



Morphological and Histological Study of Stomach (Proventriculus and Ventriculus) of Adult Partridge (*Alectoris chukka*) in South of Iraq



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Abstract

THIS study was designed to investigate both histomorphological and histochemical structures of the stomach in the partridge. Twelve healthy birds were collected from local markets at Baghdad province. All the birds were euthanized, their abdomens were dissected, and then their stomachs were identified, photographed in situ. Specimens cut from the gastric wall were fixed as well and then processed for histological and histochemical staining techniques that were H&E, Masson Trichrome, Periodic acid Schiff (PAS) and PAS -Alcian blue (AB) (pH 2.5). Gross findings indicated the presence of a stomach of two divisions that were glandular (proventriculus) and muscular (ventriculus). The proventriculus was elliptical in shape and separated from the next second part by isthmus. The ventriculus appeared circular in shape and normally covered by an amount of adipose tissue. The stomach of partridge was located at the left side of the thoracoabdominal cavity. The luminal surface of proventriculus showed longitudinal mucosal folds with the presence of rows of papillae in area between the end of isthmus and beginning of ventriculus. Ventricular luminal surface was greenish to yellowish in color; it had rough horizontally arranged folds in the center then arranged transversally in the end with presence layer of cuticle. Histologically the gastric wall was constructed by four tunicae (mucosa, submucosa, muscularis and serosa). The luminal surface of stomach was irregular due to presence of openings of superficial glands with the top of papillae. The epithelium was simple columnar invaginated in proventriculus to form superficial and deep glands lined in their basal parts by cuboidal cells. In ventriculus the surface covered by cuticle with absence of muscularis mucosa. Tunica muscularis was very thick composed from three layers in different directions to adapt the gastric functional contraction. Histochemically the epithelium and glandular ducts were reacted positively with PAS due to their neutral mucin composition.

Keywords: Partridge, Proventriculus, Ventriculus, Avian stomach, Glands.

Introduction

Chukar Partridges (*Alectoris chukar*) are a kind of Chukar birds. The Chukar habitat is mountainous areas such as north of Iraq, India, Pakistan and Afghanistan. These countries are characterized geographically by their high massive peak chains. They contain the world's highest mountainous terrains with valleys, streams and rivers. Partridges are characterized by their medium size with a length of 33 to 38 centimeters. The birds' wings and back are light grayish brown in color, whereas their belly and cheeks are pale. The throat is characterized by a black ring. It has pink eyelids, shanks and toes.

In Al-Sulaymaniyah province, Chukka's observed roosting beneath sagebrush, trees, rocky

outcrops and open rocky regions. They feed on various seeds, bugs, lawn blades, base shoots, cloves, stems, branches and vegetation buds. Grains make up the majority of their food.

Usually known that avian stomach is made of two distinct sections; the proventriculus or glandular stomach in which the gastric juices are secreted and the ventriculus (gizzard) or muscular stomach which accomplishes the subsequent mechanical function [1-5].

In fact, the morphology of avian stomach is related to their type of food intake. The stomach as unique organ in vertebrates, adapted to many types of nutrients related to the evolution [6]. Stomach morphology is followed the classification of birds.

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The birds divided to omnivorous, herbivorous, insectivorous and granivorous. In genus *Tangara*, bird's stomach is reduced to a diverticulum [7, 8]

The proventriculus or true stomach which is located caudal to the esophagus and the ventriculus or muscular portion (gizzard) is located caudal to the proventriculus. These two parts of the stomach are connected together by an intermediate zone [9]. In general, the bird's stomach has the same of gut tube of other species histologically. It formed of four layers. The inner one is the mucosa, which is the lining epithelium with underneath connective tissue lamina propria adjacent and the muscularis mucosa, next vascular connective tissue layer is the submucosa. The next layer is the muscular layer, which is composed of smooth muscles bundles. These smooth muscles are arranged as an outer longitudinal layer and inner circular layers. These muscles are responsible for the peristaltic movement and grinding processes of digestion. The outermost layer is the serosa which is composed of a thin layer of connective tissue with squamous epithelium [10, 11].

Due to sparse local studies conducted on the stomach in partridge, this work was designed to explore the histomorphological and histochemical structures of this bird i. e. the chukar stomach.

