

Streptopelia senegalensis and White-breasted Kingfisher, Halcyon smyrnensis

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Abstract

The aim of the current work was to study the histological structures of glandular stomach, the proventriculus and compare it in two types of Iraqi wild birds according to differences in diets. The birds are, Laughing dove, *Streptopelia senegalensis* which comprised the grainivorous bird and white breasted Kingfisher *Halcyon smyrnensis* which comprised the carnivorous bird. The results exhibited that the wall of the stomach (proventriculus) of two birds consists of the four layers of typical tubular organ: tunica mucosa, submucosa, muscularis and serosa. The tunica mucosa of two birds consisted of three layers, epithelium, lamina properia and muscularis , the free surface of two birds is covered with circular plicae modified from of the mucosa, The surface lining cells of this folds in both of birds is simple columnar with clear cytoplasm. The lamina propria is constituted by connective tissue with blood vessels and lymphocitary infiltration. As well as simple tubular glands. Notice that these glands are lining by simple columnar in white breasted kingfisher while simple cuboidal to columnar in laughing dove. Muscularis mucosa was a smooth muscle fibers, appears scattered along the lamina propria in laughing dove surrounds the apical part of deep proventricular glands, and extend inside the proventricular folds and entirely around the deep proventricular glands in white breasted kingfisher. Submucosa layer occupy most of the real area of the proventriculus wall containing numerous deep proventricular glands that occupy great part of submucosa. This layer is thicker in kingfisher as compared with laughing dove. Each secretory region is a well-defined unit and enclosed by a connective tissue capsule contains bloods vessels and nerves. These gland are compound tubular branched glands, each gland consists of numerous of secretory tubules. These tubule leads to ducts and open into central collecting duct that opens in the proventricular lumen. The secretory tubules of deep glands lining by simple cuboidal cells represent chief cells that secreting hydrochloric acid and pepsinogen. the tunica muscular (tunica muscularis) of two birds consist of two layers, inner circular muscle layer and outer longitudinal muscle layer, inner layer is thicker in kingfisher as compared to laughing dove. Between these two muscular layers, blood vessels and nervous plexus (Auerbach) are found. The tunica serosa consisted of connective tissue contains nerves, blood vessels and ganglionic cells of serous plexus.

دراسة نسيجية مقارنة للمعدة (الجزء الغدي) بين الحمام الضاحك *Streptopelia senegalensis* و الرفراف ابيض

الصدر *Halcyon smyrnensis*

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الخلاصة

يستهدف البحث اجراء دراسة نسيجية مقارنة للمعدة (الجزء الغدي) في نوعين من الطيور البرية العراقية تتبع الأختلاف في نوع الغذاء ، احدهما طائر فاختة النخيل او مايسمى باليمام الضاحك *Streptopelia senegalensis* حيث يعد من الطيور آكلات الحبوب والآخر طائر الرفراف ابيض الصدر *Halcyon smyrnensis* وهو من الطيور آكلات اللحوم. اظهرت النتائج ان جدار المعدة الغدية في كلا الطائرين يتكون من الطبقات الاربع التي يتكون منها العضو الانبوي النموذجي وتلك الطبقات هي : الطبقة المخاطية، تحت المخاطية، العضلية والمصلية ، وجد ان الطبقة المخاطية لكلا الطائرين تتكون

