.26 $\mu\text{m}$ ).

The surface epithelium of the proventriculus showed a strongly positive reaction with PAS-AB stains. The superficial gastric glands give red color with PAS- AB stain (Fig. 11).



**Figure 10.** Cross-section of the proventriculus wall in black shouldered kite (DGG: deep gastric glands, M: mucosa, MF: mucosal folds, MU: muscularis, SM: submucosa) (H&E) 40X.



**Figure 11.** Cross-section of the proventriculus wall in black shouldered kite (DGG: deep gastric gland, K: koilin, M: mucosa) (PAS-Alcian blue) 100X.

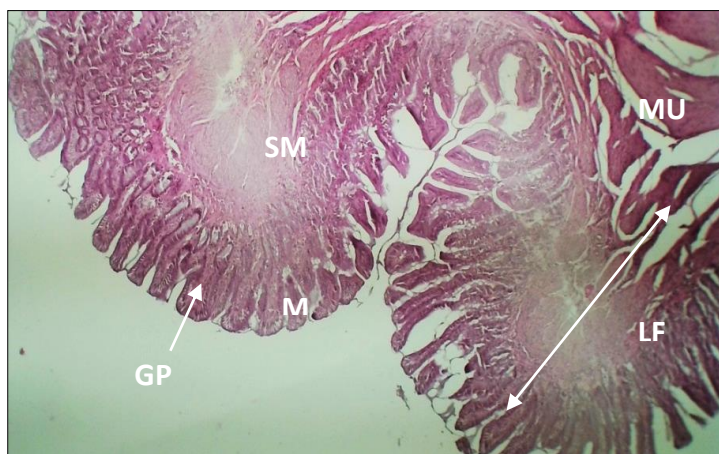


**Figure 12.** Cross-section of the proventriculus mucosa in black shouldered kite (LP: laminae propria, SE: simple columnar epithelium) (H&E) 400X.

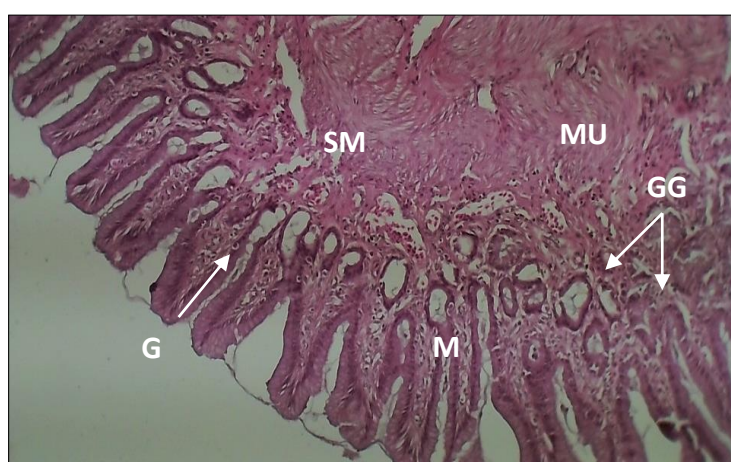
### B. Ventriculus

The mucosa showed long parallel folds, and gastric pits which occupy more than half of the mucosa (Fig. 13). The mucosa lined by simple columnar epithelial tissue consists of columnar cells representing surface mucous cells with oval nuclei located near the bases of the cells (Fig.14). The Lamina propria contained numerous long simple tubular glands with large cavities. These glands are lined by short columnar cells with oval nuclei called Chief cells. On the other hand, the glands opened in grooves between the folds. Lamina propria was based on a layer of connective tissue composed of collagen fibers (Fig.15). The muscularis externa consisted of two muscular layers, the internal arranged circularly and external arranged longitudinally extended into the cores of gastric folds (Fig.13). The mean thickness values of mucosa were  $(289.3 \pm 13.75 \mu\text{m})$ . The mean thickness values of submucosa were  $(61.46 \pm 3.00 \mu\text{m})$ . The mean thickness values of muscularis were  $(279.2 \pm 7.20 \mu\text{m})$  (Tab. 2). The serosa consisted of connective tissue with collagen fibers, blood vessels, and lymphocytes covered from the outside by a single layer of simple squamous cells, the mean thickness values were  $(181.74 \pm 6.26 \mu\text{m})$ . The mucosal epithelial cells lining the surface of the tubular glands were stained strongly with PAS-AB stain (Fig.15).

