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Comparative Anatomical, Histological, and Electron Microscopical Studies on the Cervical Region of the Esophagus in Some Birds with Different Diet Habits

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Abstract

The esophagus in bird is thin and distensible tube that extends from oropharynx to the stomach, it is divided anatomically into three sections: anterior (cervical), middle (crop) and posterior (thoracic) regions. The present work aimed to study the morphological, morphometrical, histological and scanning of the cervical region of the esophagus in three different birds (kestrel, hoopoe, rock pigeon). The current study used samples from different three species, the birds scarified, and the esophagus were dissected. The cervical part of the esophagus was analyzed grossly and by using the light and scanning electron microscope. The cervical part of the esophagus in rock pigeon is longer than the kestrel and hoopoe and its width in the kestrel is narrower than the hoopoe and rock pigeon. In lamina propria of kestrel and hoopoe had numerous mucous glands, which are rounded, branched tubular glands surrounded by a fibrous capsule. These glands open into the esophageal lumen through ducts passing within the lining epithelium. However, the lamina propria of the cervical part of the esophagus in rock pigeon was lacking the glands. The obtained results demonstrated that the type of food consumed, is greatly affect the anatomical and the histological structure of the esophagus.

KEYWORDS
Kestrel, Hoopoe, Rock pigeon, Esophagus

INTRODUCTION

The birds have different feed habits corresponding to their habitats. So, about the type of food consumed, the avian species can be classified into seed-eating birds (granivorous) as the dove, common quail, and home pigeons (O'Farrill *et al*., 2013). The omnivorous species include seeds, insects, and fruits in their diets as ducks, rock pigeons, and gulls (Rodenburg *et al*., 2005). The carnivorous birds have consumed insects, frogs, and fish (Hussein and Rezk, 2016). The alimentary tract of the bird is a canal starting from the beak and ending with the anus (King and McLelland, 1984).The avian esophagus has a thin wall and distensible tube that extends from oropharynx to the stomach, it is divided anatomically into three sections; anterior (cervical), middle (crop) and posterior (thoracic) regions (Hodges, 1974). Its cervical region has a role in food lubrication and differs from that of mammals. It is located on the right side of the neck (King and McLelland, 1984). The alimentary canal in birds showed physiological and morphological discrepancies in relation to food habits (Kadhim *et al*., 2011). Kestrel (*Falco tinnunculus*) is a carnivorous bird whose diet depends mainly on rodents and insects (Riegert *et al*., 2009). The digestive system of carnivorous birds supplies the nutrition required for hunting and flight (Alsanosy *et al*., 2021). Hoopoe (*Upupa epops*) is a hole-nesting bird. Its diet is insects and some plant materials as seeds and berries (Kristin, 2001). Generally, the esophageal wall is divided into four layers tunica (mucosa, submucosa, muscularis, and adventitia) (Alsanosy *et al*., 2021). There is a lack of data about the structural adaptation of the esophagus between the different birds according to the nature of diet habits. So, the present investigation aimed to study the morphological, morphometrical, histological and scanning electron microscope (SEM) of the cervical region of the esophagus in three different food habits of birds to give a detailed explanation of how the esophageal structure accommodates several types of food.

MATERIALS AND METHODS

Samples collection

In this investigation, 30 specimens were conducted on ten birds of each species (kestrel, hoopoe, and rock pigeon) collected from the Iraqi city of Erbil. The birds were collected from bird cage stores. Sample collection was done according to the guidelines of the Institutional Ethical Committee of Salahaddin University, Erbil, Iraq and following the Iraq Animals' laws.

Light microscopy

Each specimen was removed directly after slaughter and washed with normal saline. Each order's cervical region of the esophagus is then cleaned of waste and fixed in 10% neutral, buffer formalin for histology processing. During the fixing process, the tissue was dehydrated by passing through a series of

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alcohols with increasing concentrations, cleaned with xylene, and embedded in paraffin. Sections of 5–8 µm in thickness. The sections were stained by Harris's Hematoxylin and Eosin (H&E) staining for histology characteristics. Periodic Acid Schiff (PAS) for mucopolysaccharides (neutral mucins), Masson's trichrome for connective tissue (C.T), and Alcian blue (pH 2.5) for acidic mucins. (Bancroft *et al*, 2013). Photomicrographs of the sections was done by using a digital microscope LECA DM 2700 P.

Scanning electron microscopy

Specimens in the cervical region of esophagus for a scanning electron microscope (SEM) were fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.3. Then, the specimens were washed several times in phosphate buffer for 10 minutes each, and then fixed in 1% buffered osmium tetroxide for 2 hours at 4°C. Finally, the specimens were washed in phosphate buffer, pH 7.3 (three times). Then, they were sputtered with gold, and a JEOL, JSM 5200 scanning electron microscope at Egypt Mansoura University Faculty of Agriculture, was used to take pictures of them.

Statistical analysis

The data were investigated using SPSS software. The mean and standard deviation are used to express the results of the numerical data.

RESULTS

Gross (macroscopic) morphological features

Esophagus was a tubular muscular organ divided into three regions in some birds, represented by cervical, crop, and thoracic, beginning from the posterior end of the oropharynx and attached to the trachea, respectively. The food movement from the esophagus to the stomach (proventriculus and ventriculus).