Material and Methods

The studied birds (twelve healthy adults) were collected from the local markets in Baghdad for anatomical, histological and histochemical studies. The birds were euthanized by xylazine (80 mg/kg) then dissected by making a mid-line incision in the abdominal wall of each bird to view the coelom viscera. The stomach of each bird was identified and photographed in situ by using digital camera. Location and relationship of the stomach to the surrounding organs was made. For histological study, the specimens of proventriculus and ventriculus were washed gently with normal saline after emptying them from their contents. Then pieces from these specimens were fixed in formalin 10% for 48 hrs [12] Then dehydrated with a series of ascending ethyl alcohols (70%, 80%, 90% and 100%) and subsequently cleared with xylene for half hour after that embedded in paraffin wax and then the blocks were made and sectioned at 6 μ m thickness by rotary microtome. The prepared sections were stained with either one of the following stains: Mayer's Hematoxylin and Eosin as a routine stain for general structures identification, Masson Trichrome stain for the staining of the collagen and muscles fibers and PAS - Alcian blue (AB) pH 2 for the determination of neutral and acidic mucin. The PAS alone was used for the neutral mucin [13].

Results and Discussion

The current study found that, stomach of partridge was made of two parts; the cranial part the proventriculus, which was the caudal continuation of the esophagus. The caudal part is the ventriculus which was located caudally to the proventriculus; in another aspect it was continuous with the initial part of the duodenum. The stomach anatomically located at the thoracoabdominal cavity. It lies ventrally to the lung and covered partially by the left lobe of the liver. The stomach was well protected by the presence of keel and the ribs (Fig.1). These observations were recorded in other avian; [14-17]. They studied crossly the structures of stomach in broilers chicks, starling and duck. They found that the stomach of these birds, had the same divisions and location but with few differences. The proventriculus was elliptical in shape with light brown coloration, while the ventriculus was large circular or rounded in shape with brown blue coloration. The latter consists of two pairs of muscle masses on the sides and central white area of tendon. Narrow region was found situated between the two stomach parts termed the isthmus. It connected them together (Fig.2). The unique muscular-tendons structure of the ventriculus gave it the capability of grinding and mixing of food. The presence of isthmus as transitional zone provides easy distinguishing matter between the two parts of the stomach in the partridge. The current anatomical findings in partridge's stomach were different to those recorded in the stomach of *Elanus caeruleus* [3]. The latter reference found that the stomach of this meat eating bird was large and pear-shaped. The two parts, the proventriculus and ventriculus were not easily distinguished from each other. The reason was lack of the isthmus between proventriculus and ventricular in this bird. In the starlings, also the isthmus did not appeared clearly, causing difficulty in distinguishing between the two stomach's portions [18].

The surface, in proventriculus mucosa was thrown into longitudinal folds with presence of papillae. The mucosal folds became shallower in the direction toward the isthmus, where, the papillae disappeared from its luminal surface (Fig.3). The presence of folds and gastric papillae in the mucosa of partridge proventriculus were similarly reported in Large-billed and Carrion Crows internal surface of proventriculus, ostrich, zebra finch and starling [18-20]. The gastric papillae were absent in the proventriculus of carnivorous birds. These facts were documented in the stomach waders and falcon, there were no papillae found in their luminal surface [21, 22]. The luminal surface of ventriculus (gizzard) was rough and irregular, transverse high folds noticed in the area facing the muscular masses, in the center low longitudinal folds were present. The entire ventriculus internal surface covered with thick

yellowish layer of koilin, stones and greenish colored materials (Fig. 3). The presence of thick layer of high rough surface folds and cuticle or koilin in the ventriculus of partridge is characteristic feature of the seed eating birds. These findings were in agreement with results of [23-25] in the quail, waders, Laughing Dove, starling and zebra finch, respectively. They found in the internal surfaces of ventriculus in these birds a thick, yellowish to greenish layer of koilin or cuticle; with many parallel longitudinal folds. In fact, this structural arrangement facilitated the grinding