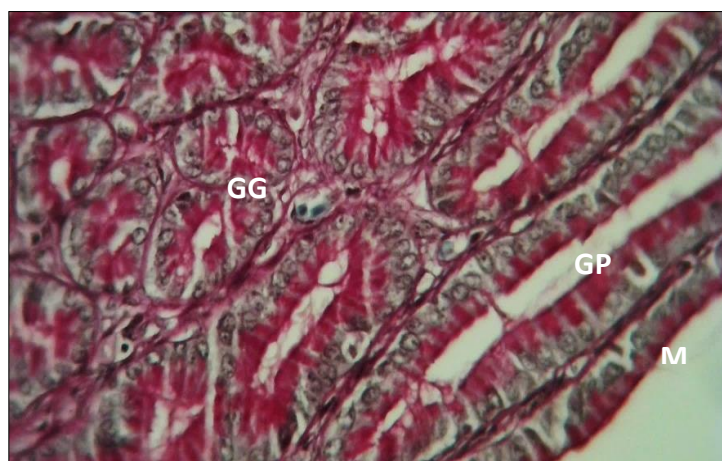




**Figure 13.** Cross-section of the ventriculus folds in black shouldered kite (GP: gastric pits, LF: long fold, M: mucosa, MU: muscularis, SM: submucosa) (H&E) 40X.



**Figure 14.** Cross-section of the ventriculus wall in black shouldered kite (GG: gastric glands, GP: gastric pits, M: mucosa, MU: muscularis, SM: submucosa) (H&E) 100X.



**Figure 15.** Cross-section of the ventriculus wall in black shouldered kite (GG: gastric gland, GP: gastric pits, M: mucosa) (PAS-Alcian blue) 400X.

## Discussion

The present study showed clear differences between the stomachs of two species of birds, the ring necked parakeet proventriculus was a fusiform shape tube separated from the gizzard by an isthmus. This result was in agreement with the finding of Al-Juboory *et al.* (2016) in the common wood pigeon, while in the black shouldered kite, proventriculus was pear in shape, wider and longer than that of the parakeet and no isthmus

separated proventriculus from the gizzard (Hamdi *et al.*, 2013). Gizzard in the parakeet appeared with disc or biconvex lens shape, lined with yellowish-green tissue (koilin). Moreover, this result was in agreement with the finding of Al-Juboory *et al.* (2016) in the common wood pigeon. The Kite gizzard appeared pear-shaped with a vertical and longitudinal pattern of the inner folds and grooves, lined with a very poor yellowish koilin layer (Abumandour, 2014). The koilin layer was a grinding surface, enabling the muscular ventriculus to mechanically action for digesting food. Granivorous birds possess a thick cuticle layer with a well-developed muscular ventriculus. In contrast, Carnivores birds possess a thin-walled ventriculus and soft cuticle (Al-Saffar and Al-Samawy, 2015). The differences in stomach shape in birds are probably due to the feeding habits or perhaps the amount of food engulfed by birds (Abd-Elnaeem *et al.*, 2019). The birds feed on relative large soft items of food, it required a stomach adapting more for storage than digestion as in the stomach of Kite under investigation, by contrast, in the parakeet which feeds on hard items of food, stomach adapting for more digestion than storage (Al-Juboory *et al.*, 2016).

The histological study showed that the proventriculus wall of two species of birds consisted of the mucosa, submucosa, muscularis, and serosa; the same result was observed by Al-Taai and Hasan (2020) however disagree with the finding of Zhu (2015) in Black-tailed crane who mentioned that the proventriculus is composed of three layers, mucosa, muscolosa, and serosa while submucosa was absent.