The morphological study of the present work showed that the esophagus in the kestrel was divided into the same three regions, similar to rock pigeon (Fig. 1 A, C), but in the hoopoe, the crop region was lacking (Fig. 1 B). While the esophageal regions displayed some differences in shape and size among the birds in the study. The crop region in kestrel was fusiform in shape, but in Hoopoe (*Upupa epops*), it was smaller or nearly indistinct. In common rock pigeon, the crop was well developed, and it consisted of two lobes.

The esophagus in rock pigeon is longer than the kestrel and hoopoe but with a very thin wall and leads into a small proventriculus. The means the total length of the esophagus in kestrel was (6.18 cm), the range of length (5.65-6.65), in hoopoe was (7.91 cm), the range of length (7.2-8.3 cm), in pigeon, was (12.84 cm), the range of length (12.25-13.40).

The first esophageal region is a cervical part, which begins at the anterior part of the neck to the anterior end of crop, and it occurs dorsally to the trachea. It is considered a connection between the oropharynx and the crop.

The length of the cervical region in the kestrel was shorter than that of the hoopoe, and rock pigeon and the width in the kestrel is narrower than the hoopoe and rock pigeon.

The mean of length of the cervical region in the kestrel was (1.84 cm), the range of length was (1.5 – 2.1 cm), the mean diameter (0.489 µm), and the range of diameter was (0.45– 0.51 µm).

The mean length of the cervical region in hoopoe was (2.53 cm), the range of length (2-3 cm), the mean diameter was (0.75 cm), and the range of diameter was (0.68-0.80 cm).

The mean length of the cervical region in rock pigeons was (4.96 cm), the range of length was (4.50-5.25 cm), the mean diameter was (0.795 cm), and the range of diameter was (0.70-0.90 cm).

Histological and histochemical studies

The histological observations of the esophagus were not the same between the different regions of the esophagus. There were no differences between males and females in histological

Fig. 1. A photograph of the ventral view of the dissected alimentary canal of Kestrel (A), Hoopoe (B)and Rock Pigeon (C). Notice, CE: cervical part of the esophagus, CR: crop, TH: thoracic part of esophagus.

features of the esophagus in kestrel, hoopoe, and rock pigeon.

The esophageal wall in kestrel, hoopoe and rock pigeon was composed of four concentrical layers arranged from inside to outside: mucosa, submucosa, muscularis extrena and adventitia (Fig. 2). The mucosa was assembled in longitudinal folds.

The lamina epithelialis mucosae of the cervical region consisted of keratinized stratified squamous epithelium in kestrel and pigeon (Fig. 2), which gave the epithelial papillae wave-like shape. But in hoopoe, the epithelium was non-keratinized stratified squamous epithelium (Fig. 2C), and epithelial papillae give raise finger-like consists of numerous folds arranged in longitudinal folds. The mucosal folds become elongated, leaving only a narrow lumen.

The epithelial cell in the three birds have oval nuclei, and granular cytoplasm. These cells and their nuclei contain flatted progressively as they migrate toward the dorsal free surface of the epithelium (Fig. 3). The lining epithelium in kestrel and pigeon was thicker than that of the hoopoe.

The lamina propria (LP) in the three birds was represented by connective tissue containing blood vessels (BV) and nerve fibers (NV). In LP of kestrel and hoopoe, numerous mucous glands are round, branched tubular glands surrounded by a fibrous capsule. These glands are opened into the esophageal lumen through ducts passing within the lining epithelium.

In kestrel, LP consists of dense connective tissue which binds together, forming bundles of collagen fibers, and simple branched alveolar mucus-secreting glands (Fig.4A), the gland covered by CT thin membrane. In hoopoe LP consisted of many collagens' fiber and numerous simple tubular mucus-secreting glands covered by CT thin membrane (Fig.4B). Still, pigeon LP had few collagen fibers and was devoid of mucosal glands (Fig.4C). The mucosal glands in kestrel and hoopoe gave a strong Alcian and PAS positive reaction. These glands in kestrel were larger and contained more Alcian (Fig. 5 A-D) and PAS (Fig. 6 A-D) positive secretory materials than in hoopoe. The muscularis mucosa was composed of thin layers of smooth muscle fibers.

Submucosa, the second layer in kestrel was thick and consists of loose connective tissue and contained large arteries, small veins and nerve fibers (Fig. 2 A & B). In rock pigeon was thinner than kestrel and hoopoe consists of loose connective tissue was

