Original Article

Comparative anatomical, histological, and scanning electron microscopical investigations on the thoracic region of the esophagus in three avian species

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ABSTRACT

The avian esophagus is a narrow and flexible tube that runs from the oropharynx to the stomach. The anatomical division it consists of three sections: the anterior thoracic, middle crop, and posterior thoracic areas. The objective of this study is to examine the structure, size, and composition of the esophagus in the thoracic region of three bird species (kestrel, hoopoe, rock pigeon) with diverse dietary habits. This will involve analyzing the morphology, measurements, histology, and scanning of the esophagus. In the present investigation, we examined samples from three different species. The birds were slaughtered, and their esophagi were dissected. The thoracic section of the esophagus was examined macroscopically using both light and scanning electron microscopy. The length of the thoracic section of the esophagus in rock pigeons is greater than that of kestrels and hoopoes. Additionally, the width of the esophagus in kestrels is narrower than that of hoopoes and rock pigeons. The lamina propria of the kestrel and hoopoe has numerous mucous glands, which are spherical, branched tubular glands that are enveloped by a fibrous capsule. The glands are connected to the esophagus lumen through ducts that traverse the lining epithelium. However, the thoracic section of the esophagus in rock pigeons lacks glands in its lamina propria. The results of our studies have shown that the type of food ingested strongly affects the anatomical and histological structure of the esophagus.

Keywords: Anatomical, Histological, SEM, Esophagus, Thoracic.

Introduction

The anatomical of the alimentary canal differs between the various avian species according to the food habits of every species (1). Therefore, the birds species are divided according to their food: granivorous birds, such as pigeons, graze on a diet of seeds and grains. (2); As cattle egrets, carnivorous birds live on insects and fish (3). And omnivorous birds, such as ducks and gulls, whose diet consists of seeds, insects, and fruits (4). The esophagus of birds is situated on the right side of the neck and dorsal to the larynx, which is considered a thin-walled muscle transfer feed from the mouth to the stomach. The esophagus of theavian is divided into three regions S-shaped thoracic, crop, and thoracic (5). The internal esophageal surface is separate by similar longitudinal folds that differ in size according from the nature of the diet (6). The avian esophageal wall composed of four tunica: mucosa, submucosa, muscularis, and adventitia (7). The epithelium is covered by stratified squamous epithelium depending on the species (8). The submucosa is a connective tissue that contains the mucus gland. (9). Muscularis composed of outer longitudinal and inner circular muscle fibres (10).

There have been numerous studies on the morphological structure of the esophagus. However, still a little is known about the differences in the histological structure depending on the type of diet (9, 11, 12), particularly in kestrel, hoopoe and rock pigeon. Therefore, this study investigates the morphological, histological and ultrastructure of the thoracic esophageal region among the three birds with different food habits.

Materials and Methods

Sample collection:

For the present investigation, an average of thirty samples were collected from ten individuals of each species (kestrel, hoopoe, and rock pigeon) in the city of Erbil, Iraq. The birds were obtained from avian shops, and their well-being was examined through euthanasia. The sample collection was conducted according with the parameters set by the Institutional Ethical Committee of Salahaddin University, Erbil, Iraq, and in compliance with the applicable animal welfare legislation in Iraq.

Light microscopy:

Each specimen was removed directly after slaughter and washed with normal saline. Each order's thoracic 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 alcohols with increasingly higher 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 (13). 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 & Gamble, 2008; Humason, 1979). Photomicrographs of the sections using a digital microscope LECA DM 2700 P.

Scanning electron microscopy

Specimens in the thoracic 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 they were post-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.

Results and Discussion

Gross morphological (macroscopic feature):

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

The morphological study showed that the esophagus in the kestrel was divided into the same three regions similar to rock pigeon (Fig. 1 A, C), but that of hoopoe lacks the crop region. (Fig. 1 B). While the esophageal regions displayed differences in shape and size between kestrel and hoopoe. But the shape of the thoracic region is similar in both birds. The thoracic wall was thick, overfilled folds than the crop region (Figure 1a,b and c). The thoracic region of kestrel is slightly dilated than in hoopoe and rock pigeon.