The mucosa of the proventriculus in the kite was thrown into numerous finger-like shape folds with flat apex; the simple columnar epithelial cell was lining the proventriculus folds; this finding is identical with many previous studies such as El-Shammaa *et al.* (2019) in Ostrich and Hassan and Moussa (2012) in duck.

The lamina propria of mucosa appeared as connective tissue with blood vessels and lymphocytes. Simple tubular glands were found in the base of proventriculus folds, this finding is identical with many previous studies such as Jassem *et al.* (2016) in Common moorhen and Beheiry (2018) in turkey. The Simple tubular glands in the Kite were lined by low columnar epithelial, similar observations mentioned by AL Sheshani (2006) on the carnivorous bird, *Accipiter nisus*.

The parakeet mucosa glands of the proventriculus were lined by simple cuboidal epithelial tissue. Previous studies have mentioned the same result such as Taher *et al.* (2020) in moorhen and Hassan and Moussa (2012) in pigeon. The histological results showed that these glands were larger and deeper in the kite when compared with parakeet proventriculus glands; muscularis mucosa was not observed under the mucosa in the parakeet, while in kite the muscularis mucosae appeared as a smooth muscle layer extending inside the proventriculus folds; this finding was in agreement with Batah *et al.* (2012).

The submucosa was composed of connective tissue containing blood vessels in both species of birds. Submucosa occupied most of the proventriculus wall. It was contained numerous deep proventricular glands. Submucosal glands were compounded tubular branches lined by cuboidal epithelial cells. The results are by El-Nahla *et al.* (2011), who confirmed the presence of this gland in the submucosa layer as in the present study, while other studies mentioned the presence of glands between the muscularis mucosa of quail (Ahmed *et al.*, 2011). These results are in disagreement with the present finding. These differences in the location of the glands may be due to species variation or due to developmental stages differences. Each gland enclosed by a connective tissue contains blood vessels and nerves (El-Nahla *et al.*, 2011).

The muscularis externa was composed of two layers of muscle as mentioned by Kausar *et al.* (2019) in domestic pigeons; these findings were in disagreement with Rodrigues *et al.* (2012) in blue and yellow macaws. The inner circular layer in the kite is thicker than the outer longitudinal layer such as in pheasant (Parisa *et al.*, 2019). The findings were in disagreement with El-Nahla *et al.* (2011).

The tunica serosa in the present study was composed of connective tissue containing blood and lymph vessels as agreed with the study of Salih *et al.* (2019).

From the results above it can be concluded that the kind of food reflects some histological differences in the proventriculus between two species of birds appear in the thickness of the layers that composed the wall of the glandular stomach in kite and the distribution of deep submucosal glands.

The histological examination in the present study showed that the gizzard wall of the two species of birds consisted of four layers (Taher *et al.*, 2020); this finding is in disagreement with Zhu (2015) in Black-tailed crane who mentioned that the ventriculus is composed of three layers, mucosa, muscolosa, and serosa while submucosa was absent.

The mucosa of the kite gizzard had three sub-layers, epithelium, lamina propria, and muscularis mucosa; these findings are in disagreement with the findings of Wilkinson *et al.* (2018), while the mucosa of parakeet consisted of two layers, epithelium and lamina propria. The type of feeding leads to the absence of muscularis mucosae in the parakeet while the muscularis externa more develop which replace from this layer; this result was in agreement with Zaher *et al.* (2012).

The present findings showed that the surface lining of the tunica mucosa in kite studied was simple columnar epithelium; this finding is in agreement with the results of Hussein *et al.* (2020). In the parakeet, the surface lining of the tunica mucosa was simple cuboidal epithelium. This finding is in agreement with the results of Taher *et al.* (2020) in Moorhen.