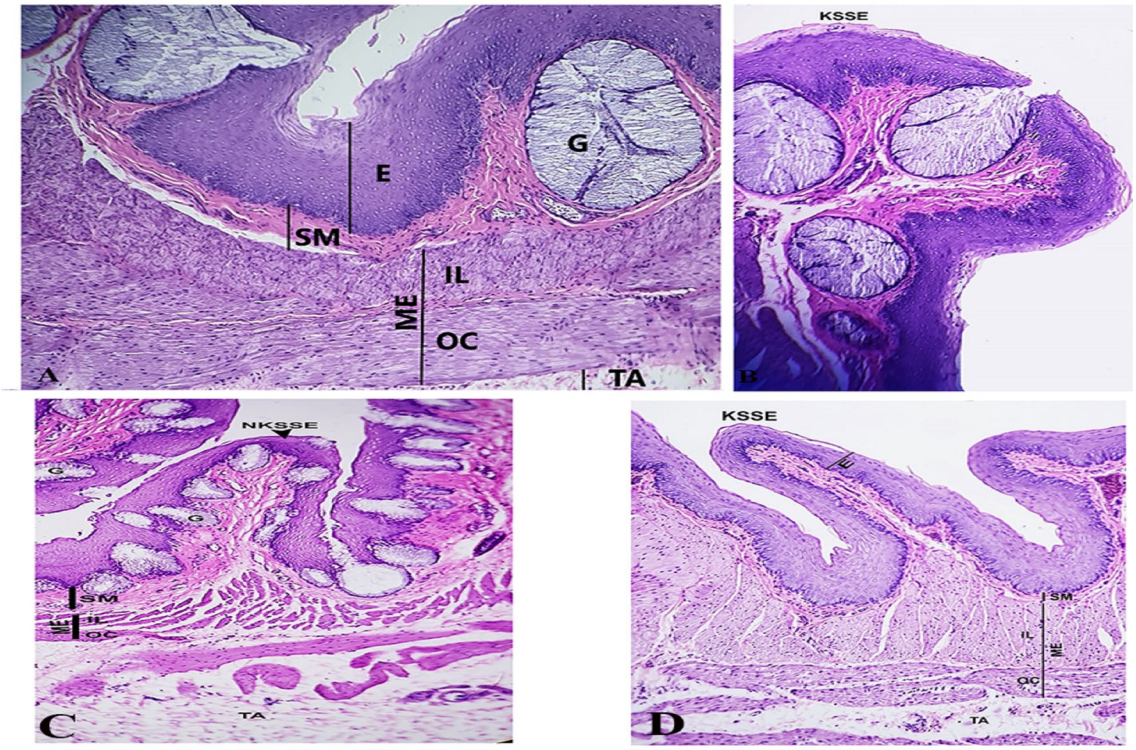

Fig. 2. A: Cross section of the cervical region stained by H&E in Kestrel showed it consisted of four layers: M: mucosa,SM: submucosa, ME: muscularis extrena (IL: inner longitudinal and OC: outer circular) and TA: tunica adventitia. B: The epithelium of the mucosa is keratinized squamous epithelial tissue (KSSE). 100x. C: In hoopoe showed it consisted of four layers: M: mucosa, SM: submucosa, ME: muscularis extrena (IL: inner longitudinal and OC: outer circular) and TA: tunica adventitia. The epithelial cell of the mucosa is nonkeratinized squamous epithelial tissue (NKSSE). 100x. D: In pigeon, M: mucosa, SM: submucosa, ME: muscularis extrena (IL: inner longitudinal and OC: outer circular) and TA: tunica adventitia. The epithelial cell of mucosa is keratinized squamous epithelial tissue (KSSE) 100x

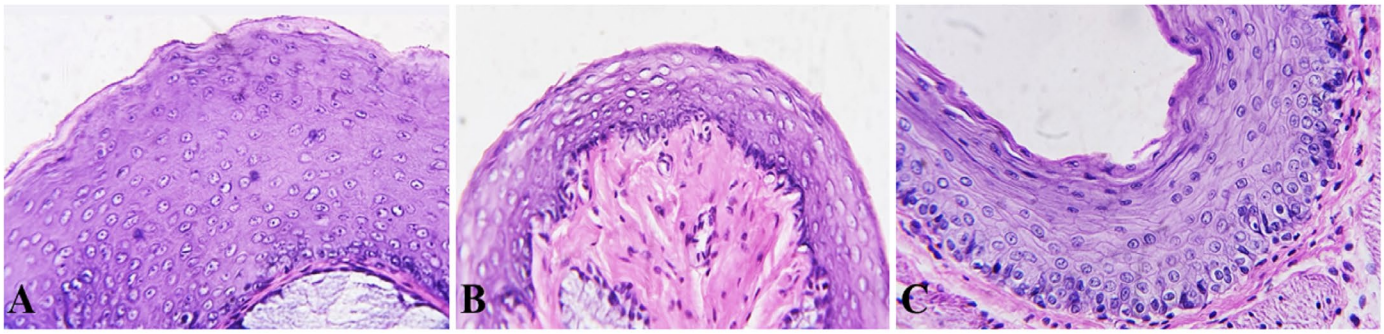

Fig. 3. Higher magnification of the of epithelium of the esophageal cervical region in Kestrel (A), hoopoe (B) and rock pigeon (C) showed it was keratinized squamous epithelial tissue KSSE in kestrel (A) and pigeon (B) but non keratinized squamous epithelial tissue (NKSSE) in hoopoe (C).

associated with submucosal plexus, nerve fibers, blood vessels, fibroblasts, fine reticular, elastic, and collagen fibers (Fig. 2 C&D).

The staining affinity against bromophenol stain differed in esophageal layers according to the protein contents. Lamina propria revealed deeply stained protein materials in the three birds, also in muscular externa layers (IL, OC) in kestrel and hoopoe but not in rock pigeon. The mucosal glands (G) of kestrel and hoopoe demonstrated no staining (Fig. 7).