The mean total length of the esophagus in kestrel was (6.18 cm), the mean length of the thoracic in **kestrel** was **(1.945 cm),** with a range of (1.75-2.10 cm) while the average diameter is (0.416 cm), and the range of diameter (0.25-0.50 cm). The mean total length of the esophagus in hoopoe was (7.905 cm), the mean length of the thoracic in **hoopoe** was **(1.975 cm),** the range of length was (1.75-2 cm) while the average diameter is (0.2 cm), and the range of diameter (0.15-0.25 cm). The mean total length of the esophagus in rock pigeon was (12.84 cm), the mean length of the thoracic in **rock pigeon was (3.85 cm),** the range of length was (3.50-4.25 cm) while the average diameter is (0.312 cm), and the range of diameter (0.25-0.40 cm), (Table 1-1).

The total length of esophagus in kestrel and hoopoe is slightly difference between them. However, the length of the rock pigeon is longer than the kestrel and hoopoe.

Table 1.1: Morphometrical comparison between the three studied birds Kestrel, Hoopoe and Rock pigeon (first line: minimum and maximum. Second line: mean ± SD)

Thoracic	Kestrel	Hoopoe	Rock pigeon
Length of esophagus	5.65 – 6.65 6.18 ± 0.47	7.2 – 8.3 7.905 ± 0.395	12.25 – 13.40 12.84 ± 0.56
Length	1.75-2.10 1.945 ± 0.155	1.82-2.2 1.975 ± 0.025	3.50-4.25 3.85 ± 0.4
Width	0.25-0.50 0.416 ± 0.084	0.15-0.25 0.2 ± 0.05	0.25-0.40 0.312 ± 0.088

Histological studies:

The histological investigation of the thoracic region, posterior region, of the esophagus showed some differences, represented by the number of the folds, the length of these folds, the thickness of the wall layers, the presence of the esophageal glands, and the number of these glands. There were no differences between males and females in histological features of the esophagus in kestrel, hoopoe and pigeon.

The results determined the wall of the esophagus in kestrel, hoopoe and rock pigeon was composed of four layers: mucosa, submucosa, muscularis extrena and adventitia (Figs. 2,3&4).

The mucosa thoracic region of the mucosal epithelium was keratinized stratified squamous epithelial in kestrel and rock pigeon (Figure 2 and 4) but non-keratinized stratified squamous epithelial in hoopoe (Figure 3).

The mucosal folds in the thoracic were given raise finger-like consists of numerous folds which were arranged in longitudinal folds. (Figure 2) In kestrel, it has leaf-like elongated numerous folds and a narrow lumen (Figure 3). In the hoopoe, the mucosal folds are small wave-like shapes but in rock pigeons wide folds (Figure 4), (Table 1.3).

Also, the lining of epithelium is thick in kestrel (fig.5), but thin in hoopoe (fig.6) and rock pigeon (fig.7).

The epithelial cell is covered by keratinized stratified squamous epithelium in kestrel and rock pigeon (fig.5&7), but in hoopoe epithelium cell is covered by Non-keratinized stratified squamous epithelium (fig.6). The lining epithelial cells in the three species have oval nuclei and granular cytoplasm, these cells and their nuclei contain flatted progressively as they migrate

toward the dorsal free surface of epithelium. The lining epithelium in kestrel is thicker than rock pigeons and hoopoes.

In kestrel, the surface of the epithelium has few openings of the compound alveolar glands, which opened into the lumen (fig.2). In hoopoes have numerous simple tubular mucus-secreting glands (fig.3). But rock pigeons have numerous large alveolar submucosal glands were recognized like-series (fig.4), the glands covered by (CT) thin membrane in all species.

Moreover, the glands positively reacted to AB and PAS (Figure 11A,11B,12A, 12B,13A,13B) in all studied species.

The lamina propria consists of dense collagenous connective tissue below the epithelium layer containing blood vessels (BV) and nerve fibers (NV), with glands in kestrel and hoopoe (Figure 8 and 9) but in rock pigeons less dense collagenous(Figure 10). then the muscularis mucosa, which was composed of smooth muscle fibers.

The means the thickness of the mucosa in the thoracic region in the kestrel was (26.405 µm) with a range of (22.593-31.0949µm), and in common hoopoe was (8.524 µm) with a range of (7.814-10.132µm),

in the rock pigeon was (9.833 µm) with a range of (9.316-10.595 µm), (Table 1.4).

2-Submucosa, the second layer, consists of loose connective tissue and contains large arteries, small veins and nerve fibers. In kestrel was thick (fig.2)**,** and rock pigeon was thinner (fig.4) than kestrel and hoopoe (fig.3) consists of loose connective tissue was associated with submucosal plexus, nerve fibers, blood vessels, fibroblasts, fine reticular, elastic, and collagen fibers (Table 1.3).