The microscopic examination of the stained histological sections of partridge proventriculus showed that the wall of the organ was built up of four layers (mucosa, submucosa, muscularis and serosa). The mucosa made the inner membrane. It formed of one layer of tall columnar epithelial cells. The mucosal surface was irregular because of the presence of papillae. The epithelium invaginated deeply in the lamina propria to form the simple tubular superficial proventriculus glands (Fig.4). The muscularis mucosa was scattered which was interrupted by the presence of deep glands. The Superficial proventriculus glands were simple tubular lined with cuboidal cells. The secretion of these glands were clearly noticed (Fig.5). These results were in agreement with those recorded in broiler chick, Japanese quail and domestic pigeon [14, 27], respectively. The gastric papillae that were noticed in the partridge were absent in the proventriculus of carnivores species such as Eurasian Hobby [28]. The submucosa made of connective tissue; in this layer the deep proventriculus glands were numerous take hexagonal shape between them connective tissues. They were compound tubular glands divided into many lobes by a fibrous connective tissue. Each lobe consisted of numerous secretory tubules, which were lined by simple cuboidal epithelium. The tubules were opened in central canals then lead to the luminal surface (Fig. 6). These results were parallel with the previous, [29] in prey birds *Elanus*

Examination of the ventriculus wall revealed three layers that were mucosa, muscularis and serosa([31, 32]. The lamina propria overlaps with the connective tissue submucosa together forming one layer. The ventriculus mucosal surface coated with thick layer of cuticle, it formed of simple columnar epithelium and underneath connective tissue lamina propria-submucosa. The luminal epithelium invades the connective tissue lamina propria-submucosa, forming simple branched tubular ventriculus glands which lined by simple cuboidal epithelium. The muscularis mucosa was absent (Fig.9). Similarly to this columnar lining epithelium, it was found in other avian species such as quail, in falcon. The lamina propria-submucosa was occupied by numerous simple branched tubular glands, where the lining epithelial cells of gland were cuboidal with round nucleus. The ventriculus mucosa had thick layer of cuticle, because partridge feed on seeds which need

process of seeds and other foods staff, which were consumed by such avian species. Oppositely, the birds which feed on soft high protein food didn't have such modification in their gizzard's luminal surface, as reported in *Elanus caeruleus* bird and Falconidae family. The koilin either absent or present in a very thick consistency beside those there are other avian species such as cattle egret showed soft greenish-yellowish cuticle in the luminal surface of their ventriculus [26, 27]. There is no differences between this bird with other birds in same species.

caeruleus and [30] in Turkey and in laughing dove. They stated that the submucosa layer made of vascular connective tissue and glands. Almost all the submucosa engaged with the deep glands lobes.

The muscularis layer was constructed of smooth muscle bundles; it took two directions with the long axis of the proventriculus, inner longitudinal layer and outer thick circular layers the muscles directed in different wards which help in digest the fibers. Tunica serosa coated the proventriculus from the outside contain loose connective tissue covered the mesothelium. (Fig.7). In the histochemical study of the proventriculus wall, the study identified that combined PAS and Alcian blue gave positive reaction toward the mucous coating the epithelial surface. The secretion gave positive reactions with of PAS. The positive reaction was clearly noticed in the cells of the central canals and those of the superficial glands epithelium (Fig. 8). These observed reactions explained that epithelial cells secrete neutral mucine material which neutralized the acidity of Hcl that produced during the digestion. These findings were similarly found in raptor birds; in common moorhen, in *Elanus caeruleus*, in falcon and in Black-Tailed Crake. They stated that cells lining the tubular glands and the lining epithelium of deep glands were secreted mixed mucous. The mucous protect the proventriculus tissues from the effect of deep proventriculus glands hydrochloric acid and enzymes.

grinding or mechanical digestion. Similar to current findings, the thick cuticle layer in seeds eating birds such as Pigeon and quail ventriculus, were recorded by [23, 24,27]. They stated that thick cuticle layer secreted by ventriculus glands. The current results were disagreed with results of Abumandour et al. [28] in Eurasian Hobby. This reference observed the luminal surface without cuticle layer, in the examined bird. The muscular layer of the partridge's ventriculus was very thick which included muscle bundles runs or arranged in different directions. The different muscular bundles were separated by obvious connective tissue contents [33-35]. The outermost layer was the serosa; the serosa consisted of connective tissue with blood vessels[36, 37]. It was covered with a layer of mesothelium (Fig.10). The muscular arrangement with the serosa in partridge ventriculus was similarly described in other birds studied previously by Zhu [38]) to the

proventriculus wall of black tailed crane, quail and moorhen stomach, respectively. The ventriculus glands were simple branched tubular type. They were opened into the surface epithelium by tubular ducts. The secretion of these glands gave positive reaction

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

with PAS, and the koilin which cover the internal surface react strongly positive with PAS. but negative reaction with Alcian blue (Fig.10) due to presence of neutral mucin which have protection role to this area from the acidity of Hcl.