من الظهارة ، الصفيحة الأصيلية والعضلية المخاطية ، وان السطح الحر للخلايا الطلائية في كلا الطائرين مغطى بطيات دائرية Plicae circulars متحورة عن الطبقة المخاطية، حيث تُبطن هذه الطيات في كلا الطائرين بواسطة خلايا طلائية عمودية ذات سايتوبلازم رائق او شفاف. اما الصفيحة الأصيلية فتكونت من نسيج ضام يحتوي على أوعية دموية ومواد لمفية مترشحة، فضلا عن وجود عدد انبوية بسيطة. لوحظ ان هذه الغدد مبطنه بخلايا عمودية في طائر الرفراف اما في طائر الفاخنة وجد انها تبطن بواسطة خلايا مكعبة الى عمودية. اما بالنسبة للطبقة العضلية المخاطية اظهرت النتائج انها عبارة عن الياف عضلية ملساء تظهر بصورة مُتقطعة على طول الصفيحة الاصيلية تحيط الجزء القمي للغدد تحت المخاطية العميقة لطائر فاخنة النخيل ، بينما تمتد داخل طيات المعدة الغدية وتحيط بالغدد تحت المخاطية العميقة تقريبا بصورة كاملة في طائر الرفراف . شغلت الطبقة تحت المخاطية اغلب مساحه جدار المعدة الغدية واحتوت على عدد كبير من الغدد العميقة التي تحتل جزء كبير من هذه الطبقة. اظهر الوصف النسيجي ان هذه الطبقة تكون سميكة في طائر الرفراف مقارنة بطائر فاخنة النخيل. وان كل غدة عميقة تمثل منطقة محددة محاطة بمحفظة من نسيج ضام يحتوي على اوعية دموية واعصاب. تكون هذه الغدد من النوع المركب الانبوي المتفرع وتكونت كل غدة في نوعي الطيور من ثنبيات افرازية عديده تؤدي الى قنوات اخرى لتفتح في قناه مركزية جامعه تُفتح في تجويف المعدة الغدية. تبطن هذه الثنبيات بخلايا مكعبة بسيطة تمثل الخلايا الرئيسية التي تفرز حامض الهيدروكلوريك وانزيم الببسين . اما الطبقة العضلية فلوحظ انها تتكون من طبقتين من الالياف العضلية تترتب الداخلية منها بصورة دائرية اما الخارجية فتترتب بصورة طولية. واتصفت الطبقة الداخلية في طائر الرفراف بكونها اسمك مقارنة بطائر فاخنة النخيل ، ولوحظ وجود أوعية دموية وظيفية اوربخ العصبية بين هاتين الطبقتين. اما الطبقة الخارجية المصلية فظهرت مكونه من نسيج ضام يحتوي اوعية دمويه واعصاب وخلايا عصبية للضفيرة المصلية.

Introduction

Animal's diet may change dramatically due to changing food availability, or differing requirements for energetically demanding reproduction or moult (Moore & Battley, 2006). Digestive system and gut morphology show considerable variation among animals, depending on phylogenetics, diet quality, the size of the animal and differing environmental pressures (Davis, 2007). The most active part of the digestive system of birds is a stomach which is formed of two distant parts; proventriculus or true stomach and the muscular portion ventriculus or gizzard (Ahmed *et al.*, 2011). Histologically the wall of the stomach (proventriculus and gizzard) is constituted by the following layers: tunica mucosa, submucosa, muscular and tunica serosa (Catroxo *et al.*, 1997). The proventriculus serves as the glandular stomach of birds (Wideman *et al.*, 1996) which presents an elliptical shape, relatively thick-walled structure located at the distal end of the oesophagus. The tunica mucosa of proventriculus presents folds (plicae) in its luminae surface. The lamina propria is constituted by connective tissue with blood vessels and lymphocitary infiltration. In its interior, simple tubular glands are found. The submucosa is constituted by connective tissue and deep proventricular glands are located in it (Catroxo *et al.*, 1997). Tunica muscularis is slightly modified and the inner longitudinal, circular and outer longitudinal layers are found, the tunica serosa is composed by connective tissue and cuboidal cells layer (Rossi *et al.*, 2005). The proventriculus varies in size

between species, being relatively small in graminivores, but often quite large and distensible in carnivores that ingest large food items and in ostriches use it for water storage (Duke, 1997). So the aim of this work was to investigate and comparative the histological differences of stomach (proventriculus) in two species of Iraqi wild birds, Laughing dove, *Streptopelia senegalensis*, belongs to family Columbidae, (Schleucher, 2001) with distribution extends across Africa (Hockey *et al.*, 2005), and found locally in Palestine, Syria, Lebanon, Egypt and Iraq (Lahony *et al.*, 2008). This species is a generalist granivorous and consider an important model for physiological and behavior studies (Johnson *et al.*, 2001). The other bird, White-breasted Kingfisher *Halcyon smyrnensis* (Aves: Coraciiformes) which is a common species of a variety of habitats, mostly open country in the plains with trees, electric wires. It is the most common carnivorous (Ali & Asokan, 2010) which feed upon fish, grasshoppers and mice, also upon large insects, crustaceans forms a major part of their diet (Soud *et al.*, 2010). This species is found in Turkey, Palestine, Egypt, Iraq, Afghanistan.

Materials and Methods

Birds and feeding

Twenty healthy birds laughing dove (*Streptopelia senegalensis*) and white breasted kingfisher (*Halcyon smyrnensis*) (males and females) were captured in the city of Kut, Iraq (ten birds from each species) and kept in individual cages, for a short period. They were fed

with grains, insects and fishes similar to those existent in the area of capture. The birds were prepared for histological study.

