

Morphometrical and Histochemical study of glandular stomach (Proventriculus) in local domestic male ducks (*Anase Platyrhchos*)

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Abstract

The present work explores morphometric and histochemical foundations of the wall of proventriculus in adult domestic male ducks. To achieve this study, six healthy adult ducks were purchased from local vendors in Mosul province. All birds were sedated. Their stomachs were removed after being dissected to achieve the gross description and measurements of the proventriculus, including the length and diameter of cranial, middle, and caudal regions of the proventriculus. Small tissue pieces 6-8 mm in length from three regions of proventriculus were processed for histomorphological studies using hematoxylin and Eosin stain, combined PAS-AB pH 2.5, toluidine blue, and Masson's trichrome stains. The finding showed that the proventriculus connects with the esophagus from the cranial side and the muscular stomach from the caudal side. It was a thin elongated oval tubular organ. Histologically, the mucosa of the proventriculus revealed the presence of folds and sulci-like structures covered by simple columnar epithelium. The tunica submucosa consisted of many signs of profound and straightforward sub-mucosal glands supported by connective tissue septa and extended from the basal part of the diverged gland through the lamina propria, which was organized in a conical manner (adenomere). Tunica Muscularis appeared as a double-layer coordinated in the form of inner longitudinal and outer circular ones. Finally, serosa consists of fibrous tissue, vessels, fatty tissue, and nerve plexuses studded by the mesothelial cells. The highest thickness of the four layers of mucosal, submucosal, muscular, and serosal layers in the distal part of the proventriculus. Histochemical PAS staining positively reacted with epithelial tunica mucosa and wall of proventricular glands.

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Introduction

The essential elements of the birds' alimentary tube are the oral cavity, esophagus (containing the crop in several types of birds), the proventricular and ventriculus), and intestinal segments. Anatomically, the gastric portion of domestic ducks is allocated as two main segments: the glandular part, known as gastric proventriculus (glandular stomach), which is linked to the esophagus cranially (1), and the muscular portion, known as the ventriculus (gizzard), that is divided by an isthmus or intermediary zone

(2). The proventriculus varies in size according to the species variation of birds. It looked to be very small in the graminivorous, whereas found to be more significant in carnivorous (3). The length and breadth vary among regions and species (4). The wall of the proventriculus comprises the four general layers for the most digestive organs, which are tunica mucosa, submucosa, muscular layer, and serosa (5). The surface mucosa of glandular stomach was described by the presence of papilla with abundant tiny folds (plicae) ranging in its elevation and grouped in concentric manner round the solitary duct opening at the

papilla's apex (6). Liman *et al.* (7) mention that the mucosa is markedly wrinkled, creating flat folds split by fissures or grooves where the tubular mucous glands opened in the bases of the furrows, elevation of Tmuscularis (papilla) contains the opening of leaving duct of submucosal proventricular glands (8). The stomach proventriculus mucosa liberating pepsinogen enzyme, hydrochloric acid, and mucin (7). The mucosal layer is lined with simple columnar cells. These cells extend to the interior of the furrows (9,10). The submucosa is studded by many tubular submucosal glands, ramified or complex types; only a primary simple cuboidal exocrine cell and a few stomachs argyrophil endocrine cells make up the glandular epithelium. (9). Most of the deep proventriculus wall thickness was taken up by glands that were conic or pear in form (11). T muscularis is somewhat adapted where the innermost layer is oriented longitudinally and the external circularly. The tunica serosa is constructed from connective tissue supported by mesothelium (8).

This work explores histological, morphometrical, and carbohydrate histochemical foundations in the proventriculus in adult domestic male ducks. It also aims to provide an adequate anatomical and histological database about the proventriculus of local male ducks to fulfill their necessities of food and energy.

Materials and methods

This study used six healthy adult male ducks (*Anas Platyrhchos*). The Birds were purchased from local sellers in Mosul province's animal market with body weights ranging between 2.5-3 Kg. After using chloroform inhalation, the birds were slaughtered, and the digestive tube was exposed (Figure 1). The proventriculus is harvested and divided equally into three segments (proximal, middle, and distal). The entire length of the proventriculus was measured using a measuring tape, as well as the length and width of each segment were measured with the aid of a vernier caliper.