Muscularis externa consists of two layers of smooth muscle, separated by connective tissue inner longitudinal and an outer circular layer in all studied birds. In kestrel inner longitudinal muscle is thinner than the outer circular fibers (Fig.8 A), and in

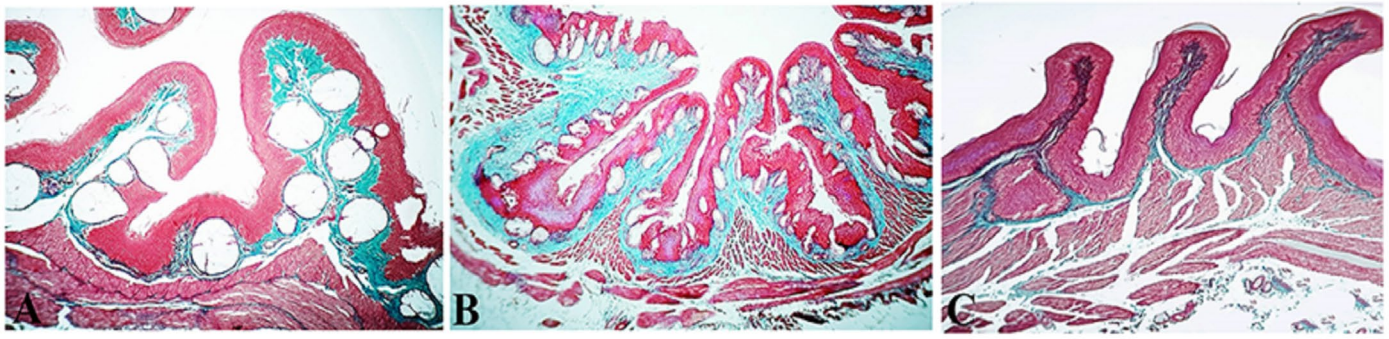

Fig. 4. Cross section of cervical region stained by Masson trichome in Kestrel (A), hoopoe (B) and pigeon (C) showed of the connective tissue of the lamina propria and that between the muscularis externa-stained green color. 40x.

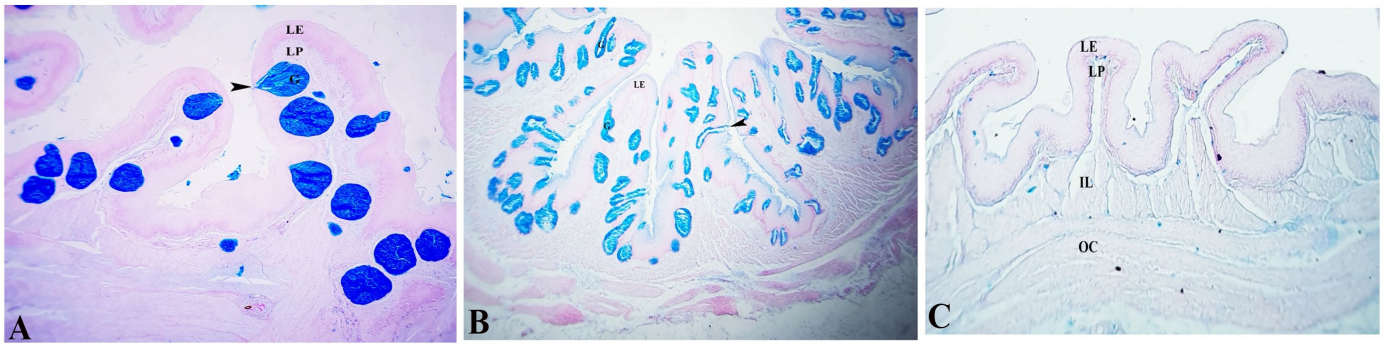

Fig. 5. Cross section of cervical region stained by Alcian blue in kestrel (A), hoopoe (B) and pigeon (C) showed; A: many and large mucosal gland (G) within the cervical region mucosa which exhibited strongly positive reaction against the AB-stain. 10x. B: in hoopoe showed many but smaller mucosal gland (G) within the mucosa of the cervical region which exhibited positive reaction against the AB-stain. Notice, the opening of the gland (arrowhead) within the lamina epithelialis (LE) 4x. C: in pigeon, lack of the mucosal glands. Notice, LE (lamina epithelialis), LP (lamina propria), IL (inner longitudinal of muscularis externa) and OC (outer circular of muscularis externa),4x