Processing for light microscopy

Twenty healthy birds were collected and body weight was recorded. The birds were anesthetized with chloroform inhalation. Anatomical observation (weight, position and dimensions) of stomach for each species was recorded. For the histological study, the digestive tube was exposed and fragments of stomach (proventriculus) were immediately fixed in 10% buffer neutral formalin for 72 hours. The specimens were dehydrated in ascending grades of ethyl alcohol and clearing in xylol, then embedded in paraffin wax, sectioned at (5-6 μm) thickness and mounted, then stained by haematoxylin-eosin stain for general examination (Bancroft & Stevens, 1982). Later, histologic sections were examined in light microscope, analyzed and described histologically.

Results

The stomach of birds consists of a glandular proventriculus and muscular ventriculus. The glandular portion is a fusiform organ lying dorsal to the liver between the esophagus and the gizzard. The histological analysis, under light microscopy, revealed that the wall of the stomach (proventriculus) of two birds laughing dove (*Streptopelia senegalensis*) and white breasted kingfisher (*Halcyon smyrnensis*) has all the four layers of typical tubular organ: tunica mucosa, submucosa, tunica muscularis and tunica serosa (fig. 1, 2). The tunica mucosa of two birds consisted of three layers, epithelium, lamina propria, and Muscularis mucosa as in other birds; the free surface of two birds is covered with circular plica of the mucosa (fig. 2). The surface lining cells of this folds in both of birds is simple columnar with clear cytoplasm (fig. 3). The lamina propria is constituted by connective tissue with blood vessels and lymphocitary infiltration. And extend inside the folds. Simple tubular glands are found in this propria (fig. 4, 5). In kingfisher these glands are lining by simple columnar epithelium and opened in the bases of mucosa folds (fig. 4), furthermore they were numerous, larger and extend deeper in the lamina propria of kingfisher as compared to laughing dove in which they were lining with simple cuboidal to columnar epithelium and distributed in the apical part

of lamina propria(fig.5).Muscularis mucosa is a smooth muscle fibers, Appears scattered along the lamina propria in laughing dove and surrounds the apical part of deep proventricular glands(fig. 1) , While in kingfisher appears as smooth muscle layer extend inside the proventricular folds and entirely around the deep proventricular glands that lies beneath the muscularis mucosa (fig. 2). The second tunic, submucosa was constituted by connective tissue, containing blood vessels, This tunic occupy most of the real area of the proventriculus wall containing numerous deep proventricular glands that occupy great part of submucosa(fig1,2). Each secretory region is a well-defined unit and enclosed by a connective tissue capsule contains bloods vessels and nerves, which separate glands from each other to outlines and defines the limit of gland. Deep proventriculus gland are compound tubular branched glands with secretory tubules(fig. 6, 7), This glands appears as conical shape in laughing dove (fig. 8), and long cylindrical shape in white breasted kingfisher(fig.9). Each gland consists of numerous of secretory tubules. These tubule leads to ducts and open into central collecting duct that opens in the proventricular lumen (fig. 6, 7). The secretory tubules of deep glands lining by simple cuboidal cells present chief cells that secreting hydrochloric acid and pepsinogen. The collecting duct of deep glands open inside the gastric lumen through main duct lining by simple columnar cells with clear cytoplasm in white breasted kingfisher (fig.7) while open directly into stomach in laughing dove(fig.6). The tunica muscularis of two birds consist of two layers, inner circular and outer longitudinal. The result shows that inner layer is thicker than outer in kingfisher (fig. 11). While in laughing dove, the outer layer is thicker than the inner (fig. 10). Between these two muscular layers, blood vessels and nervous plexus (Auerbach) are found. The tunica serosa is consist of connective tissue contains nerves, blood vessels and ganglionic cells of serous plexus (fig. 9).

