Histomorphological and histochemical study of stomach of domestic pigeon (*Columba livia domestica*)

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Summary

The study aimed at describing the histomorphological and histochemical structures of the stomach wall in domestic pigeon. Twelve healthy birds were collected from local suppliers at Baghdad province. Birds were divided into three equal groups for gross, histological and histochemical approaches. All birds were euthanized and their abdomens were dissected, then their stomachs were identified and photographed in situ. Specimens from stomach were fixed as well and then processed for histological and histochemical staining techniques using Hematoxylin-eosin, Masson Trichrome, Periodic acid Schiff (PAS) and PAS -Alcian blue (AB) (pH 2.5). Grossly the stomach of the pigeon consisted of two distinguishable chambers, that were glandular or proventriculus and muscular or ventriculus. The proventriculus was tubular-shaped organ. Whereas, the ventriculus appeared fusiform in shape and surrounded by considerable amount of fatty tissue. Histologically the proventriculus mucosal lining was simple columnar epithelium cells. The submucosal consists of numerous and voluminous deep submucosal glands which occupied the greater part of the organ wall. The internal lining of the ventriculus was simple columnar cells. Muscularis mucosa appeared circularly arranged bundles interrupted by the presence of mucosal glands in the lamina propria. The tunica muscularis appeared as a very thick structure of smooth muscles fibers of three layers, thin inner, outer longitudinal and very thick intermediate circular layers.

Keywords: Pigeon, Proventriculus, Ventriculus, Histochemistry.

Introduction

The stomach is considered the most active part of the digestive system in birds. It comprises two parts that are proventriculus and ventriculus (gizzard). The gizzard contains gravel or grit, which facilitate alongside with muscles in grinding up the food. Birds have an extremely high metabolism to correspond with their requirements, consequently bird must eat a large amount of food (1). Accordingly, such characteristic high metabolic rates lead them to consume greater quantity of food in proportion to their size and such a trait was differently greater compared to those reported in other animal species (2). The size and shape of the two stomach parts (proventriculus and ventriculus) in birds found based on type of diet was found distensible and different in shapes to distinguish between the two parts in carnivorous as well as piscivorous birds. Whereas, the wall of the proventriculus was thin and glandular in the birds depend on solid food. But the latter, possess a thick and muscular ventriculus. This gastric arrangement was typically found in granivorous, omnivores, insectivores and herbivores (3 and 4). Whereas, in vegetarian fowls, the gizzard was found markedly developed in birds that depend on hard foods in their feeding such as granivorous in which the organ considered as a masticator structure (4 and 5) and in insectivores (6). The proventriculus is varied in the size in different species of birds in which it appeared small in size in the graminivorous, whereas, it was found large in the carnivorous (7). Abumandour (8) divided birds according to their type of stomach, into three types. First of them the soft eating birds as in kestrel and owl in which the main function of the gizzard was the storage of food. Second type was hard diet eating birds represented by turkey and sparrow in which the main function of the gizzard was mechanical action on the food. Whereas, the third type, intermediate diet eating birds as in goose, hoopoe and darter in which the role of gizzard was storage and physical digestion. There were paucity of work to investigate the histomorphological and histochemical structures of the stomach of the domestic

pigeons and to preent knowledge there were no local studies have been conducted yet. Accordingly the current investigation intended to conduct such investigation.

Materials and Methods

Twelve domestic pigeon (*Columba livia domestica*) were used to conduct the current study. These birds were bought from specific markets at Baghdad province from the local suppliers. Birds were housed at animal house of the Veterinary Medicine College/ Baghdad University in suitable cages. They were fed as well and giving them water before their euthanasia and dissection. The total number of birds were divided into three equal group each of four. For gross, histological and histochemical approaches.

