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ORIGINAL ARTICLE

MORPHO-HISTOLOGICAL STUDY OF THE PROVENTRICULUS OF EURASIAN MARSH HARRIER *CIRCUS AERUGINOSUS* (LINNAEUS, 1766) AVES, ACCIPITRIFORMES, ACCIPITRIDAE)



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ABSTRACT

The study aimed to describe the anatomical, histological, and histochemical structure of the proventriculus of adult male Eurasian marsh harrier, *Circus aeruginosus* (Linnaeus, 1766) (Accipitriformes, Accipitridae). Six birds were used for this study. The histological sections of proventriculus were stained with hematoxylin and eosin, periodic acid Schiff, and alcian blue. The proventriculus, representing the upper stomach, appeared as a conical structure with a thick wall connecting the caudal end of the thoracic esophagus to the gizzard. It had a light brown color. Microscopic examination revealed that the proventriculus wall consists of the outer tunica serosa, the tunica muscularis, tunica submucosa, and the inner tunica mucosa.

The mucous layer exhibited folds, and the sulci represented many longitudinal folds with uniform lengths on its luminal surface. A single layer of columnar cells with oval-shaped nuclei made up the lining epithelium. The second layer was the lamina propria, composed of a relatively dense network of connective tissue. A thin, discontinuous layer of circularly arranged smooth muscle fibers in the muscularis mucosa separates the lamina propria from the submucosa. The submucosa appears as a very thick layer of dense vascular connective tissue, sub-mucosal compound tubuloalveolar glands form the major bulk of this tunica. These glands are arranged in one to three series of lobules that appear in different shapes. Muscularis externa consists of smooth muscle fibers arranged in a thick inner circular layer and a thin discontinuous outer longitudinal layer. The tunica serosa was a well-developed layer constituted by loose connective tissue covered by a single layer of flattened epithelial cells of the mesothelium. The proventriculus has some similarities to other bird species as well as some variations to other species, which may be explained by its nourishing food and behavior.

Keywords: Harrier bird, Histology, Morphology, Proventriculus, Stomach.

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INTRODUCTION

The bird of the Eurasian marsh harrier, *Circus aeruginosus* (Linnaeus, 1766), belongs to the Accipitridae family, which typically hunts by flying low over open ground, and consuming small animals, birds, or reptiles. Sometimes the young species are referred to as "ring-tail harriers" (Ferrarini *et al.*, 2023).

The stomach is a large part of the gastrointestinal tract and is responsible for storing, digesting, and transporting food to the duodenum; its structure presents variations that depend on the alimentary habits of the bird (Saran and Meshram, 2021). The proventriculus, a glandular portion of the stomach in birds, secretes the gastric juices, while the gizzard, a muscular portion, performs a mechanical role. The proventriculus is cranially continuous with the esophagus and presents an elliptical shape (AL-Taai and Hasan, 2020). The glandular stomach is distinguished by its spindle shape and direct emergence from the esophagus, with an intermediate zone between it and the gizzard (isthmus) (Abdel Maksoud *et al.*, 2022). The proventriculus differs in size in different species, typically being rather large and distensible in carnivores that consume large food items while being quite tiny in granivores, compared to other bird species that consume seeds, the black-winged kite, *Elanus caeruleus*, had a stomach that was enormous in size and had a thin wall (Al-Saffar and Al-Samawy, 2015).

Histologically, the stomach is composed of four tunics: the mucosa, sub mucosa, muscularis, and serosa (Khaleel *et al.*, 2021). The three layers of the mucosa are the lining epithelium, lamina propria, muscularis mucosa, simple columnar epithelium, and several gastric glands, which meet with the stomach lumen through gastric pits. The tunica of the sub mucosa is composed of connective tissue, having blood and lymph vessels, while the muscularis tunica consists of smooth muscles, and the tunica serosa, which appears as a thin layer of connective tissue lined by mesothelium, covers the stomach from the outside (Al-Samawy *et al.*, 2021). The current study aimed to describe the anatomical, histological, and histochemical structure of the proventriculus of adult males of *Circus aeruginosus* from Iraq.

MATERIALS AND METHODS

Specimens collections: Six adult males Harrier *Circus aeruginosus* (Linnaeus, 1766) were used in the current study, and collected from the commercial market. The birds were checked for their health status before being euthanized. Identification of bird species: The birds were diagnosed and classified based on the field guide to the birds of Iraq (Salim *et al.*, 2006).