The present histological examination revealed that there was a hardened membrane yellow to green in color resting on the surface of the epithelium in the parakeet. This cuticle or koilin membrane is made from carbohydrates and proteins, as mentioned by Zhu (2015). This layer shows great variation and is most elaborate in granivorous. On the other hand, the cuticle or koilin layer did not found covering the epithelium of the gizzard in the kite; this finding was in agreement with Al-A'araji (2007) and AL Sheshani (2006) who mentioned that the koilin layer did not found in kestrel and *Accipiter nisus*. The presence of the koilin layer in the gizzard of parakeet, and disappeared in the gizzard of the kite could be related to the kind of food on which the function of gizzard in these birds depends. The species with well-developed muscle and hard thick membrane protects the gizzard's epithelium (Das *et al.*, 2018). The main function of the muscular stomach in granivorous species is to triturate the food in preparation for gastric proteolysis. This is achieved by powerful asymmetrical contractions of the muscles aided by the tough internal cuticle layer, while in species with thin sac-like gizzard, the gizzard function mainly as a storage organ where the gastric juice can act.

Lamina propria thickness in parakeet was less than that of the kite, which was loose connective tissues with many tubular glands also appeared in love birds (Al-A'araji, 2007). The differences found between the lamina propria of gizzard in parakeet and that in kite might be due to the variation in the number of tubular glands that present in their lamina propria. Submucosa consisted of connective tissue containing blood vessels and nerves in both studied birds; this finding was in agreement with the result of Madkour and Mohamed (2019). The histological studies showed that the muscularis externa of the ventriculus was composed of two muscular layers in the two studied birds arranged into the inner circular layer and outer longitudinal layer. Also, these findings showed the difference in thickness of this layer between two species of birds in the current study. Parakeet gizzard showed the larger thickness in its muscularis externa, while the less thickness in the gizzard of the kite. The muscular part of the stomach in birds is extremely variable in its muscularity dependent on the type of diet that the bird consumed (Al-Saffar and Al-Samawy, 2015).

The differences in the thickness of the wall of gizzard that appeared in the present work among the two species of birds were in agreement with Al-Juboory *et al.* (2016) that observed in carnivorous birds, that ingested soft food items, the gizzard sac-like structure with a thin wall; this is a marked contrast to the gizzard of granivorous birds that ingest harder food items, and therefore it requires a well-developed muscular gizzard; these results were confirmed by Al-Kinany (2019). The gizzard of granivorous bird play important role in the physical digestion of food while in carnivorous it functions mainly as a storage organ where the gastric juice can act.

Histochemically, on applying PAS-AB (pH 2.5) stain, the mucous cells lining the epithelium and pits of the proventriculus were reacted strongly with PAS-AB (pH 2.5) stains. This reaction indicated the presence of high content of neutral and acidic mucins, similar to findings of Hamdi *et al.* (2013) in the proventriculus of the mallard. The deep gastric glands of the proventriculus in the two studied birds give red color with PAS-AB (pH 2.5) stain due to the presence of neutral mucin secretions. This finding is identical with many studies in many avian species, such as *Vanellus spinosus* (Taki-El-Deen, 2017) and common starling (Sayrafi and Aghagolzadeh, 2019)

In the ventriculus (Gizzard), the cuticle layer in the ventriculus showed a pink-colored reaction for PAS and no reaction with Alcian blue (pH 2.5), due to the presence of neutral mucin (Taher *et al.*, 2020). The mucosal epithelial cells lining the surface of the tubular glands in the ventriculus were stained strongly with both PAS-AB (pH 2.5) stains. This reaction indicated the presence of neutral and acid mucin (Al-Saffar and Al-Samawy 2015, 2016). The neutral and acid mucin may protect the ventriculus mucosal surface and forms a resistant barrier (Taher *et al.*, 2020).

### Conclusion

The stomach of birds is different depending on the diet; in carnivorous birds, the stomach was simple with single-chamber and thin-walled; in granivorous birds, the stomach is complex with two chambers and a thick wall in the muscular part covered with a koilin layer. There were a high number of longitudinal folds and gastric glands in the Carnivorous stomach.

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### Conflict Of Interest Statement

The results of the current study are part of the requirements of Ph.D. in Department of Biology, College of Sciences, Mustansiriyah University in Zoology, for the first author. Also, we are the authors of this manuscript, declare and confirm that no significant financial or other relationship with any official institution.

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