Representative specimens of 6-8mm were taken as follows: esophageal proventriculus junction (proximal), proventriculus (middle), and proventricular gizzard junction (distal). All specimens were washed with buffered saline and then fixed immediately in standard neutral buffer formalin 10% for about 48 hours. They were dehydrated progressively by ethyl alcohol and cleared with xylene before embedding in wax of paraffin. Formerly, they were cut by rotatory microtome to obtain a histological section of 4-6 micrometer thickness and applied to the routine histological stain H&E for common inspection, Masson's Trichrome stain to recognize fibrous tissue, Toluidine blue, and Periodic acid Schiff reagents (PAS) for identification of mucins and glycosaminoglycan respectively (12-14).

The included micromorphometric measurements were included the tunica mucosa thickness as well as that of

tunica submucosa, tunica muscularis, and tunica serosa of the studied areas measured in micrometer (unit/ μm). The length or span of the folds in the studied areas / μm . Dimensions (length * width) of the compound gastric glands in the submucosal region and the simple tubular gastric glands in the mucosal region, and the duct diameter of the studied areas measured / μm .

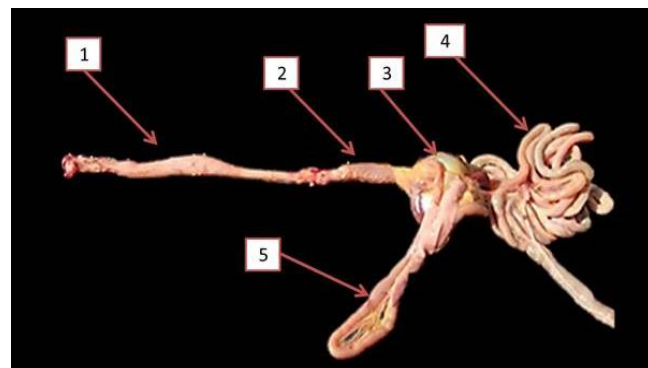


Figure 1: Photograph illustrates the tubular organs of the digestive tract of male ducks showing: 1esophagus, 2 proventriculus, 3 ventriculus (gizzard), 4 small intestines, and 5 duodenum.

All tissue sections we re-photographed, and the micromorphometric parameters were analyzed with the aid of an OMAX 8.0MP digital USB microscopic camera and sophisticated picture analysis software (OMAX Toup view 3.7), the camera was fitted to Microscope-Olympus-CX31, and the aid of stage micrometer did the calibration of objective lenses to the software.

Statistical analyses were carried out with the SigmaPlot 12.5 (Systat Software Inc 2016)(software package. All numerical numbers were expressed as mean \pm SE. Then ANOVA with Duncan's test were assessed at $P \leq 0.05$.

Ethical approve

This work was conducted in the laboratory of anatomy, College of Veterinary Medicine, University of Mosul under reference number UM.VET.2021.011.

Results

Anatomical approach

Gross anatomical results showed that the proventriculus in ducks was positioned cranial to esophagus and caudal to ventriculus in the left ventral region of the abdominal cavity (Figure 2). The liver's left lobe partly buried the organ. Conversely, well-allocated proventriculus from the esophagus and ventriculus were stated, probabilmene delineation of the esophageal-proventriculus junction.

The gross anatomical investigation of the stomach of local breed duck revealed that the stomach of the examined

ducks looked to be a thin-walled part, noticeably composed of two segments which are the glandular one (proventriculus) and muscular one (ventriculus), these two segments separated by intermediate zone (Figure 2). The proventriculus, according to observations, of local duck was large, fusiform, or tubular with a mean length of 52.91 ± 1.83 mm and mean width or diameter in three regions proximal, middle, distal 7.804 ± 0.21 , 12.522 ± 1.18 and 10.56 ± 0.19 mm, respectively (Figure 3).

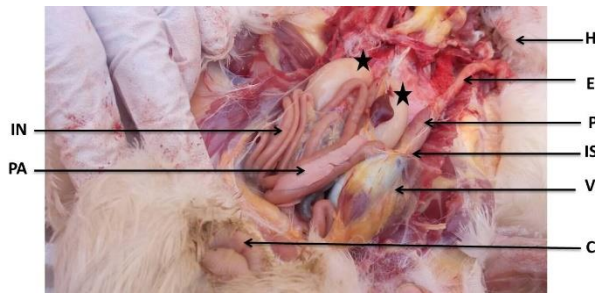


Figure 2: Photograph illustrates a ventral side of duck bowl presenting: (H) Site of head, (E) Esophagus, (P) Proventriculus, (IS) Isthmus, (V) Ventriculus (gizzard), (C) Cloaca, (IN) intestine (PA) Pancreas, (Black stars) Testes.