Laboratory techniques: The birds were euthanized with ethyl ether. After anesthesia, the digestive tube was dissected, and specimens of the glandular stomach (proventriculus) were directly fixed in Bouin solution for 24h followed by dehydration with ascending series of ethyl alcohol (50-100%). All the specimens were embedded in paraffin wax, and transverse sections were taken using a rotary microtome at 5 μ m. Haetamoxylin and eosin stain were used to determine the general structure (Suvarna *et al.*, 2018), and periodic acid Schiff (PAS) for differentiating acidic and neutral mucins, and alcuin blue for connective tissue. The selective sections were photographed by using a light microscope with a digital camera.

RESULTS AND DISCUSSION

Gross examination showed that the stomach of the Harrier *Circus aeruginosus* (Linnaeus, 1766) was in the left part of the abdominal cavity, and divided into two portions: proventriculus and gizzard which were not clearly defined from each other, the proventricular ventricular isthmus was not present so that the two organs form one large conical shape, there was no clear division internally as there was no demarcation line between proventricular and ventricular part of the stomach but differentiation from each other by their structure in which each one had its characteristic structure (Pl.1A). This result was in agreement with Abdel Maksoud *et al.* (2022) in hooded crow *Corvus cornix*, while it was in contrast with Al-Saffar and Al-Samawy (2015) who recorded that there was a short intermediate zone (isthmus) separating between these two portions of the stomach in the mallard *Anas platyrhynchos*. The proventriculus had a light brown colour of conical shape that distinguished it from the esophagus whitish colour, it had a number of well- developed longitudinal folds reach to 5 longitudinal folds which were separated from each other by clear longitudinal grooves (Pl.1B), where the researchers differentially described the proventriculus as being large, fusiform-shaped in mallard (Al-Saffar and Al-Samawy, 2015) and appeared as a tubular shaped in common moorhen (Jassem *et al.*, 2016), while it was showed as a longitudinal expansion of esophagus with an apparent external gastric papillae in starling bird (AL-Taai and Hasan, 2020), and as a spindle shape in the barn owl (Maher and Hussein, 2023). These features of the proventriculus are possibly related to the dietary habits.

Microscopic examination revealed that the wall of the proventriculus consisted of four classical tunics: outer tunica serosa, tunica muscularis, tunica sub mucosa, and inner tunica mucosa (Pl.2); a similar arrangement was described by previous studies (Al- Nakeeb *et al.*, 2019; Saran and Meshram, 2020; Umar *et al.*, 2021). The current findings were not in line with Zhu (2015), who reported that the wall of the proventriculus in black-tailed crane porzana bicolor and yellow-billed grosbeak consisted of three tunics; mucosa, muscularis, and serosa.

The mucous layer exhibited folds and sulci that represented many longitudinal folds with uniform lengths on its luminal surface (Pl.3), the presence of these folds may increase the secretory surface epithelium and facilitate the digestion of foods (Zhu, 2015). A single layer of columnar cells with oval-shaped nuclei located in the basal half of the cell built up the lining epithelium of the mucosa. The foamy cytoplasm of these cells was intensely stained with eosin, especially in the supra-nuclear region (Pl. 4). This observation was supported by other researchers (AL-Taai and Hasan, 2020; Khaleel *et al.*, 2021). The second layer of mucosa was lamina propria, composed of a relatively dense network of connective tissue extending immediately inferior to the lining epithelium and also forming the central core of the mucosal folds to provide mechanical support, blood vessels, nerves, lymphoid elements, and simple tubular glands were seen within the connective tissue of lamina propria (Pl.5). These results are in line with Saran and Meshram (2020). The mucosal gland is lined by a single layer of cuboidal mucous secreting epithelial cells, and their ducts open at the bases of the sulci. These results are likely in Mallard (Al-Saffar and Al-Samawy, 2015). A thin, discontinuous layer of circularly arranged smooth muscle fibers known as the muscularis

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mucosa separates the lamina propria from the sub mucosa (Pl.6).The current observations were in agreement with other researchers (AL-Taai and Hasan, 2020; Khaleel *et al.*, 2021), while the results were disagreement with Ahmed *et al.* (2011) who showed that the muscularis mucosae of Japanese quail were formed of two small layers of smooth muscle fibers, inner isolated longitudinal bands, and outer circular bands.