Birds were euthanized prior to their dissection with an intravenous injection of sodium pentobarbitone sodium (80 mg/kg), after fixing them on a dissecting board. A mid-line incision was made in the abdominal wall to view the coelomic viscera. The proventriculus and ventriculus (gizzard) were identified and photographed in situ using digital camera (pupil cam, ken-a-vision). Histological specimens (whole proventriculus, gizzard) from each dissected birds were washed gently by normal saline then immersed in 10% neutral buffered formalin. After well fixation the specimens from the stomach routine histological was done the sections stained by: Mayer's Hematoxylin and Eosin routine stain for general features identification, Masson Trichrome stain for the staining of the collagenous and smooth muscle fibers (9). The histochemical study for the stomach, specimens were fixed in Bouin's solution and then sections of 6 µm thickness were prepared and then after were stained with one of the bellow stains and subsequently examined and photographed by Olympus BH-2 microscope, using Dino-eye camera. For the determination of neutral and acidic mucin, PAS - Alcian blue (AB) pH 2.5 was used. The PAS alone was used for the illustration of the neutral mucin and basement membranes of the epithelial lining of the stomach (10).

Results and Discussion

Grossly the stomach in the studied pigeons appeared as an organ distinctly divided into two parts that were proventriculus and ventricular (Fig. 1) and such feature similarly observed in the majority avian species such as domestic fowl (*Gallus gallus*) (11), partridge (*Rhynchotus rufescens)* (12) and Japanese quail (13). Hodges, (14) mentioned that stomach in some birds consists of three compartments; namely proventriculus, ventriculus and pyloric part.

Current observations revealed that the proventriculus or the glandular stomach was tubular-shaped organ (Fig. 1). Similar shape was observed previously (15) in the proventriculus of the chicken, pigeon and duck. Whittow (16) stated that the shape of the proventriculus is fusiform in the most avian species but with the differences in their sizes. Macroscopic examination showed in pigeon stomach distinctly thicker wall of proventriculus than that of esophagus giving rise to well demarcation between them (Fig. 1). Similarly, the proventriculus-ventriculus constriction or isthmus was a well demarcation between both organs. The gross demarcation between esophagus and proventriculus in pigeon stomach was by the existence of numerous projections in its internal surface which represent the proventricular glands, whereas, the internal surface of esophagus was smooth. The well demarcation proventriculus in pigeon was in accordance with previous findings observed by (17 and 18) in the seed eater birds such as Streaky and Paramo. Whereas, not well demarcated proventriculus from the esophagus and gizzard was dissimilarly described in meat eater birds such as owl (19). Difficult demarcation between the esophagus and proventriculus was also found in case of most fowl (20), in bustards (21) and partridge (12).

The second parts of the stomach which is the ventricular appeared fusiform in shape and surrounded by considerable amount of fatty tissue in pigeon (Fig. 1). Differently, the organ described as an ovoid muscular sac in owl the (19) and in the carnivorous birds such as kestrel and owl (22). The constricted area between the two parts of the stomach which was clearly visible in pigeon was previously

also recorded in seed eater avian species such as brown-rumped (*Serinus tristriatus*) and white-collared (*Sporophila torqueola*) (17 and 19) in partridge (12). Conversely the constriction not observed in carnivorous and piscivorous avian species (20 and 23).

Differently, the cuticle layer not observed in other birds such in owl's ventriculus (19) and the wall of this bird was thin and such different might be due to the nature of prey in owl need less physical digestion. Additionally, the thick layer of cuticle that observed in pigeon was almost similar to (24) in the gizzard's mucosa of the red-capped cardinal species. These findings were also in accordance with those of (25) who observed well developed muscularity and cuticle in both granivorous and herbivorous species such as domestic fowl, pigeon, ducks and geese and differently they documented thin walled ventriculus in both owls and hawk.

Microscopic examination proventriculus wall revealed the presence of four layers of the typical tubular organ, tunica mucosa, submucosa, muscularis and serosa (Fig. 2). The layers which structured the wall of proventriculus was similarly documented in the proventriculus in many avian species such as ostrich (*Struthio camelus*) (26), Guinea fowl (*Numida meleagris*) (27), quail (28), Japanese quail (13) and Coot bird (29). Whereas, in the wall of the proventriculus of Asiatic swiftest (*Collocalia spp*.), (30) observed only three layers in which only mucosa, muscularis and serosa were detected. Tunica mucosa showed longitudinal branched folds that were lined by simple columnar epithelium (Fig. 4). The underlying lamina propria was constructed of loose connective tissue filled with blood vessels and infiltrated lymphocytes (Fig. 3).