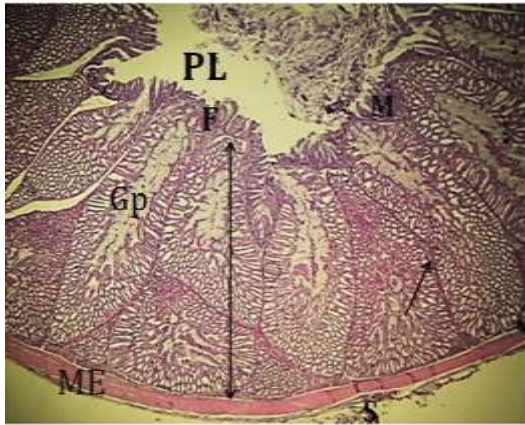


Fig 1: Photomicrography of the cross section of laughing dove proventriculus wall: Proventriculus lumen (PL), folds of mucosa (F), mucosa (M), deep proventricular glands (GP), capsule of gland (arrow), submucosa (Sm), muscularis externa (ME), serosa(S), Hematoxylin and Eosin stain (40x)

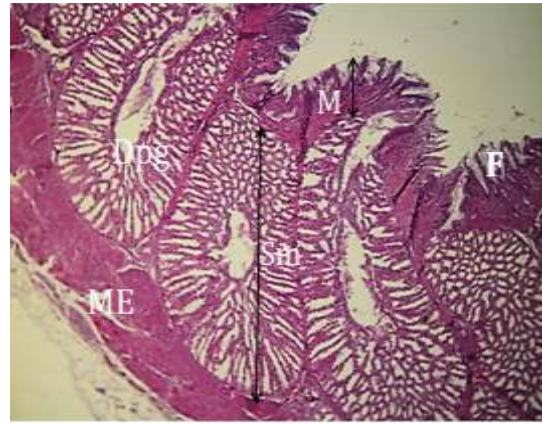


Fig2: Longitudinal section of white breasted kingfisher proventriculus wall: mucosa layer (M), submucosa layer (Sm), deep proventriculus glands of submucosa (Dpg), muscularis externa (ME) , H&E stain, (100X)

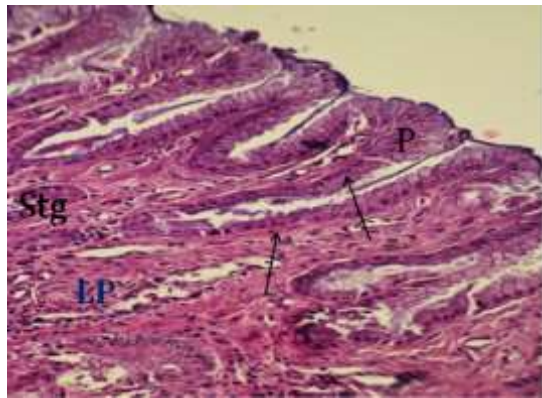


Fig 3: Photomicrography of the longitudinal section of mucosa in white breasted kingfisher show the mucosal folds, Plica, (P), surface lining cells (arrow), simple tubular glands (Stg), lamina propria (Lp), H&E stain, 400x

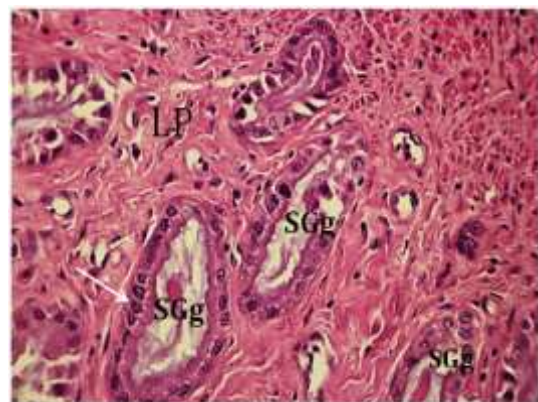


Fig 4: Photomicrography show the simple tubular glands of mucosa proventriculus in white breasted kingfisher, lamina propria (LP), superficial gastric glands (SGg), nucleus (arrow), H&E stain, 400 x



Fig 5: Photomicrography of the cross section of mucosa in laughing dove show the mucosal plica (P), surface lining cells (SLc), simple tubular glands (arrow), proventriculus lumen, H & E stain, 100 x

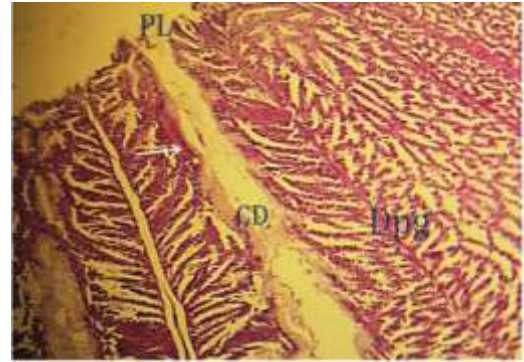


Fig 6: Photomicrography of the central duct of laughing dove: proventriculus lumen (PL), lining columnar cells of central duct (arrow), central duct (CD), deep proventriculus glands (Dpg), H&E stain, 100X