The mean of thickness in kestrel was (8.911µm) with a range (8.0357-10.348 µm), and the mean of thickness in hoopoe was (7.414 µm) with a range (6.0559-8.1522µm). The mean thickness in rock pigeons was (3.272 µm) with a range of (2.9115-3.5704 µm). (Table 1.4).

In the kestrel, submucosa was composed of loose connective tissue containing round-shaped branched tubular glands surrounded by a fibrous capsule (Fig. 2), whereas they were tubular shape in hoopoe (Fig. 3), and larger gland in rock pigeon (fig. 4).

The mean diameter of the large gland was (40.226 µm) in kestrel, whereas the hoopoe was smaller (13.165 µm). While the rock pigeon has a larger gland (52.051 µm) respectively, (Table 1.2).

Table 1.2 showed of the morphometrical compare the glands of the thoracic region between the kestrel, hoopoe, and rock pigeon

Gland	Kestrel	Hoopoe	Rock pigeon
Diameter	35.170-42.333	12.577-13.982	50.426-54.207
	40.226±2.107	13.165±**0.817**	52.051±2.156

3-**Tunica Muscularis** appeared in two layers separated by thin connective tissue inner longitudinal and outer circular smooth muscle fibers (fig.14, 15, and 16). Statistically, the tunica mucosa of the hoopoe was thicker than kestrel and rock pigeon. (Table 1). The inner longitudinal muscle of the rock pigeon is thinner than that of the kestrel and hoopoe. Still, the outer circular muscle of the kestrel with the same thickness as the hoopoe, while in rock pigeon is thinner than that of the two species. The outer circular muscle is composed of three bundles of muscle fibers in three species, and each other is separated by a thin layer of connective tissue (fig.14,15 &16). In the hoopoe, the inner longitudinal muscle is thinner than the outer circle.

The mean of thickness in kestrel was (35.985 µm) with a range (35.752-37.963 µm), the mean of thickness in hoopoe was (41.554 µm) with a range(38.383-43.672µm), and the mean of thickness in rock pigeon was (30.994 µm) with range(28.856-32.226 µm) (Table 1.4).

4-**Adventitia,** the outer layer composed of a single white layer of connective tissue around the muscular externa was rich in loose connective tissue contained in adipose tissues, nerve fibers, and blood vessels.

In the kestrel, the thickness is similar to the rock pigeon (Figs. 2&4), but in the hoopoe, few are thicker than the kestrel and rock pigeon (Figs. 3).

The mean of thickness in kestrel was (274.370 µm) with a range of 252.283-289.634 µm), the mean of thickness in hoopoe was (291.518 µm) with a range of 248.940-275.705µm), and the mean of thickness in rock pigeon was (270.893 µm) with range (248.940-275.705 µm) (Table 1.4).

Table 1.3: the length and width of folds with the lumen of the thoracic region in kestrel, hoopoe, and pigeon.

Folds	kestrel	Hoopoe	pigeon
Length	148.567-166.948	86.861-101.236	214.805-220.469
	160.421±6.527	94.913±6.323	216.997±3.472
Width	92.003-99.404	59.977-66.819	160.417-171.429
	95.031 ± 4.373	63.121±3.698	163.976±7.453

Table 1.4: The morphometric of histological thoracic layers in kestrel, hoopoe, and pigeon.

Width	Kestrel	Hoopoe	Rock pigeon
epithelium	22.593-31.0949	7.814-10.132	9.316-10.595
	26.405± 4.6899	8.524±1.608	9.833±0.762
Mucus	22.593-31.0949	7.814-10.132	9.316-10.595
	26.405± 4.6899	8.524±1.608	9.833±0.762
submucosa	8.0357-10.348	6.0559-8.1522	2.9115-3.5704
	8.911±1.437	7.414±0.7382	3.272±0.2984
IL	10.248 -10.830	13.694-15.955	7.609-8.585
	10.364 ±0.466	15.173±0.782	8.114±0.471
OC	25.388-27.133	24.689-27.717	21.247-23.641
	25.621±1.512	26.381±1.336	22.880±0.761
Muscularis	35.752-37.963	38.383-43.672	28.856-32.226
Externa	35.985±1.978	41.554±2.118	30.994±1.232
adventitia	252.283-289.634	261.333-329.491	248.940-275.705
	274.370±15.264	291.518±37.973	270.893±4.812

Scanning Electron Microscopic observation (SEM)