Tunica submucosa prominently, this layer found occupying most of the wall thickness of this organ. It was formed of loose connective tissue containing pear-shaped branched tubular proventriculus glands surrounded by a fibrous capsule (Fig. 2 and 3). Such findings were not agreed with (31) in the burrowing owl (*Speotyto cunicularia*) who described these glands as oval and lined by tall columnar epithelium. The glands consists numerous secretory tubules which were lined by cuboidal cells and each tubule continued by one duct opened into the main collecting duct which subsequently opened into luminal surface of the organ. Current findings were almost similar to those recorded in other birds such as Red-Capped Cardinal (*Paroaria gularis gularis*) (24) and Coot bird (*Fulica atra*) (29) but different to those found in the red jungle fowl (32). The current findings concerned presence of proventriculus glands were not parallel with those of (33) and (34) who referred to the absence of these glands in the submucosa of the proventriculus in chickens.

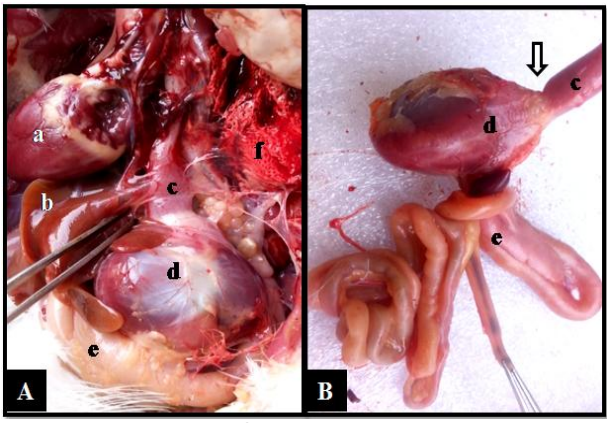
Tunica muscularis was constructed of two layers, inner thin longitudinal and an outer thick circular layers. Between such layers, fine connective tissue was observed filled with blood vessels (Fig. 3). Differently to current findings, in parrots (3) observe only one layer of smooth muscle fibers circularly arranged. In other birds found three layers of smooth muscle bundles constituting the muscular layer such as the red-capped cardinal birds (24). The layers were inner longitudinal, intermediate circular and an outer longitudinal in which nerves and ganglion cells were distributed. However, in the falcon proventriculus, similarly to current studied pigeons, (8) who found two layers but the inner longitudinal layer was well developed that constructs most thickness of the wall of this organ in this bird.

Tunica serosa was constructed of loose connective tissue in which nerves, blood vessels, adipose cells were observed and such structures were covered by a layer of mesothelium (Fig. 3). These findings were also observed by (13) in Japanese quail and (29) in Coot bird (*Fulica atra*). Similarly to the proventriculus, the microscopic structure of ventriculus also showed the four known tunicae forming its wall (Fig. 4). Same findings regarding the wall structure were recorded in most avian species such as Red-Capped Cardinal (*Paroaria gularis gularis*) (24) and guinea fowl (*Numida meleagris*) (27).

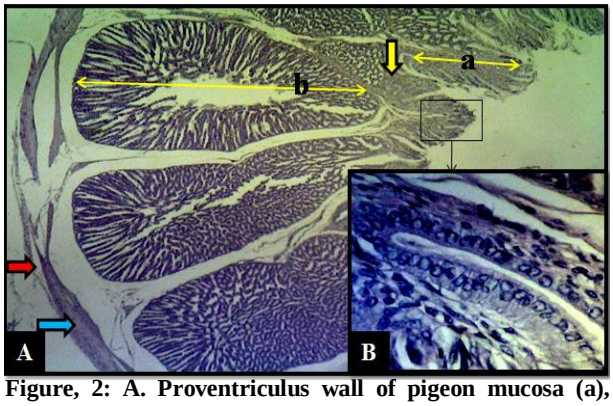
The color and the presence or absence of the cuticle was previously documented in avian species. Indicated a relationship between the cutical and the type of food consumed by the bird. As in the current pigeon the presence of cuticle was similar to other avian species that possessed thick cuticle layer with well-developed muscular stomach. Researchers (35-