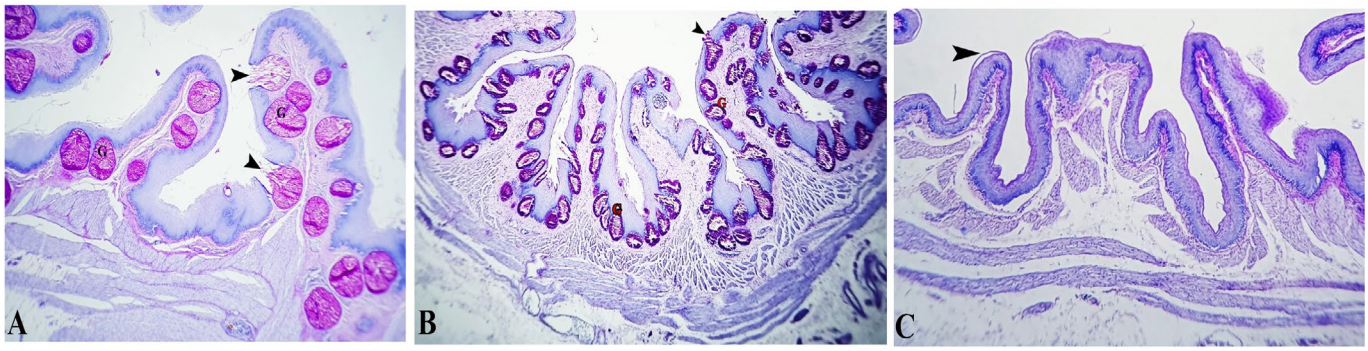

Fig. 6. Cross section of cervical region folds in; A: kestrel showed the secretory material of the gland (G) displayed strong PAS reaction. Notice, the gland empties its content within the lumen (arrowhead).4x. B: hoopoe showed the esophageal folds contained numerous glands (G) which contain PAS positive secretory materials. 4 X. C: rock pigeon showed the esophageal folds lack of the mucosal glands, the cornified layer of the epithelium exhibited positive PAS reaction. 4x

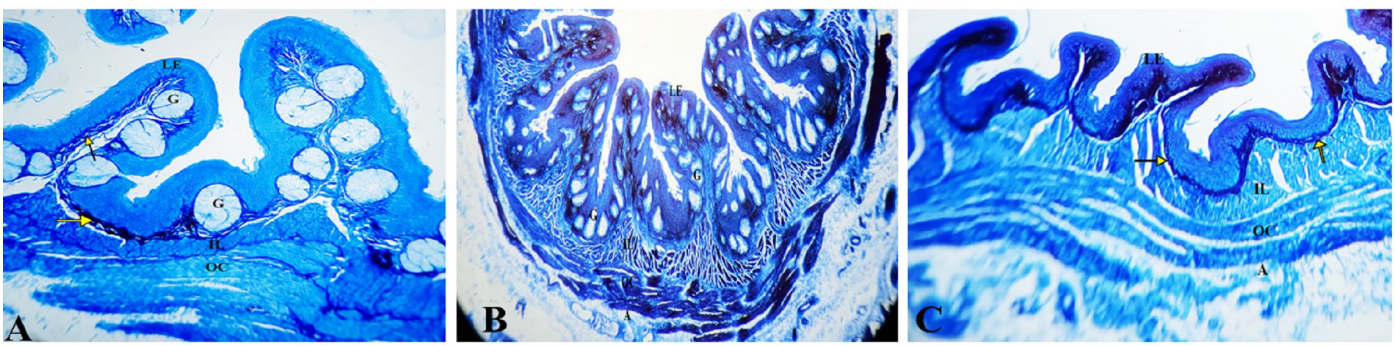

Fig. 7. Cross section of the cervical region in Kestrel (A), hoopoe (B) and rock pigeon (C) stained by bromophenol stain, showed deeply stained in lamina propria (arrows) in the three birds and in layers of muscular externa (IL, OC) in kestrel (A) and hoopoe (B) but not in pigeon (C). Notice, the mucosal glands (G) in first two birds showed no stain

hoopoe, the inner longitudinal muscle thinner than the outer circular (Fig. 8B), while in rock pigeon the inner longitudinal muscle thinner than the outer circular (Figs. 8C).

Adventitia is the outer layer composed of single layer around the muscularis externa was rich in loose connective tissue contained in adipose tissues, nerve fibers, and blood vessels.

The morphometrical studies

The mucosal folds in kestrel were elongated and had finger-like, shorter than the hoopoe and pigeon. The kestrel consisted of 10 folds with an average length (927.67 µm) and width (552.70 µm). In hoopoe consisted of 12 folds mucosal folds leaf-like with length (640.52 µm) and width (244.23 µm), but in rock pigeon wave like consisted of 38 folds, the length (329.26 µm) and width (206.23 µm) (Table 1).

The mean of thickness of the mucosa in the cervical region in the kestrel was (167.06 µm) with a range of (167.06-209.04 µm), and in common hoopoe was (71.458 µm) with a range of (56.19-87.68 µm), in the rock pigeon was (88.54 µm) with a range

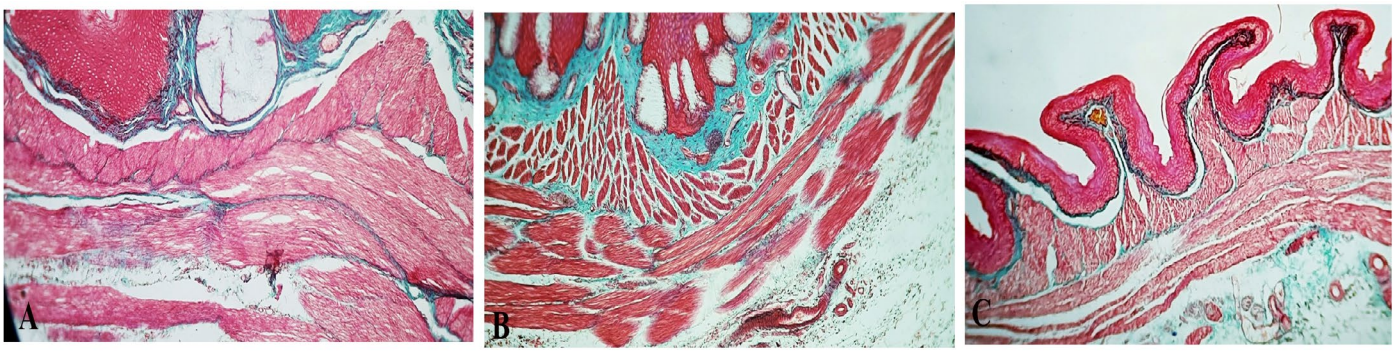

Fig. 8. Cross section of cervical region stained by Masson trichome in Kestrel (A), hoopoe (B) and pigeon (C) showed the muscularis extrena with thin inner longitudinal (IL), and thick outer circular 100x