The sub mucosa appears as a very thick layer of dense vascular connective tissue with a few muscle fibers extending from the muscularis mucosae and running through the deep or sub mucosal compound tubuloalveolar glands that formed the major bulk of this tunica (Pl.7), such finding agreed with AL-Taai and Hasan (2020). These glands are arranged in one to three series of lobules that appear in different forms like conic, pearl, ovoid, and elongated forms (Pl.7). The researchers did not agree in their observations on these glands in different birds; Al-Jubory (2016) observed one row of glands in *Tyto alba*, while Al-Saffar and Al-Samawy (2015) showed rounded branched tubular glands in Mallard, submucosal glands arranged in two rows surrounded by connective tissue in Iraqi male Guinea fowls, and barn owl, respectively (Khaleel *et al.*, 2021; Maher and Hussein, 2023). These differences can be explained by their nourishing food and behavior. Each gland lobule is surrounded by a connective tissue capsule rich with blood vessels and contains numerous secretory units lined by a row of cuboidal cells. These cells have rounded, centrally located nuclei and deeply stained eosinophilic granular cytoplasm (Pl.8).

The epithelium of the secretory units is organized in an oblique direction on the thin strands of connective tissue that form the septa between the tubules, and these cells are closely applied one to another only towards their bases, where they provide to the epithelium a dentate appearance (Pl. 9), as stated by Al-Nakeeb *et al.* (2019) and Beheiry (2018). Each secretory lobule drained into the central cavity, which released the gastric secretion to the lumen of the proventriculus through an excretory duct (Pl.10). One layer of columnar cells made up the lining epithelium of the excretory ducts and the central cavities that characterized by their lightly stained cytoplasm in which, basally located and oval shaped nuclei surrounded by a well-marked nuclear membrane and contain one or more clear nucleoli (Pl.11). These findings were in coincidence with previous studies (Beheiry, 2018; Al- Nakeeb *et al.*, 2019; Abdel Maksoud *et al.*, 2022).

Microscopical examination for the proventriculus sections revealed that the sub mucosal gland's ducts, mucous gastric glands, and lining epithelium of the mucosal folds all exhibited PAS-positive reaction in their apical regions (Pl.12) indicating the presence of neutral mucopolysaccharide in these cells, on the other hand, staining with alcian blue gave a strong reaction in the whole cytoplasm of the ducts of the sub mucosal glands while the lining cells of the mucosal folds and the mucous gastric glands only their apical region reacted positively to this stain indicating the presence of acid mucopolysaccharide in these cells (Pl.13). Both stains also react positively with discharging secretory material outside the cells. The current results are strengthened by the results of previous researches (Ahmed *et al.*, 2011; Zhu, 2015), but they were not supported by Khaleel *et al.* (2021) in Guinea fowl, who reported that sub

mucosal glandular epithelium showed a negative reaction to mucins. Al-Jubory (2016) suggested that the mucous secretion of these cells formed a barrier to protect the epithelium.

Muscularis externa consists of smooth muscle fibers arranged in a thick inner circular layer and a thin, discontinuous outer longitudinal layer (Pl. 14). These findings disagreed with Al-Nakeeb *et al.*, (2019), who found the muscularis externa in Magpie *Pica pica* consists of three layers: very thin muscle fibers supported deep gastric glands, the middle thick longitudinal layer, and the outer circular layer. The thickness of the tunica muscularis can help for empty the secretion of the deep periventricular glands. The tunica serosa of the proventriculus in Harrier was constituted by loose connective tissue, containing many blood vessels and nerves, and covered by a single layer of flattened epithelial cells represented by the mesothelium (Pl.15). This finding was similar to results in other species of birds (Saran and Meshram, 2020; Al-Samawy *et al.*, 2021). Areas of aggregation of adipose cells were also observed in this layer. Sultan *et al.* (2023) observed the presence of melanin in the connective tissue of the serosa layer.