Histological approach

Histological investigations exhibited the three parts' proventricular wall constructed from the main tunicae of any classic tube-like structure: tunica mucosa, tunica submucosa, tunica muscularis, and serosa (Figure 4). The tunica mucosa varied in thickness among the three segments of the proventriculus, the highest thickness was recorded in the distal part $650.85 \pm 60.65 \mu\text{m}$ (Table1), and it consisted of three layers epithelium, lamina propria, and muscularis mucosa. The mucosa appeared thrown with folds (plicae

proventricular) and sulcus at its luminal surface. The folds diverse in length. The highest lengths were recorded in proximal region $412.11 \pm 7.3 \mu\text{m}$ (Table 2). Some of the folds combined, and the lining epithelium was simple columnar with clear cytoplasm and intermingled mucous-secreting cells (goblet cells) may be present. The cells of the basal region of the folds were low and stained intensely more than those of the superficial region (Figure 5). Mucin granules were shown in supranuclear parts of columnar cells (Figure 6). The proventricular glands at the base of these folds consist of both superficial and profound gastric glands. The superficial glands were dispersed in propria of mucosae, while profound glands existed in submucosa. The outer most superficial glands are short, tubular simple glands extended through the lamina propria (Figure 6). The glandular cells in the basal portions and body of the superficial gastric glands consisted of many pepsinogen granules (Figure 6).

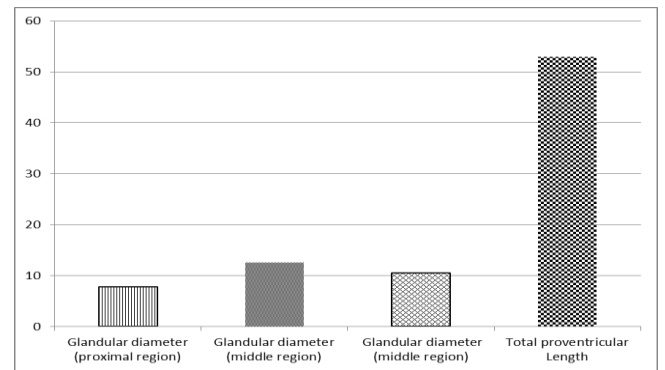


Figure 3: Histogram showing the length and diameter of proventriculus in mail ducks.

Table 1: The thickness of proventriculus in different regions of male duck

Region	Proventricular (thickness/μm)		
	Proximal part	Middle part	Distal part
Mucosa	$572.85 \pm 20.47a$	$589.72 \pm 59.86a$	$650.85 \pm 60.65b$
Submucosa	$1170.33 \pm 37.90a$	$1356.88 \pm 52.62b$	$1765.20 \pm 50.86c$
Muscularis	$320.01 \pm 5.48a$	$439.84 \pm 24.69b$	$756.87 \pm 43.93c$
Serosa	$10.78 \pm 0.55a$	$26.46 \pm 0.74b$	$65.60 \pm 3.30c$

The similar letters among region in revealed no statistically significant difference at $P \leq 0.05$.

Table 2: The diameter of proventricular glands, canals and lengths of folds of proventriculus in different regions of male duck

Region	Proventricular (thickness/μm)		
	Proximal part	Middle part	Distal part
Diameter of proventricular glands	$605.68 \pm 14.9a$	$804.84 \pm 18.9B$	$706.12 \pm 20.1c$
Diameter of canals of proventricular	$39.88 \pm 0.89a$	$34.88 \pm 1.10B$	$42.96 \pm 1.9a$
Length of the folds	$412.11 \pm 7.3a$	$213.50 \pm 8.8B$	$161.23 \pm 3.3c$

The similar letters among region in revealed no statistically significant difference at $P \leq 0.05$.