The SEM investigation of the thoracic region of the esophagus in the three types of birds (Kestrel, hoopoe, and rock pigeon) appearance some morphological differences (Fig.17-19). In kestrel, the mucosal epithelium, which is covered by keratinized stratified squamous epithelium (Fig. 17A, B), well distinct openings of glands, the few glands openings are surrounded by

folds (Fig. 17A). In hoopoe, the thoracic mucosa is characterized by wide mucosal folds and depressions between them, with numerous openings of the mucous glands (fig. 18A, B and C). The circular glands openings contain mucous secretion (fig. 18B). The mucosal folds which covered by Non-keratinized stratified squamous epithelium (fig.18C). In rock pigeons, the mucosa of the esophagus showed numerous wave-like longitudinal short mucosal folds which are separated by depressions (fig.19A). Mid-section view showed the esophageal layers; a mucosa which contained thin layer of keratinized stratified squamous epithelium layer and lamina propria, submucosa (SM), muscular externa (ME) and Tunica adventitia (Fig. 21).

Birds have varying feeding behaviors that are closely aligned with their respective habitats. Regarding the dietary preferences, avian species can be categorized as granivorous, which includes birds such as doves, common quails, and domestic pigeons, that mostly consume seeds(14). The omnivorous birds, such as ducks, rock pigeons, and gulls, consume a variety of food items including seeds, insects, and fruits(15). The carnivorous avian species have ingested worms, amphibians, and piscine mammals(16).

The anatomical structure of the alimentary tract of birds varies depending on the specific types of food they consume and their flight-related needs(17). The avian esophagus is a flexible and stretchy muscular tube situated to the right of the neck, positioned between the oropharynx and proventriculus. The structure consists of two distinct components, namely the thoracic and thoracic regions (18). The present study involved a comparison of the morphological and histological distinctions in the thoracic section of the esophagus across three avian species with varying dietary patterns. Specifically, the kestrel, which is a carnivorous bird, primarily consumes rodents and insects (19), The hoopoe is a species that primarily feeds on insects (20), while the rock pigeon has a diet that includes both plants and animals (21). Through the use of light microscopic and scanning electron microscopy (SEM), variations in the structure of the esophagus mucosa have been observed. In kestrels, the mucosa is characterized by short folds, while hoopoes have large and thin folds. On the other hand, rock pigeons have numerous longitudinal folds that resemble leaves. The variations in the morphology and quantity of the esophageal folds can be ascribed to the esophagus lumen's capacity to expand its breadth while swallowing. The kestrel's mucosal folds are short, which increases the diameter of its lumen in order to accommodate the massive meat bolus of its prey (22).

Furthermore, the thickness of the esophageal folds is determined by the thickness of the mucosal epithelium, the density of the lamina propria, and the presence of the muscularis mucosa layers (22).

Furthermore, the present investigation showed that the outer layer of cells in kestrel and rock pigeon is composed of keratinized epithelium. This may be attributed to the rock pigeon's diverse diet, which includes dry seeds that can potentially cause damage to the esophagus lining if it is not keratinized. This study documented the presence of mucous glands on the lower eyelid of kestrels and hoopoes. The presence of mucous glands in the esophagus can be considered as an adaptation to the specific nature of the food consumed(23).

The glands showed a strong Alcian and PAS-positive response, indicating that the secretory material in the glands consisted of acidic and neutral mucopolysaccharides. This discovery aligns with the prior research conducted on the rock dove and house sparrow, which documented the secretion of both acidic and neutral mucopolysaccharides by the glands. The presence of acid and neutral mucins in the glands, which are secreted into the lumen through the epithelium, serve as a barrier that aids in protecting the mucosa from potential mechanical harm during the process of swallowing(24). The presence of esophageal glands varied across the three species examined; hoopoe and kestrel had a substantial number of mucosal glands. However, the rock pigeon lacked the glands. One possible explanation for these observations is that these birds have a short or absent crop, which necessitates a long thoracic esophageal region with a significant number of glands in order to facilitate the swallowing of their food(25).