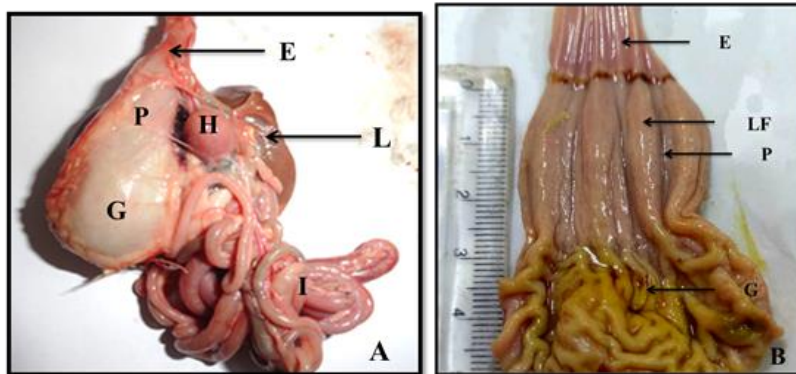


Plate (1): The alimentary canal in Harrier bird; (A) and (B) The anatomy of digestive tract. The longitudinal folds (LF) in proventriculus, (E) Esophagus, (P) proventriculus, (G) Gizzard, (H) heart, (L) Liver, (I) Intestine.



Plate(2): Section in Harrier proventriculus showing the four tunics. [Tunica mucosa (TM), Sub mucosa (SM), Muscularis externa (EM), Tunica serosa (TS), H &E (10X)].

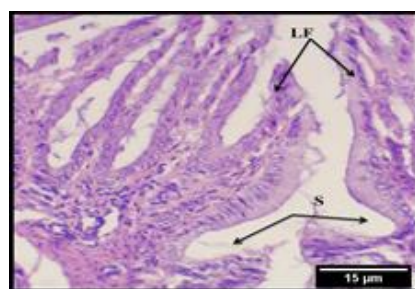


Plate (3): Section in Harrier proventriculus showing the folds and sulci. [Longitudinal folds (LF), Sulci (S), H & E (40X)].

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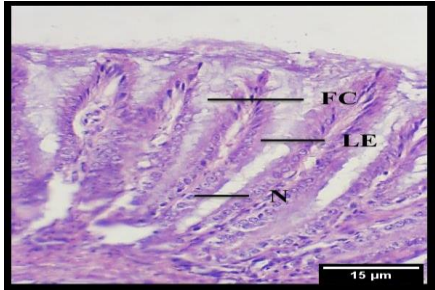


Plate (4): Section in Harrier proventriculus showing: Lining epithelium (LE), Foamy cytoplasm (FC), Nucleus (N), H&E (40X)

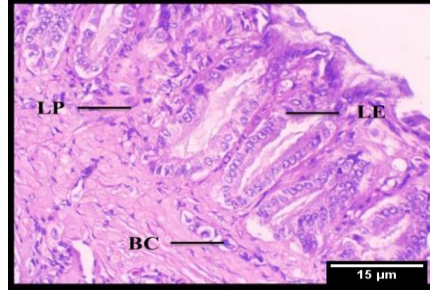
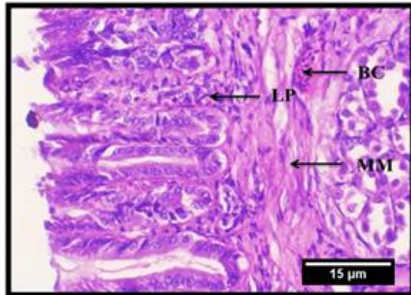


Plate (5): Section in Harrier proventriculus showing: Lamina propria (LP), Lining epithelium (LE), Blood capillaries (BC), H&E (40X).



Plate(6): Section in Harrier proventriculus showing: Muscularis mucosae (MM), Lamina propria (LP), Lining epithelium (LE), Blood capillaries (BC), H&E (40X)].

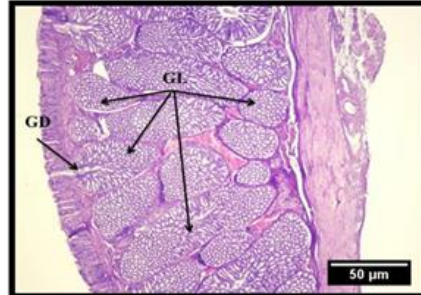


Plate (7): Section in Harrier proventriculus showing sub mucosal glands. [Glandular lobules (GL), Gland duct (GD), H&E (10X)].