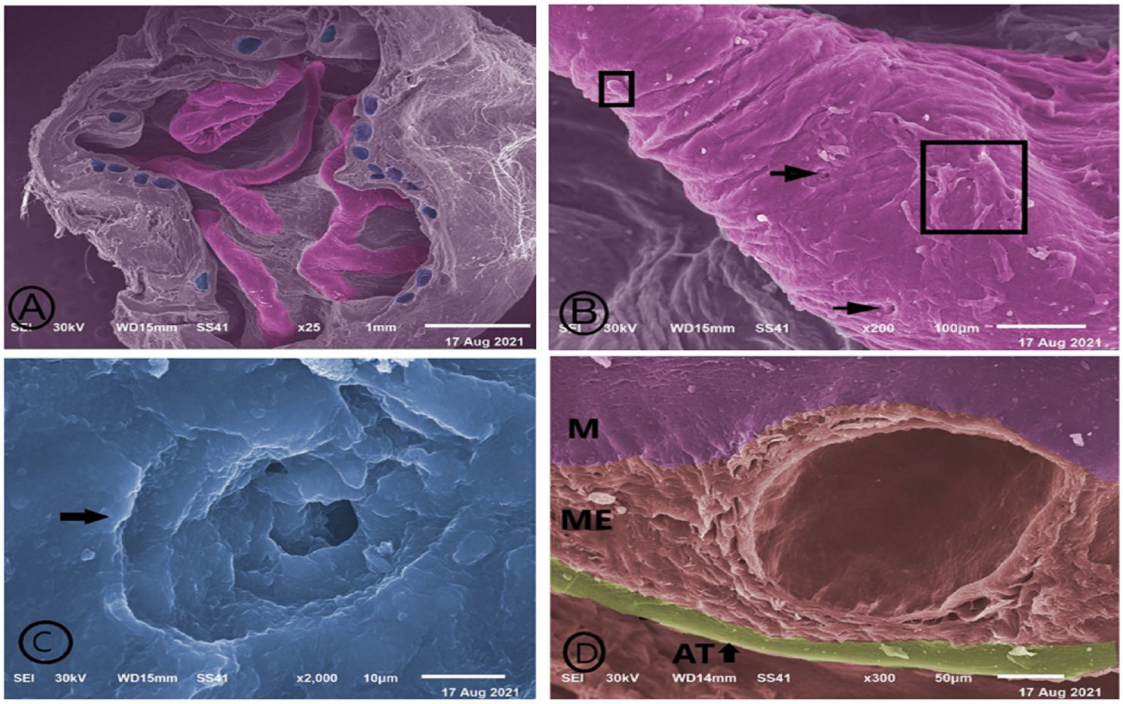

Fig. 9. A: Colored Scanning electron micrograph (SEM) of cervical region of kestrel esophagus. Cross section of the luminal surface of the esophagus which lined with short and thin folds and numerous esophageal glands within the mucosa (blue color). B: Colored Scanning electron micrograph (SEM) of cervical region of kestrel esophagus. Higher magnification of the mucosal fold showing small opening of the esophageal glands (arrows) and mucous secretion (squares). (C): Colored Scanning electron micrograph (SEM) of cervical region of kestrel esophagus. High magnification of the large mucosal gland (arrow). D: Colored Scanning electron micrograph (SEM) of cervical region of kestrel esophagus. surface view of the esophageal fold showing the mucosa(M)which lined by stratified squamous epithelium (violet color), lamina propria contains large esophageal gland extends in muscularis externa (ME) and tunica adventitia (AT).

Table 1. The length and width of folds with lumen of cervical region in kestrel, hoopoe and pigeon.

Folds	Kestrel	Hoopoe	Pigeon
Lumen (um)	large	365.85-420.70	512.19-548.78
(width)		376.29±44.41	542.19±6.65
Length (um)	890.53-950.34	597.11-646.71	315.42-471.56
	927.67±22.66	640.52± 6.19	329.26±142.30
Width (um)	249.06-570.96	231.26-354.70	146.33-227.10
	552.70±18.26	244.23±110.48	206.23±20.87

Data are presented as Minimum and maximum, Mean±SD.

of (86.94-114.99µm), the hoopoe thinner than kestrel and rock pigeon (Table 2-2). lumen centrum in kestrel is width and rock pigeon (548.78-512.19), but in hoopoe appeared narrow (365.85-420.70µm) (Table 1).