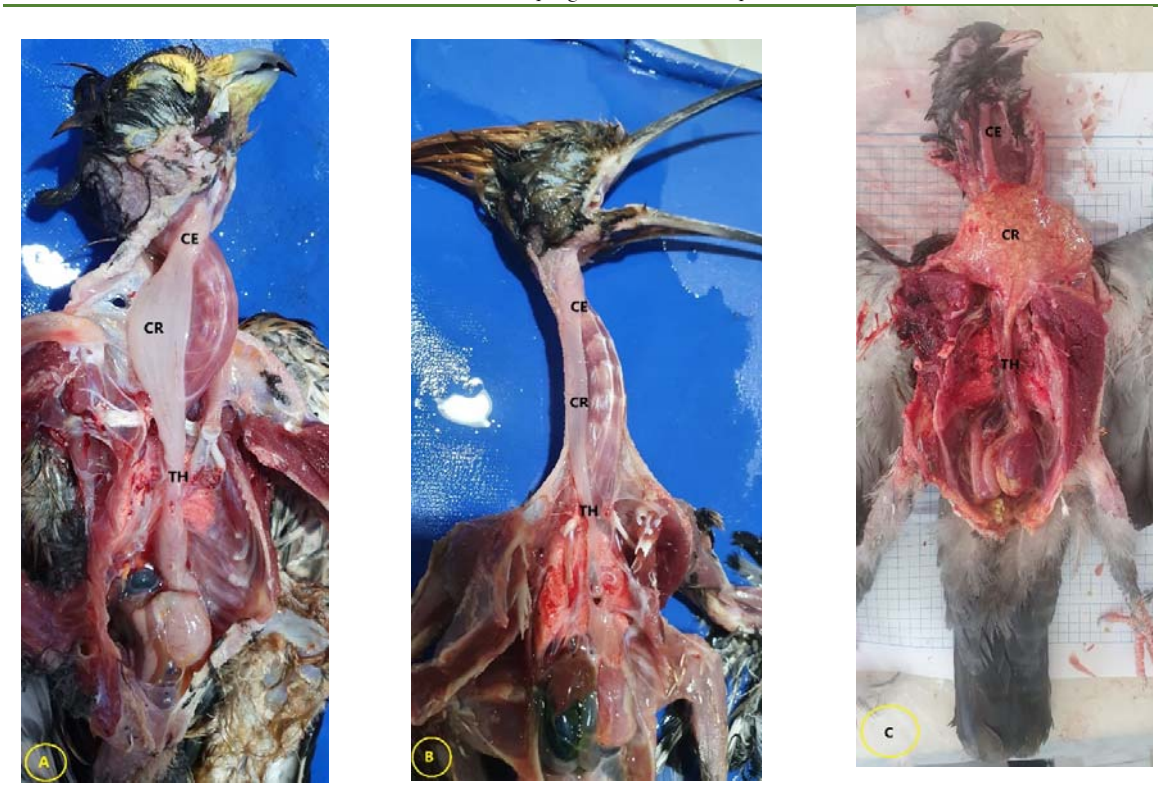


Fig. 1: A photograph of the dissection of the alimentary canal of A-Kestrel, B-Hoopoe and C-Rock Pigeon.

Fig.2 Cross section of the Thoracic region in Kestrel showed, the mucosa fold (MF), epithelial cell(E), keratinized squamous epithelial tissue (KSSE), mucosa (M), lamina propria (LP), submucosa(SM), inner longitudinal muscularis (IL) and outer circular muscularis (OC) and tunica adventitia (TA), secretory glands(G), the duct of the glands (arrowhead) .H&E 40x.

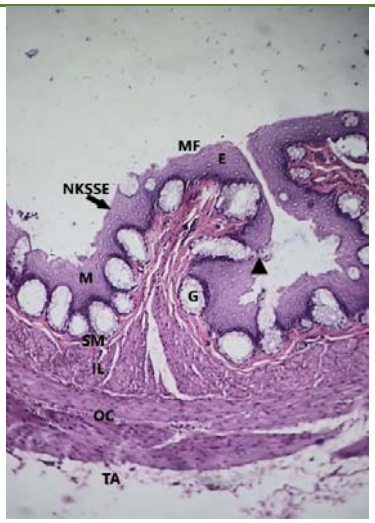


Fig. 3 Cross section of Thoracic region in hoopoe showed, the mucosal folds (MF), epithelial cell (E), the keratinized squamous epithelial tissue (NKSSE). Mucosa (M), submucosa (SM), inner longitudinal muscularis (IL), and outer circular muscularis (OC), and tunica adventitia (TA). Secretory glands (G), the duct of the glands (arrowhead). H&E 100x