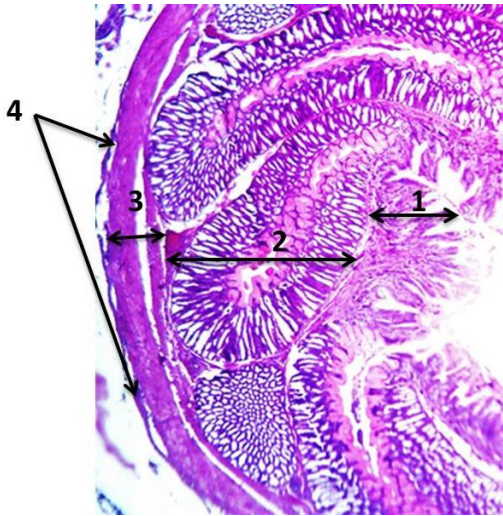


Figure 4: Photomicrograph of proventricular wall in duck presented (1) mucosa, (2) submucosa, (3) muscularis and (4) Serosa, H and E, (X100).

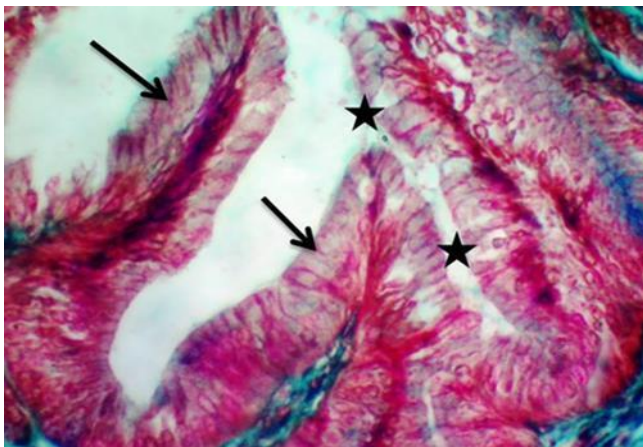


Figure 5: Photomicrograph of proventriculus of local duck showed the folds lining epithelium was simple columnar (Black arrow) with clear cytoplasm cells, (Black stars) Goblet cells, Masson's trichrome (X 400).

Muscularis mucosa, which was made up of circular smooth muscle fibers establishing a net appearance and neighboring the units of the large number of deep gastric glands (Figure 7). All of the branched tubular glands are divided by septa of connective tissue then each tubule connected to one duct opened into the central main duct, that opens into the proventricular luminal surface. The lobules of the glands were extended ovoid in the duck and were determined from each other by well-vascularized collagen fibers (Figure 7). Each glandular lobule consisted of tubuloalveolar units. Tunica submucosa was made up of a thin layer of loose connective tissue containing several fine blood capillaries and nerve endings.

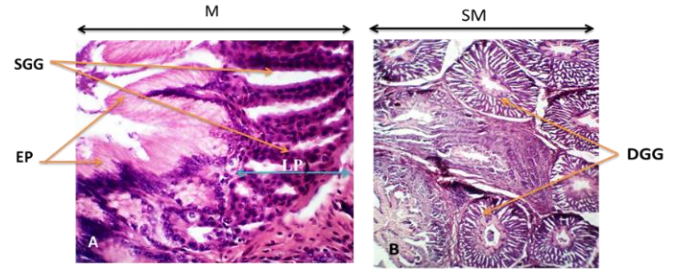


Figure 6: Microphotograph of crosswise section of proventriculus of duck (A and B) showing the (M) Mucosa, (SM) Submucosa, (EP) Epithelium, (LP) lamina propria, (SGG) Superficial gastric glands, (DGG) Deep gastric glands. H&E stain, A(X100), B (X100).

Submucosal inner glands are arranged into five longitudinal bulges or conical shapes. The lumen of the proventriculus was significantly reduced, being taken by its considerably thickened glandular walls. Tunica muscularis comprises an inner longitudinal and outer thick circular layer of smooth muscle fibers in the middle and distal parts (Figure 7). The anterior part contains inner and outer longitudinal and middle circular layers. The rich blood vessels and nerve plexus are found between such muscular layers. (Figure 8). The mean thickness of tunica muscularis varied in three different regions of the proventriculus. (Table 1). Tunica serosa consisted of connective tissue rich in blood vessels, nervous plexus, and adipose tissue, all covered by mesothelium (Figures 7 and 8). The statistical analysis revealed that the average lengths of folds in the proximal part of the proventricular are high, while the average width or diameter of the proventricular glands was high in the middle part (Table 1).