The mean thickness of submucosa in kestrel was (58.29 µm) with a range (53.38-61.41µm), and the mean thickness in hoopoe was (38.749 µm) with a range (35.42-41.81 µm). The mean of thickness in rock pigeon was (26.218 µm) with a range (23.52–32.67 µm). (Table 2).

The mean thickness of the gland was (270.06 µm) in kestrel, whereas in the hoopoe was smaller was (128.77 µm) respectively. While the rock pigeon had no glands.

The mean of thickness of the muscularis externa in kestrel was (340.65 µm) with a range (334.83-360.63 µm), the mean of thickness in hoopoe was (246.99 µm) with a range (207.44-258.26 µm), and the mean of thickness in rock pigeon was (386.26 µm) with range (350.53-431.39 µm).

In the kestrel the thickness of adventitia was like the rock pigeon (Figs. 2 and 4), but in hoopoe, few were thicker than kestrel and rock pigeon (Figs. 3).

The mean of thickness in kestrel was (274.37 µm) with a range (252.28-289.63 µm), the mean of thickness in hoopoe was (291.52 µm) with a range (248.94-275.71 µm), and the mean thickness in rock pigeon was (270.89 µm) with range (248.94-275.71 µm) (Table 2).

Scanning Electron Microscopic observation (SEM)

The SEM examination of the cervical region of the esophagus in the three types of the birds (kestrel, hoopoe, and rock pigeon) showed some morphological differences (Fig. 9-11). In kestrel, the esophageal mucosa is lined with short mucosal folds and frequent esophageal glands within the lamina propria (Fig. 9A). The glands openings are surrounded by concentric folds (Fig. 9B). The lining epithelium formed from the moderate thick layer of stratified squamous epithelium (Fig. 9 C). Also, the outermost layers of the muscularis externa and serosa were observed, and the mucosal glands could be extended within the muscularis externa layer (Fig. 9D). In hoopoe, the esophageal mucosa is lined with wide mucosal folds with numerous and large openings of the mucous glands. The circular gland openings contain mucous secretions (Fig. 10 A & B). In rock pigeons, the mucosa of the esophagus showed numerous leaf-like longitudinal mucosal folds which are separated by depressions. Mid-section view showed the esophageal layers; a mucosa which contained thin layer of keratinized stratified squamous epithelium layer and lamina propria, submucosa (SM), muscularis externa (ME) and Tunica adventitia (Fig. 11).

DISCUSSION

The structure of the alimentary tract in birds differs according to the types of food and flying requirements (Alsanosy *et al.*, 2021). The esophagus of birds is a muscular distensible tube located on the right of the neck between the oropharynx and proventriculus. It is composed of two parts, cervical and thoracic (Elshaer, 2018). In the current study, we compared the morphological and histological differences in the cervical part of esophagus in three types of birds with different diet habits. By the light microscope and SEM studies, there are discrepancies in the morphology of the esophageal mucosa, which is lined with short mucosal folds in kestrel, wide and thin mucosal folds in hoopoe but numerous leaf-like longitudinal mucosal folds in rock pigeon. The differences in the shape and numbers of the esophageal

Table 2. Histological comparison between the cervical layers in kestrel, hoopoe and pigeon

Width	Kestrel	Hoopoe	Rock pigeon
Epithelium (um)	167.06-209.04 187.53± 21.51	56.19-87.68 71.46±16.22	86.94-114.99 88.54±26.46
Mucosa (um)	167.06-209.04 187.53± 21.51	56.19-87.68 71.46±16.22	86.94-114.99 88.54±26.46
Submucosa (um)	53.38-61.41 58.29±3.12	35.42-41.81 38.75±3.07	23.52-32.67 26.22±6.45
IL (um)	108.21-133.54 129.37±4.17	66.48-87.83 68.42±19.41	111.61-153.82 143.63±10.19
OC (um)	182.45-203.81 188.76±15.05	125.44-176.15 168.38±7.77	202.83-228.56 221.28±7.28
Muscularis externa(um)	334.83-360.63 340.65±19.98	207.44-258.26 246.99±11.27	350.53-431.39 386.258±27.13
Adventita (um)	252.28-289.63 274.37±15.26	261.33-329.49 291.52±37.97	248.94-275.71 270.89±4.81

Data are presented as Minimum and maximum, Mean±SD. IL: inner longitudinal; OC: outer circular.

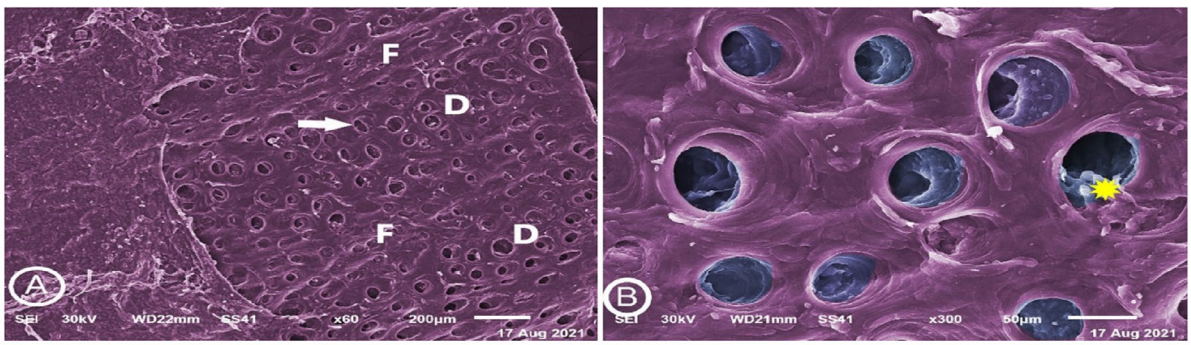

Fig. 10. A: Colored SEM of cervical region of hoopoe esophagus. lateral view of wide mucosal folds (F) and depressions (D) with numerous and large openings of the mucous glands (arrow). B: Colored SEM of cervical region of hoopoe esophagus. Higher magnification of the circular glands openings which contain mucous secretion (asterisk).