38), referred to the thickness of the cuticle which is highly correlated with food consumed. They proposed thick cuticle in granivores and a thin in frugivores. Tunica submucosa was composed of abundant connective tissue containing blood vessels and nerves (Fig. 4 and 5). This outcome in a good agreement with those observed in the Red-Capped Cardinal (*Paroaria gularis gularis*) (39), in most avian species (36) in Rock dove (*Columba livia*) (40) that described connective tissue composition in this tunic. Tunica muscularis appeared as a very thick structure of smooth muscles bundles. In the pigeon, three layers of muscles were distinguished that were thin inner and outer longitudinal and very thick intermediate circular layers (Fig. 5). There were fine collagenous fibers distributed between their bundles. The presence of three layers of muscles fibers was in accordance with the findings of (41) in the Uroloncha domestica, (42) in the Fulica armillata (granivorous species) and (32) in the ventriculus of the red jungle fowl. Conversely, two layers of muscles fibers in the wall of the ventriculus was recorded by (19, 23, 24 and 29) in the same organ of red-capped cardinal, Coot bird (*Fulica atra*) most avian species and owl, respectively.

Histochemically, microscopic examination of the proventriculus in pigeon revealed cells in its surface lining of the mucosal folds strongly positive to PAS as the reaction gave rise dark purple coloration. The observed reaction was with the granules located at the supra-nuclear area of these cells which was an indication of the presence of neutral type of mucin. These findings were almost similar to those observed in the proventriculus of the quail (43). The lamina propria extended between the gastric mucosal glands were moderately reacted with same stain. These findings were comparable to those observed by (26) in the glandular stomach of the black-winged kite (*Elanus caeruleus*). The cells that lined the ducts of the submucosal glands showed in their apical region, PAS positive reaction. The connective tissue and wall of blood vessels of submucosa and serosa give positive reaction with PAS and smooth muscle fiber in muscularis showed poor staining with PAS (Fig. 6A). The ducts cells of the proventricular glands showed also PAS positive reaction in their apical ends, but the secretory cells were negatively stained. Whereas, different findings recorded by (27) in the proventriculus of the Guinea fowl (*Numida meleagris*) in which the submucosal glandular epithelium showed negative reaction for mucins post staining with the PAS. Positive reaction toward PAS by the mucosal folds of the glandular stomach may have a protective role in avoiding the effect of hydrochloric acid on the mucous membrane of the proventriculus (44 and 45). Similar to those found in pigeon, (27) recorded neutral and acid mucins in the mucosal surface of the proventriculus of the guinea fowl. In addition to that previous comments stated that the presence of neutral and acid mucins will aid to protect the mucosal surface and forms a resistant mucosal barrier (46) in the glandular stomach of birds.



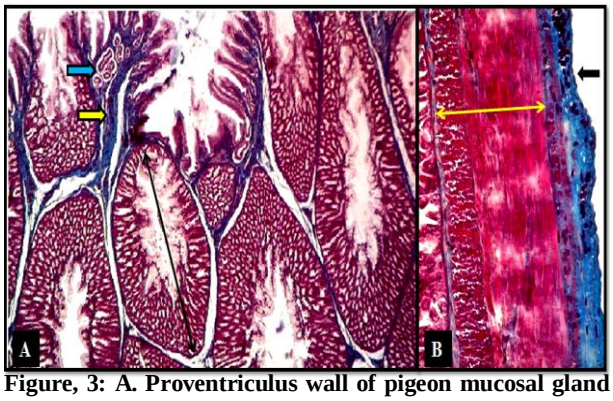
Figure, 1: A. Viscera of pigeon in situ the heart (a), Liver (b), proventriculus (c), ventriculus (d) duodenum (e). B. the isthmus (arrow).



Figure, 2: A. Proventriculus wall of pigeon mucosa (a), submucosa (b), muscularis (blue arrow), serosa (red arrow) and muscularis mucosa (yellow arrow), (H and E, X100) B. epithelial cells in mucosa, (H and E, X400).