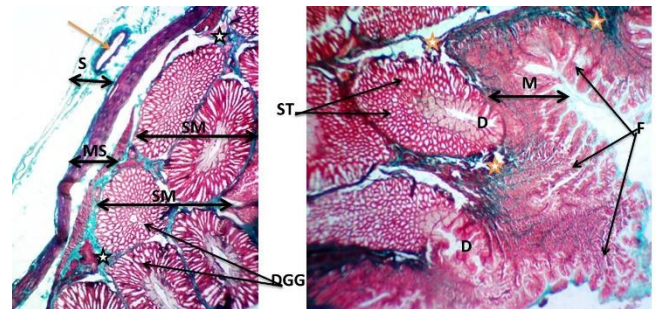


Figure 7: Microphotograph of crosswise section for Proventriculus of duck. (M) Mucosa, (F) Folds, (Orange stars) Muscularis mucosa, (D) Main collecting ducts, (ST) Secretory tubules, (DGG) Deep gastric glands, (Black stars) Connective tissue, (MS) Muscular layers of Proventriculus, (S) Serosa and (Orange row) Blood vessels, Masson's trichrome (X 40).

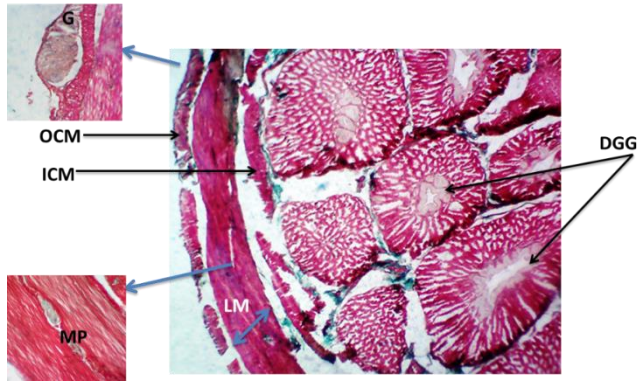


Figure 8: Photomicrograph of a transverse section from the anterior part of Proventriculus of male duck. (DGG) Deep gastric glands, (OCM) Outer circular layer of tunica muscularis in Proventriculus, (ICM) Inner circular longitudinal part of muscular tunica in Proventriculus, (LM) middle longitudinal part of muscular (MP) myenteric plexus between muscle bundle(G) ganglion plexus from large bundles of axons in tunica serosa. Masson's trichrome (X 40).

Histochemical results

In the current study microscopic examination of the glandular stomach of duck shown strong +ve reaction of surface epithelial cells of mucosal folds to PAS indicating the presence of neutral mucins, moderate to AB pH 2.5 as the representing few amounts of acidic mucins. Lamina propria tissue between mucosal folds reacts moderately to PAS/AB pH2.5. The luminal surface of submucosal acini in the proventriculus of male duck reacts strongly with PAS and mild to moderate with AB pH2.5, signifying the presence of both acidic and neutral mucins. Lamina muscularis showed weak to moderate +ve reactions with PAS and vigorous with AB pH2.5. The tunica muscularis showed mild reaction toward PAS/AB pH 2.5 respectively (Table 3 and Figure 9).

Table 3: Response of proventriculus to PAS stain, AB pH2.5, and tunica blue stains at different regions

Region	PAS	AB pH 2.5	TB
Epithelium of mucosal folds	+++	+	±
Connective tissue of the lamina propria	+	+	±
Luminal surface of submucosal glands	+++	+	±
Muscularis mucosa	±	++	±
Tunica muscularis	±	±	±

Very strong: +++ Strong: ++, Strong: +, Mild: ±, No reaction: -.

On the other hand, some structures of proventriculus showed a mild reaction against toluidine blue stain, indicating the presence of small quantities of glycosaminoglycan (Figure 10). Mast cells appeared in the connective tissue under the epithelium and showed a strong reaction when used the toluidine blue stain (Figure 10C).

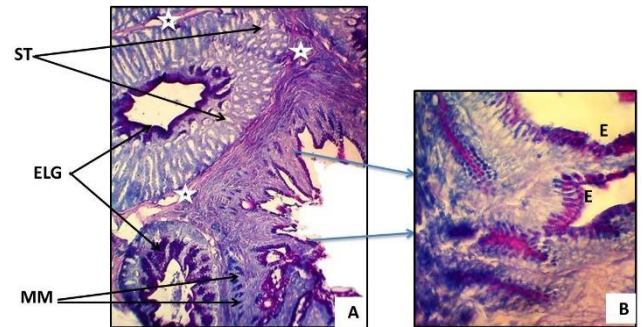


Figure 9: Microphotograph of a crosswise section of the proventricular wall of male duck (A and B). showing (Blue rows) (E) Epithelium of mucosal folds and (ELG) Epithelium of luminal gland gave a positive reaction to PAS stain. (ST) Secretory tubules, (White stars) Connective tissue, and (MM) Muscularis mucosa were positive reactions to AB pH2.5. PAS/AB pH 2.5 stain in (A&B). A (X100), B (X400).