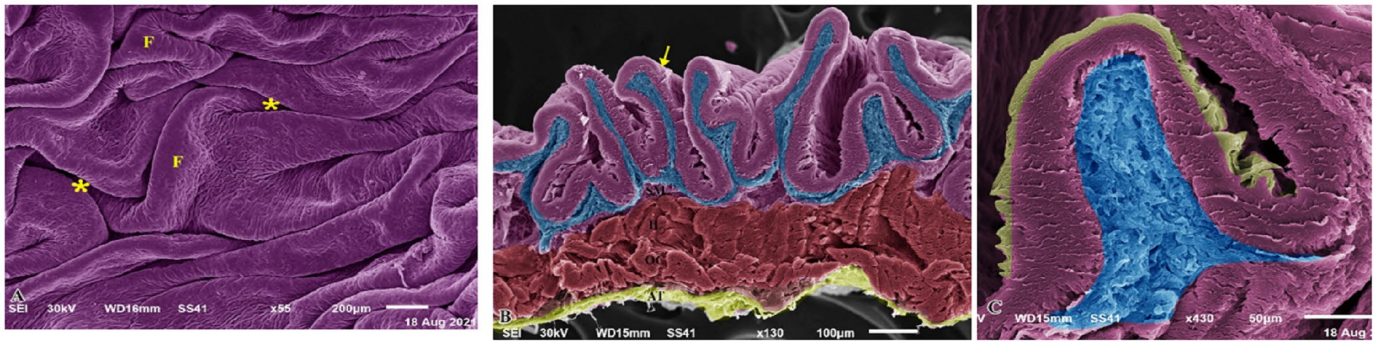

Fig. 11. A: Colored SEM of cervical region of rock pigeon esophagus. The mucosa of the esophagus showing a series of numerous undulating mucosal folds (f) which are separated by depressions (asterisk). B: Colored SEM of cervical region of rock pigeon esophagus. Mid-section view showing a finger like longitudinal mucosal folds which covered by keratinized stratified squamous epithelium (arrow). Notice, the mucosa (violet color), lamina propria (blue color) submucosa (SM), muscularis externa (IL&OC) and Tunica adventitia (yellow color). C: Colored SEM of cervical region of rock pigeon esophagus Higher magnification of the esophageal fold in the cervical region showing the epithelium (violet color) is covered by a layer of the keratin (yellow color), notice lamina propria (blue color).

folds may be attributed to the ability of the esophageal lumen to increase its width during swallowing. The short mucosal folds in kestrel make its lumen wide to accommodate the large meat bolus of the prey (Alsanosy *et al*., 2021).

Moreover, the thickness of the esophageal folds depends on the thickness of the mucosal epithelium, density of lamina propria, and the muscularis mucosa layers (Alsanosy *et al*., 2021). Additionally, the present work recorded that the covering epithelium in kestrel and rock pigeon is keratinized epithelium, which may be due to the rock pigeon eating a wide variety of foods, including seeds which are dry and can cause abrasions in the esophageal mucosa if it is not keratinized. The present work recorded the mucous glands on LP of kestrel and hoopoe. The presence of mucous glands may be regarded as an adaptation of the esophagus to the type of food (Hamdi *et al*., 2014). These glands exhibited a strong Alcian and PAS-positive reaction, which suggests the nature of the secretory material in the glands was acidic and neutral mucopolysaccharides. This finding agrees with the previous study on the rock dove and house sparrow, which recorded that the glands produce acidic and neutral mucopolysaccharides. The existence of acid and neutral mucins in the glands, which poured into the lumen through the epithelium may be act as barrier which help in the mucosal protection from any mechanical damage during the swallowing (Hamdi *et al*., 2014). The frequency of the esophageal glands differs in the three studied birds; hoopoe and kestrel have many mucosal glands. However, in the rock pigeon the glands were absent. A possible explanation for these results is those birds have small or devoid of the crop, so they need long cervical esophageal region with the large number of glands to enable to swallow their foods (Rasha and Hussein, 2015).

CONCLUSION

The morphological, morphometrical, histological and scanning of the cervical region of the esophagus revealed great differences between the three studied birds (kestrel, hoopoe, rock pigeon). The cervical part of the esophagus in rock pigeon is longer than the kestrel and hoopoe and its width in the kestrel is narrower than the hoopoe and rock pigeon. In lamina propria of kestrel and hoopoe had numerous mucous glands that are round, branched tubular glands surrounded by a fibrous capsule. The type of food consumed, is greatly affect the anatomical and the histological structure of the esophagus.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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