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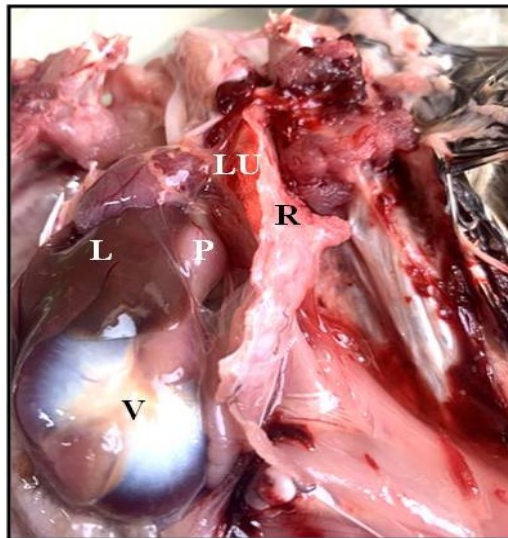


Fig. 1. Macrograph of stomach in partridge showed lung (LU), liver (L), ribs (R), Ventriculus (V) and proventriculus (P)

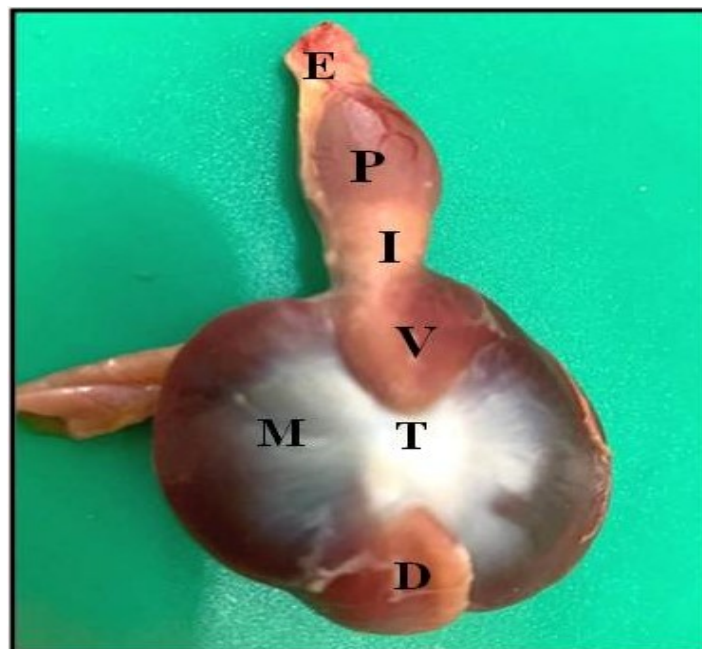


Fig. 2. Macrograph of stomach in partridge showed esophagus (E), proventriculus (P), isthmus (I), ventriculus (V) of two portions: muscular (M) and tendinous (T) and duodenum (D).

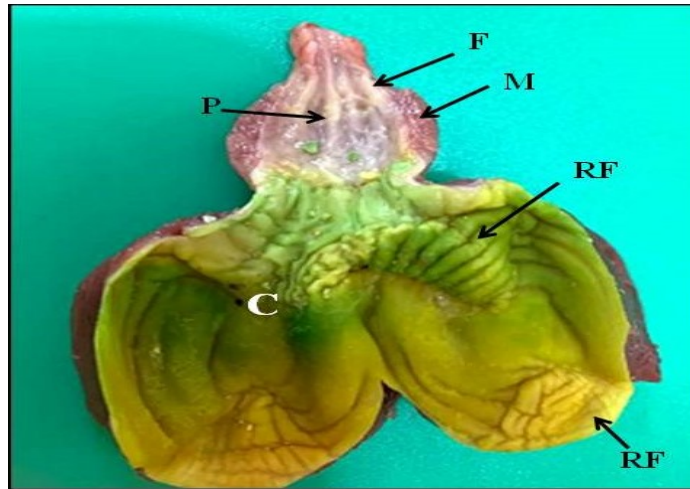


Fig. 3. Macrograph of stomach in partridge showed folds (F), proventriculus (P), mucous (M), rough folds (RF) and cuticle (C).