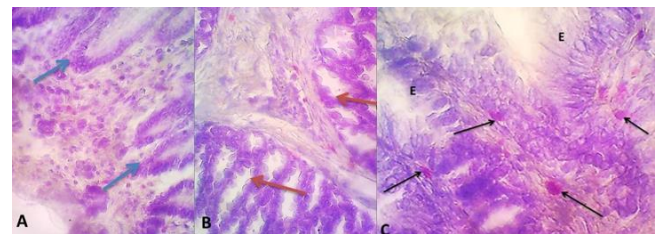


Figure 10: Microphotograph of a crosswise section of the proventricular wall in male duck. (A, B, and C) in A (Blue rows), Superficial gastric glands showed mild reaction against toluidine blue stain, B (Brown rows) caudal part glandular cells are put net to at their basal parts and presenting a dentate look. C (Black rows) Mast cells showed a strong reaction with toluidine blue, (E) Simple columnar epithelium. Toluidine blue stain in (A-C). A(X100), B (X100), C (X100).

Discussion

The stomach in the local duck looked divided into cranial proventriculus and distal ventriculus, such mannerism was detected in different types of, as Saeed and Al-Nimma (15) in pigeon, Conversely, Abumandour (16) cited that stomach of Eurasian hobby comprises third additional pyloric part. Present work shows that the glandular proventriculus appeared to be a large, ovoid organ in ducks. Comparable discoveries were previously detected

by Abumandour (16), who specified that the proventricular shape was ovoid or fusiform in different types of birds with different sizes. Contrariwise, the demarcation between proventriculus, esophagus, and ventriculus was not well-defined in the ducks of this study. The proventriculus of local breed ducks founded in the anterior aspect of ventral abdominal nook between the esophagus and gizzard. The liver covered a small part of the studied stomach. Similar findings on the location of this organ were cited by Abumandour (16) in *Falco subbuteo*, Tadjalli *et al.* (17) in male adult Ostrich and Zaher *et al.* (18) in common quail (*Coturnix coturnix*).

The apparent long glandular stomach of local duck has a role in food storage like that cited in ratite birds (19). actually, the proventriculus is devoid of papillae, and its ducts open directly on the mucosal surface. The proventriculus showed the existence of four primary tunics of a classic tubular structures, including (Tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa). These tunicae were equally recognized in the proventriculus of several birds, such as the domestic pigeon (11), Japanese quail (20), and Coot bird (21). A recent study showed that the lining cells of proventricular mucosa was columnar studded with goblet cells, and this result came in agreement with Eman *et al.* (22) when studying the proventriculus of common moorhen (*Gallinula chloropus*). The fibers of the lamina propria spread inside the folds and possessed simple tubular mucous glands. Das *et al.* (23) detected that the connective tissue of lamina propria in the stomach of Kadaknath fowl comprises nodular or diffuse lymphatic tissue. Thin, smooth muscle fibers of muscularis mucosa as bunches are scattered among the clusters of glands. Tunica Submucosa is occupied by abundant mucous compound glands, constructed of a layer of cuboidal cells and opened directly to the surface. Rossi *et al.* (24) mentioned that the glands lobules in partridge *Rhynchotus rufescens* were separated by fibrous septa. These findings were consistent with our results in this study. Das *et al.* (23) mentioned that the tunica muscularis in partridge is formed of an inner muscular layer and an outer one. In domestic ducks, the muscular was composed of an inner longitudinal and an outer thick circular layer of smooth muscle fibers in the middle and distal parts, while the anterior part contained an additional middle layer. The tunica serosa was constructed of loose connective tissue in which nerves, blood vessels, and adipose cells were observed, and a layer of the mesothelium covered such structures. These findings were similarly observed by Hussein *et al.* (8) in studies of the stomach in the Iraqi falcon (*Falco peregrinus*), while in moorhen Taher *et al.* (25) and in partridge *Rhynchotus rufescens* Rossi *et al.* (24) stated is composed of collagen fibers and a cuboidal cells layer. Histochemical, the surface of mucosal folds was intensely +ve to PAS, moderate to AB pH 2.5, indicating the presence of neutral mucins and few acidic mucins.