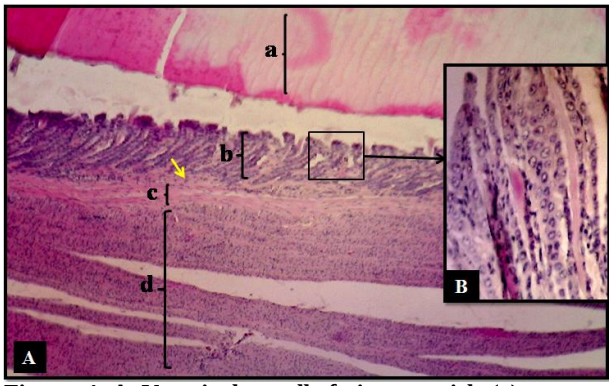
The muscularis mucosa and the walls of the blood vessels in the submucosa showed strong

positive reaction. The tunica muscularis showed mild reaction with the same stain, while the interspersed collagen fibers showed a moderate reaction (Fig. 6B). In the ventriculus (Gizzard), the cuticle covering which was detected in the ventriculus only showed positive reaction to PAS stain (pink color) as it was present above their epithelial lining (Fig. 7A). Cuticle positive reaction with this stain was similarly observed by (47) ducks, (27) Guinea fowl (*Numida meleagris*), (43) quail and (23) in the ventriculus of the black-winged kite (*Elanus caeruleus*).

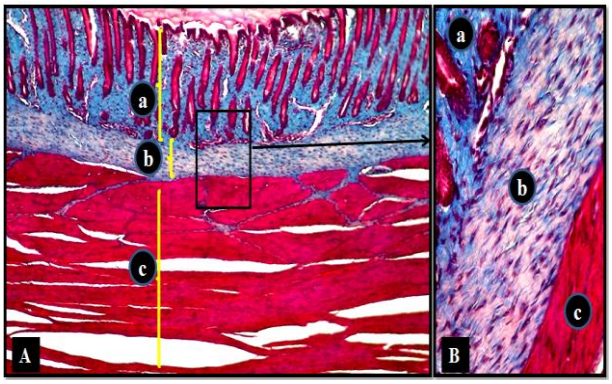


Figure, 3: A. Proventriculus wall of pigeon mucosal gland (blue arrow), connective tissue (yellow arrow) and submucosal gland (double ends arrow), (X100, Masson's Trichrome). B. Higher magnification to both muscularis (double ends arrow) and serosa (black arrow) layers, (X400).

The connective tissue in the lamina propria, submucosa and in tunica muscularis showed PAS positive reaction in ventriculus of pigeon. While the smooth muscles fibers which constructed the tunica muscularis of the organ gave rise mild reaction (Fig. 7A). The mucosal simple cuboidal epithelium lining of the surface and gastric pits of the ventriculus tubular glands were stained strongly positive with both parts of the combined PAS-AB (pH 2.5) stain (Fig. 7B). It indicated the presence of both neutral and acid mucin, respectively. Such observations were similar to those demonstrated by (48) and (41) in the propria glandular cells in the ventriculus of chicken and fowls. The presence of neutral and acid mucin might protect the mucosal surface and forms a resistant mucosal barrier in the ventriculus of the birds (46). In the ventriculus of black-winged kite which is considered one of the meat eater birds, (23) documented, positive reactions with this combined stain in the mucosa and the gastric crypts, ventriculus tubular glands and the secretory material within the lumina of these glands due to the presence of both neutral and acid mucin. The connective tissue gave the positive reaction with PAS and negative with AB, but the smooth muscle bundles of the tunica muscularis reacted weakly with PAS and negatively with AB (Fig. 7B).



Figure, 4: A. Ventriculus wall of pigeon cuticle (a), mucosa (b), submucosa (c), muscularis (d), muscularis mucosa (yellow arrow), (H and E, X100). B. showed higher magnification of epithelial cells in the mucosa, (H and E, X400).



Figure, 5: A. Ventriculus wall of pigeon cuticle (a), mucosa (b), submucosa (c), muscularis (d), (X100, Masson's Trichrome). B. higher magnification of same layers in A, (X400, Masson's Trichrome).