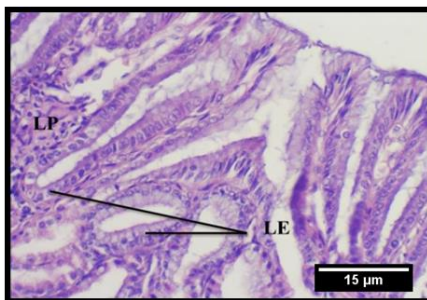


Plate (8): Section in Harrier proventriculus showing lining of mucosal glands. [Lining epithelium (LE), Connective tissue septum (CTS), Main duct (MD), H&E (40X)].

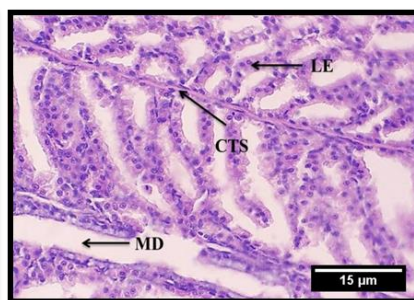


Plate (9): Section in Harrier proventriculus. [Lining epithelium of sub mucosal glands (LE), Lamina propria (LP), Main duct (MD) H&E (40X)].

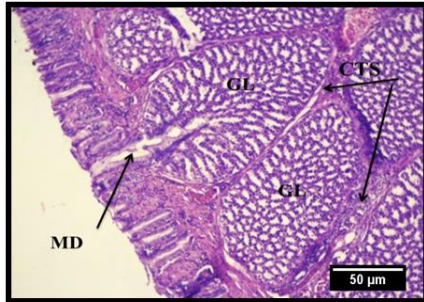


Plate (10): Section in Harrier proventriculus [Main duct (MD), Connective tissue septum(CTS), Glandular lobule(GL), H&E (10X)].

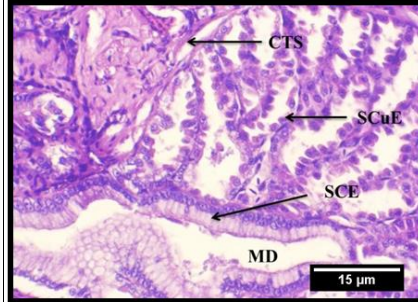


Plate (11): Section in Harrier proventriculus. [Lining epithelium of main duct (MD), Simple cuboidal epithelium (SCuE), Connective tissue septum (CTS), Simple columnar epithelium (SCE), H&E (40X)].

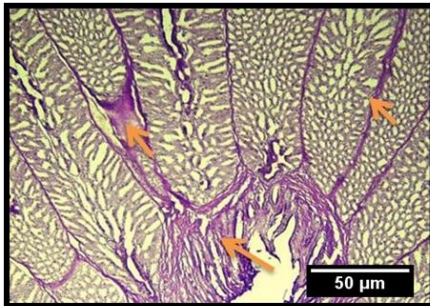


Plate (12): Section in harrier proventriculus showing: pas reaction (arrows) pas stain (10X).

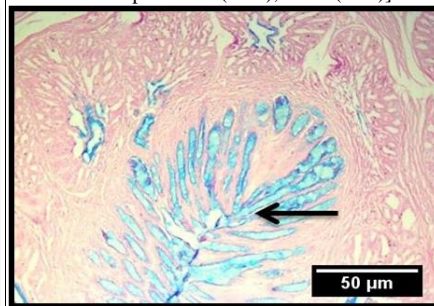
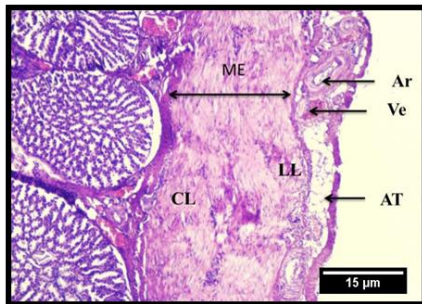
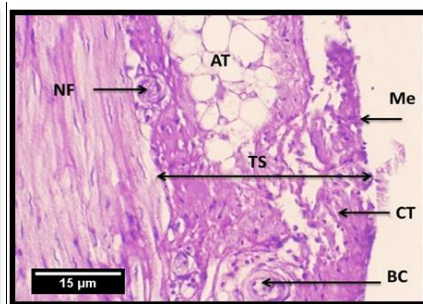


Plate (13): Section in Harrier proventriculus showing: [Alcian reaction (Arrows) Alcian blue stain (10X)].