The fibrous tissue of L. propria, which intermingled among mucosal folds, presented a moderate reaction to PAS/AB pH2.5. The luminal surface of submucosal glands in the proventriculus of male duck reacts strongly with PAS and mild to moderate with AB pH2.5, indicating the presence of both neutral and acidic mucin, and these results support those of Taher *et al.* (25) and Al-A'araji (26) in moorhen (*Gallinula chloropus*) and birds respectively and disagree with Udoumoh and Ikejiobi (27) stated that the complex tubular glands of the proventriculus of African pied crow displayed PAS adverse reactions. The muscularis mucosa and the blood vessel wall in the submucosa exhibited mild to moderate responses with PAS and strong with AB pH 2.5. Whereas the tunica muscularis showed mild reaction toward PAS/AB pH 2.5 respectively, Recently, Zaher *et al.* (18) recognized equally in the stomach of quail, rich neutral and acidic mucins in the gastric glands where they +ve reactions with PAS-AB stain. On the other hand, some structures of proventriculus showed mild reaction against toluidine blue stain, indicating the presence of small quantities of glycosaminoglycan. This is consistent with what was mentioned by Cook (28), Mahmud *et al.* (29) and Khaleel *et al.* (30) for the Gland Cells of the Stomach in different types of birds.

Conclusion

The study concluded that the domestic duck's proventriculus contains two types of glands, the superficial and profound or deep gastric glands. The superficial glands are short, simple tube-shaped glands drawn-out through the lamina propria, while its inner or deep glands are arranged into four to five longitudinal bulges or conical shapes. Histochemical PAS staining positively reacted with epithelial of tunica mucosa and wall of proventricular glands.

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Conflict of interest

The authors declared that there is no conflict of interest.

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دراسة شكلية قياسية ونسجية كيميائية للمعدة الغذائية للبط المحلي

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الخلاصة

استهدفت الدراسة الحالية إلى التحري عن القياسات الشكلية والكيمياء النسيجية لجدار المعدة الغذائية في ذكور البط المنزلي البالغ. ولإجراء هذه الدراسة، تم شراء ست بطات بالغات سليمة من السوق المحلية في محافظة الموصل. تم تخدير جميع الطيور وتشرح تجوفها البطني ثم استخراج معدتها للحصول على الوصف والقياسات العيانية والتي تشمل أطوال وأقطار المناطق (الأمامية والوسطى والخلفية) من المعدة الغذائية. تم أخذ عينات صغيرة من المناطق الثلاثة للمعدة بطول 6-8 ملم لإجراء الدراسة النسيجية باستخدام ملون الهيماتوكسيلين والأيوسين وكذلك حامض البيريودك-شيف، ملون التليودين وملون ماسون ثلاثي الصبغ. أظهرت الدراسة العيانية أن المعدة الأمامية هي عضو أنبوبي بيضاوي رفيع يتصل بالمريء أماميا وبالمعدة العضلية خلفيا. ومن الناحية النسيجية، أظهر الغشاء المخاطي للمعدة الأمامية عن وجود طيات وتراكيب شبيهة بالأخاديد تغطيها ظهارة عمودية بسيطة، وتتكون الغلالة تحت المخاطية من العديد من الغدد تحت المخاطية البسيطة والعميقة والتي كانت مدعومة بحواجز النسيج الضام وممتدة عبر الصفحة اللبائية من قاعدة الغدة الأنبوبية المتفرعة، والتي كانت مرتبة في شكل هرمي أو مخروطي. ظهرت الغلالة العضلية كطبقة سميكة من حزم العضلات الملساء مرتبة في طبقات داخلية طويلة وخارجية دائرية. وأخيرا تألفت الغلالة المصلية من نسيج ضام وأوعية دموية وأنسجة دهنية وعضلات عصبية مغطاة بطبقة من الخلايا الحرشفية. ظهر أعلى سمك للطبقات الأربع الغلالة المخاطية، وتحت المخاطية، والعضلية، والمصلية في الجزء القاصي من المعدة الغذائية. كما أظهرت دراسة الكيمياء النسيجية أن الظهارة السطحية للغشاء المخاطي وبطانة الغدد المعدية كانت موجبة لملون حامض البيريودك - شيف.