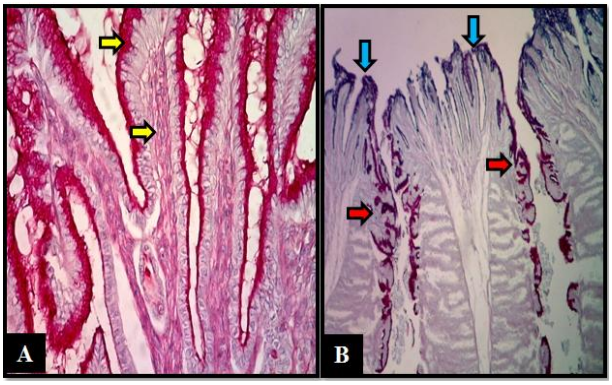


Fig. 6. A. Proventriculus wall epithelia and lamina propria (purple color) (yellow arrows), (PAS, X 400). B. Proventriculus wall neutral (red arrows) and acid mucin (blue arrows), [AB-PAS (pH 2.5), X400].

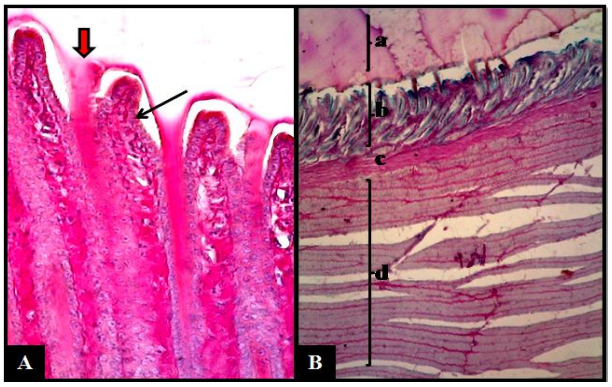


Fig. 7. A. Ventriculus wall cuticle (red arrow) and epithelial lining of the mucosa black arrow), (PAS, X 400). B. Ventriculus wall showed cuticle (a), mucosa (b), submucosa (c) and muscularis (d), neutral (purple color) and acid mucin (blue color), [AB-PAS (pH 2.5), X400].

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دراسة شكلّية-نسجية وكيميائية-نسجية للمعدة في الحمام الزاجل

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الخلاصة

هدف الدراسة هو الوصف الشكلي-النسجي والكيمياء-نسجي لتراكيب جدار المعده في الحمام الزاجل. استعمل اثنا عشر طير، حُصِل عليها من المجهزين المحليين في محافظة بغداد. قسمت الطيور على ثلاث مجاميع متساوية للدراسة العيانيه والنسجية والكيميائيه النسجية. قتلت الطيور بشكل رحيم ومن ثم فتح جدار البطن وإظهار المعدة وتصويرها في موقعها. أخذت نماذج من المعده وتثبيتها بالماده المثبته ومرّرت بالخطوات اللازمه للتصبيغ النسجي والكيمياء-النسجي باستعمال صبغات الهيماتوكسلين-أيوسين والماسون تراي كروم والباس والألشيان الزرقاء ذو حمضية 2.5. تبيّن عيانيا أن معدة الحمام تتكون من غرفتين متميزتين وهما المعدة أالغديه والمعدة العضلية. كانت المعدة الغدية أنبوبية الشكل بينما المعدة العضلية ذات شكل مغزلي ومحاطة بالنسيج الدهني. نسجياً كانت بطانة المعدة الغدية ظهارة عمودية بسيطة. في حين أنّ الطبقة تحت المخاطية كانت تحتوي العديد من الغدد كبيرة الحجم والتي تستوعب معظم جدار المعده الغدية. تبطن المعدة العضلية ظهارة عموديه بسيطه أيضاً. ووجود غدد مخاطية في الصفيحة المخاطية ووجود ألياف عضلية ملساء دائرية للعضلة المخاطية. يتكون جدار المعدة العضلية من ثلاث طبقات من الألياف العضلية الملساء وهي طبقتان داخلية رفيعة وخارجية طولية واخرى وسطية ثخينه دائرية.

الكلمات المفتاحية: الحمام، المعدة الغدية، المعده العضلية، الكيمياء النسجية.