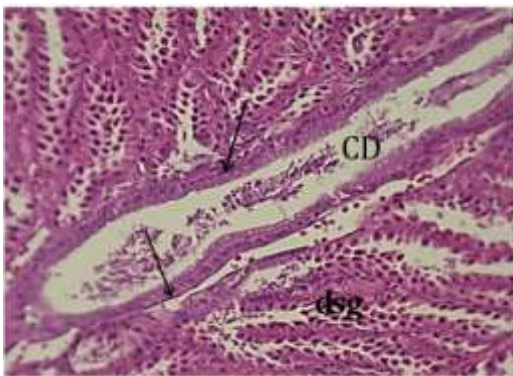


Fig 7: Photomicrography of the longitudinal section of submucosal gland in white breasted kingfisher show the central duct (CD), of deep gland (dsg), lining columnar cells of central duct (arrow), H&E stain, 400X.

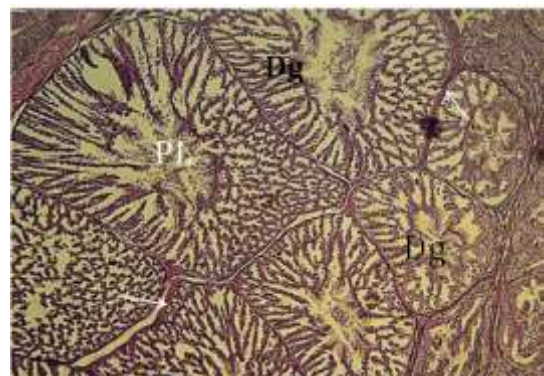


Fig8: Transverse section show the submucosal glands of laughing dove: deep proventriculus glands (Dg), connective tissue capsule (arrow), proventriculus lumen (PL), H&E stain 40 X.

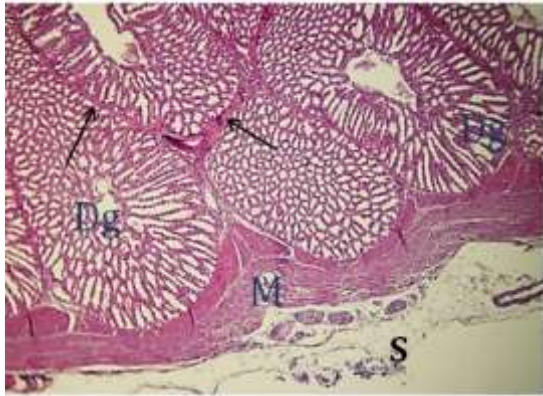


Fig9:Longitudinal section show the submucosal glands of white breasted kingfisher: deep proventriculus glands (Dg), connective tissue capsule (arrow), tunica muscularis (M), serosa (S),H&E stain, 100X

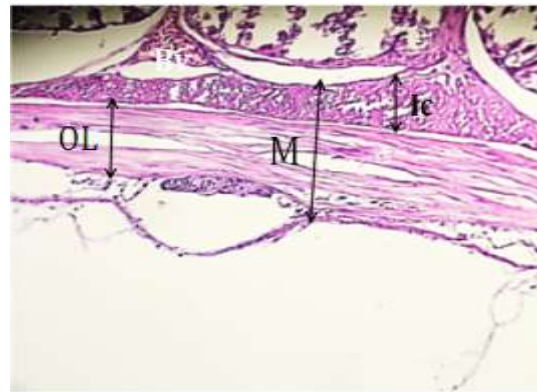


Fig 10: Photomicrography of longitudinal section of the tunica muscularis in laughing dove: total tunica muscularis (M), inner circular layer (Ic), outer longitudinal layer (OL), blood vessel (Bv), H&E stain, 400 X