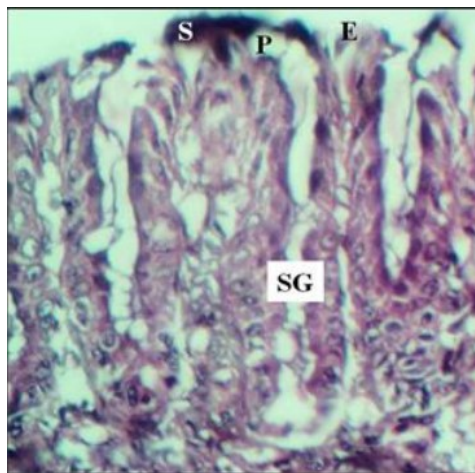


Fig. 4. Micrograph of proventriculus in partridge showed epithelium (E), papillae (P), superficial gland (SG) and mucous secretion (S).

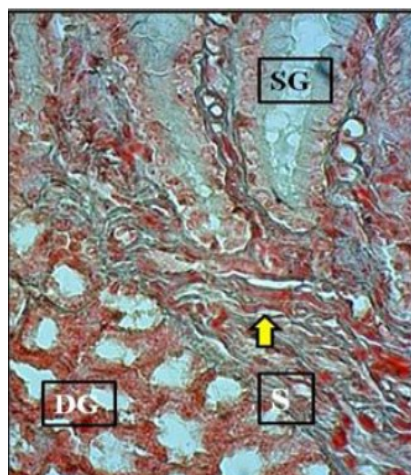


Fig. 5. Micrograph of proventriculus in partridge showed superficial gland (SG), deep gland (DG) and submucosa (S) and muscularis (yellow arrow)

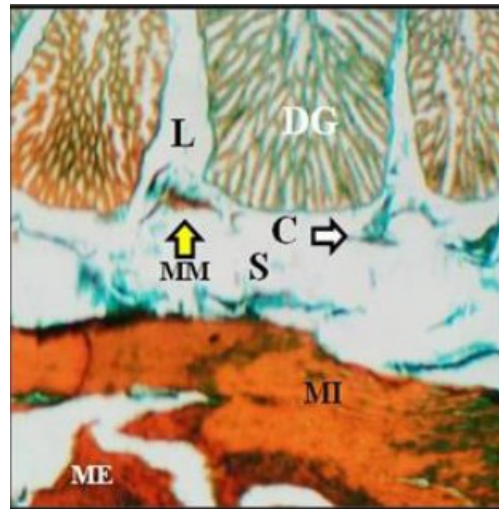


Fig. 6. Micrograph of proventriculus in partridge showed deep gland (DG), submucosa (S) and muscularis mucosa (MM), lamina propria (L) and muscularis externa.

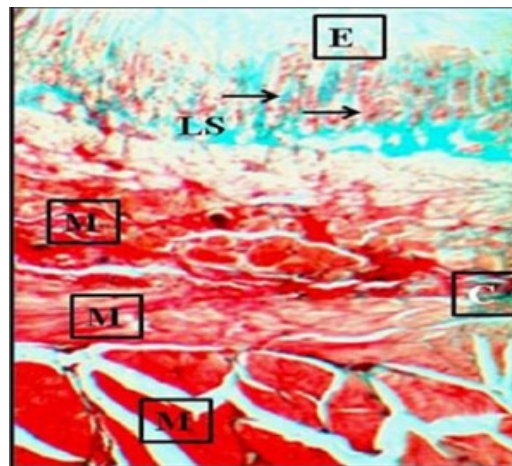


Fig. 7. Micrograph of proventriculus in partridge showed epithelium (E), lamina submucosa (LS), muscularis (M and connective tissue (C). Masson Trichome stain X40.