Plate(14): Section in Harrier proventriculus showing: Muscularis externa (ME), Circular layer (CL), Longitudinal layer (LL), Adipose tissue (AT), Arteriole (Ar), Venule (Ve), H & E (40X).



Plate(15): Section in Harrier proventriculus showing: Tunica serosa (TS), Connective tissue (CT), Mesothelium (Me), Blood capillary (BC), Adipose tissue (AT), Nerve fiber (NF), H & E (40X).

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CONCLUSIONS

The current study investigated the histological structures of the proventriculus of *Circus aeruginosus* (Linnaeus, 1766), the findings show that the proventriculus represents the upper stomach, the proventricular ventricular isthmus is not present so that the two organs form one large conical shape with a thick wall, and it has a number of well- developed longitudinal folds that reach to five longitudinal folds. Microscopic examination of the proventriculus consists of four classical tunics: outer tunica serosa, tunica muscularis, tunica sub mucosa and inner tunica mucosa. The proventriculus has some characteristics in similarities to other bird species as well as some variations from other species, which may be explained by its nourishing food and behavior.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare. We "the authors" have followed and signed the scientific research ethics announced by the journal.

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دراسة شكلائية- نسجية للمعدة الامامية لطائر مرزة البطائح
Circus aeruginosus (Linnaeus, 1766) (Aves, Accipitriformes, Accipitridae)

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الخلاصة

هدفت الدراسة إلى وصف التركيب التشريحي والنسجي والكيميائي النسجي للمعدة الامامية للذكور البالغين لمرزة البطائح (*Circus aeruginosus* (Linnaeus, 1766) (Accipitriformes, Accipitridae)). استخدم في هذه الدراسة ستة نماذج من الطائر، لونت المقاطع النسجية للمعدة الامامية بملون الهيماتوكسلين والايوسين وحمض شيف الدوري وازرق المثيل. تمثل المعدة الامامية الجزء العلوي من المعدة وتظهر كتركيب مخروطي ذو جدار سميك يربط الطرف الذيلي للمريء الصدري بالقانصة. كان لونها بني فاتح. كشف الفحص المجهرى أن جدار المعدة الامامية يتكون من الغلالة المصلية الخارجية، الغلالة العضلية، الغلالة تحت المخاطية، والغلالة المخاطية الداخلية. أظهرت الطبقة المخاطية طيات و التلم وتمثل العديد من الطيات الطولية وبأطوال موحدة على سطح التجويف. تتكون البطانة الظهارية من طبقة واحدة من الخلايا العمودية ذات النوى البيضاوية. كانت الطبقة الثانية الصفيحة الاصيلية والمكونة من شبكة كثيفة نسبيا من النسيج الضام. تفصل العضلية المخاطية المكونة من طبقة رقيقة متقطعة من الالياف العضلية الملساء المرتبة بشكل دائري بين الصفيحة الاصيلية والغلالة تحت المخاطية.

تظهر الغلالة تحت المخاطية كطبقة سميكة جدا من النسيج الضام الكثيف الوعائي، وتشكل الغدد الأنبوبية السنخية المركبة تحت المخاطية الجزء الأكبر من هذه الغلالة. يتم ترتيب هذه الغدد في سلسلة واحدة إلى ثلاث فصيصات والتي تظهر بأشكال

Morpho-histological study of the proventriculus

مختلفة. تتكون الغلابة العضلية الخارجية من ألياف عضلية ملساء مرتبة في طبقة دائرية داخلية سميكة وطبقة طولية خارجية رقيقة متقطعة. كانت الغلابة المصلية عبارة عن طبقة متطورة تتكون من نسيج ضام مفكك تغطيه طبقة واحدة من الخلايا الظهارية المسطحة من الظهارة المتوسطة. تميزت المعدة الامامية ببعض الخصائص في أوجه التشابه مع أنواع الطيور الأخرى فضلا عن بعض الاختلافات مع الأنواع الأخرى، والتي يمكن تفسيرها من خلال طبيعة التغذية والسلوك.