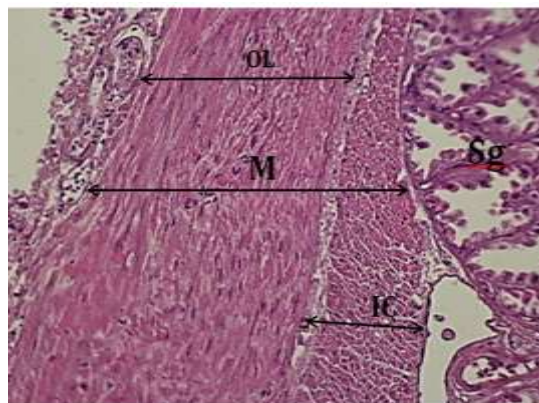


Fig 11: Longitudinal section of tunica muscularis in kingfisher: deep submucosal glands (Sg), total tunica muscularis (M), inner circular layer (Ic), outer longitudinal layer (OL), H&E , 400X

Discussion

The analysis of histological sections, under light microscopy, shows that the wall of the proventriculus of two birds is consisted of four layers: tunica mucosa, submucosa, tunica muscularis and tunica serosa, The same finding was observed by (Catroxo *et al.* , 1997) on red-capped cardinal , (Rossi *et al.*, 2005 ; Illanes *et al.*, 2006) on Partridge and *Struthio camelus*

respectively , (Selven *et al.*, 2008) on Guinea fowl, (Tadjalli *et al.*, 2011) on Ostrich, but disagree with (Marshall& Folley, 1965) who considered the existence of only three tunics. The present work showed that the tunica mucosa of two birds consisted of three layers, epithelium, lamina properia, and muscularis mucosa as in other birds; the mucosa of the proventriculus was thrown into folds of varying height termed plicae circulars. This finding was in agreement with the results of Rocha and Lima, (1998); Catroxo *et al.*, (1997) and (Ahmed *et al.*, (2011) who mentioned the presence of

these folds. The surface lining cells of proventriculus folds was simple cuboidal to columnar with clear cytoplasm. As revealed by Rocha and Lima, 1998; Selven *et al.*, 2008) this cell secrete mucus. This mucous secretion acts as a protective lining for the surface of epithelium from the action of acids (Bacha and Bacha, 2000). The lamina propria is constituted by connective tissue with blood vessels and lymphocitary infiltration. In its interior, simple tubular glands are found. These findings agree with many previous studies such as Rocha and Lima, (1998); Catroxo *et al.*, (1997); Rossi *et al.*, (2005) that confirm the presence of these glands. In white breasted kingfisher these glands are lining by simple columnar epithelium similar histological observations mentioned by Catroxo *et al.*, (1997) on their study on red-capped cardinal and Alsheshani,(2006) in his study on carnivorous bird, *Accipiter nisus*, while lining by simple short columnar to cuboidal in laughing dove and opens in the bases of mucosa folds, This finding is in agreement with Alsheshani, (2006) in his work on *Columba Livia*, Some authors Rocha and Lima, (1998); Farner, (1960) mention that the lining cells of these glands are simple cuboidal. The histological analysis showed that these glands that are numerous, larger and extend deeper in the lamina propria as compared to laughing dove, where distributed in the apical part of lamina propria. Beneath lamina propria, as revealed in present study isolated smooth muscle of muscularis mucosa was found as scattered fibers along the lamina propria in laughing dove and surrounds the apical part of deep proventricular glands, while in white breasted kingfisher appeared more clearly as smooth muscle layer extend inside the proventricular folds and entirely around the deep proventricular glands that lies beneath the muscularis mucosa. This finding is in agreement with El-Banhawy *et al.*, (1993); Alsheshani, (2006), who confirmed the presence of muscularis mucosa. The tunica muscularis is composed of two muscle fibers layers as mentioned by Rocha & Lima, (1998), Rossi *et al.*, (2005); Attia, (2008); Ahmed *et al.*, (2011), Albideri *et al.*, (2011) on the other hands Banks, (1992), Catroxo *et al.*, (1997); El-Banhawy *et al.*,(1993) who mentioned the presence of three layers of muscle fibers. The inner layer was arranged circularly, where the outer layer have longitudinal disposition in both of birds as described by Rocha & Lima,(1998); Ahmed *et al.*,(2011) , the histological analysis showed that the inner layer in kingfisher is thicker than the outer while it was reverse observe in the laughing dove . Between these two muscular layers, blood vessels and

nervous plexus (Auerbach) are found. The tunica serosa in both of birds was consisted of connective tissue contains nerves, blood vessels and ganglionic cells of serous plexus as found in previous studies Rocha and Lima,(1998); Catroxo *et al.*,(1997). It is concluded that the kind of diet reflects some histological differences in the glandular stomach of the two studied birds appears in the thickness of the layers that composed the glandular stomach.

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