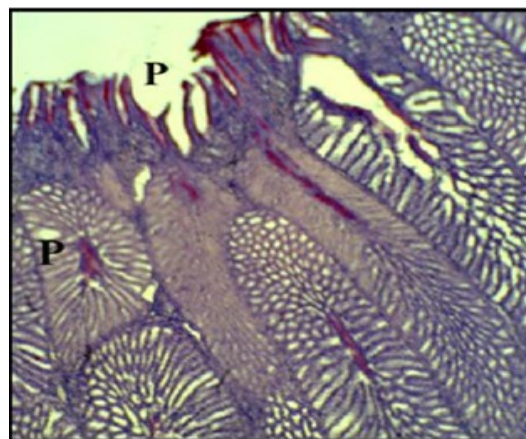


Fig. 8. Micrograph of proventriculus in partridge showed PAS positive reaction (P+) X40.

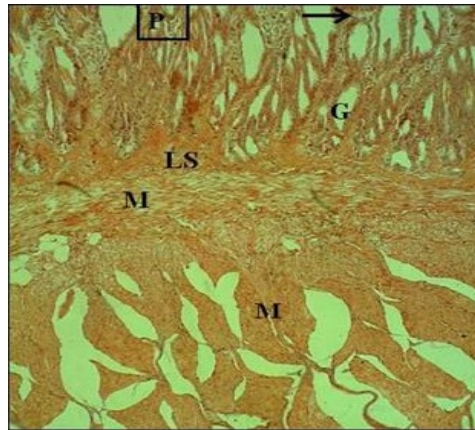


Fig. 9. Micrograph of ventriculus in partridge showed epithelium (E), gland (G), lamina submucosa (LS) and muscularis (M). Crossman stain X100.

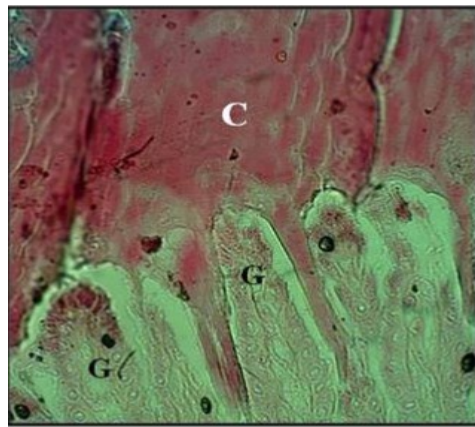


Fig. 10. Micrograph of ventriculus in partridge showed cuticle (C) and gland (G) PAS stain, X40

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قسم التشريح والأنسجة - كلية الطب البيطري - جامعه بغداد - العراق.

المستخلص

صممت هذه الدراسة لتعيين الدراسة النسيجية والتشريحية والتراكيب للكيمياء النسيجية للمعدة في القبج. تم جمع اثني عشر طائر صحي من الاسواق المحلية في بغداد. وتم قتلها بعد تخديرها وتصويرها وهي بداخل التجويف. ثم تثبت العينات وتممر طبقات لتقنيات التلوين النسيجي والكيميائي النسيجي مثل الهيماتوكسيلين والماسون وتفاعل شيف الحمض البيريودي. وجدنا ان المعدة تتكون من قسمين غديه وعضليه تكون الغديه مفصولة عن العضلية بتخصر واضح، الغديه شكلها متطاوول بينما العضلية دائريه ومغطاه بنسيج دهني. تقع المعدة في الجزء الايسر من التجويف الصدري البطني يحتوي السطح الداخلي للمعدة الغديه على طيات بشكل صفوف بينها الحليمات، اما السطح الداخلي للمعدة العضلية فتحتوي على طيات مستعرضه في المركز وطوليه في الاطراف ومغطاه بطبقه سميكة تتلون بماده خضراء مصفرة نسيجا تتكون المعدة من اربع طبقات (المخاطية وتحت المخاطية والعضلية والمصلية)، السطح الخارجي يكون غير منتظم لوجود فتحات الحليمات مع فتحات الغدد السطحية والغدد لعميقه البطانة مبطنه بنسيج طلائي عمودي بسيط ويبطن الغدد من الاعلى بينما الخلايا القاعدية تكون مكعبه ولا يوجد الغشاء المخاطي العضلي تكون الطبقة العضلية سميكة من ثلاث طبقات في اتجاهات مختلفة للتكيف مع الانكماش الوظيفي في المعدة، من الناحية الكيمياء النسيجية تفاعلت الظهارة والقنوات الغديه بشكل ايجابي مع كاشف الحمض بسبب تكوينها المحايد.

الكلمات المفتاحية: طائر القبج، القانصة، البطين، معدة الطيور، الغدد.