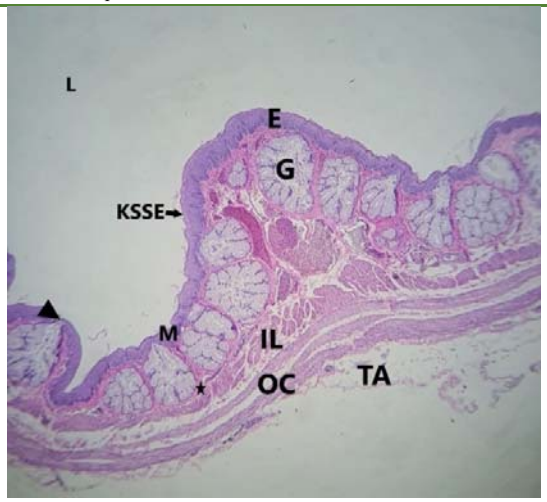


Fig. 4 Cross section of Thoracic region in pigeon showed, the mucosal folds (MF), epithelial cell (E), the keratinized squamous epithelial tissue (KSSE). Four layers: M: mucosa, SM: submucosa (star), muscularis extrena: inner longitudinal (IL) and outer circular(OC) and tunica adventitia (TA). Secretory glands (G), the duct of the glands (arrowhead).. H&E 40x

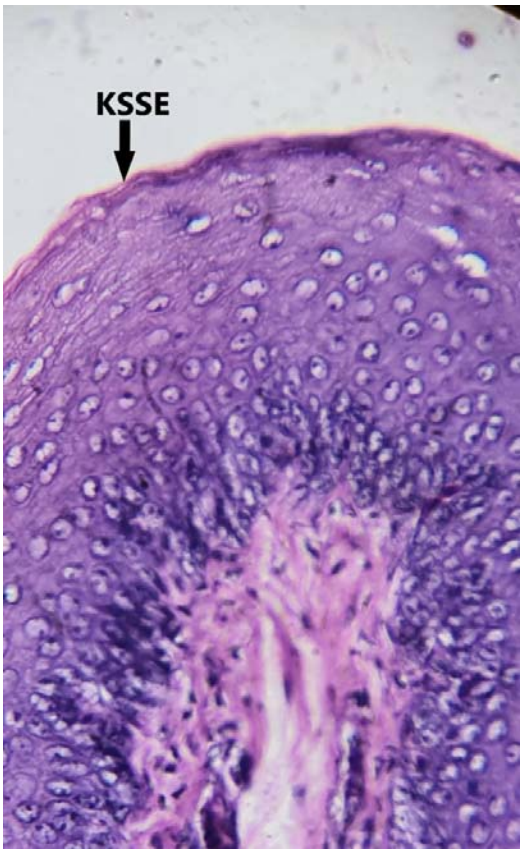


Fig.5 Cross section of thoracic region in Kestrel showed the mucosa epithelial cell the keratinized squamous epithelial tissue KSSE. 400X

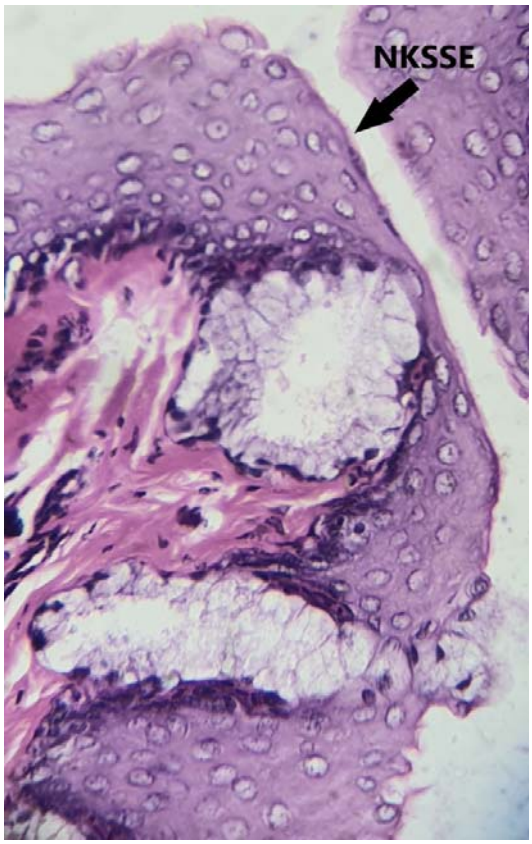


Fig.6 Cross section of thoracic region in HOOPOE showed the mucosa epithelial cell the Non-keratinized squamous epithelial tissue NKSSE. 400X

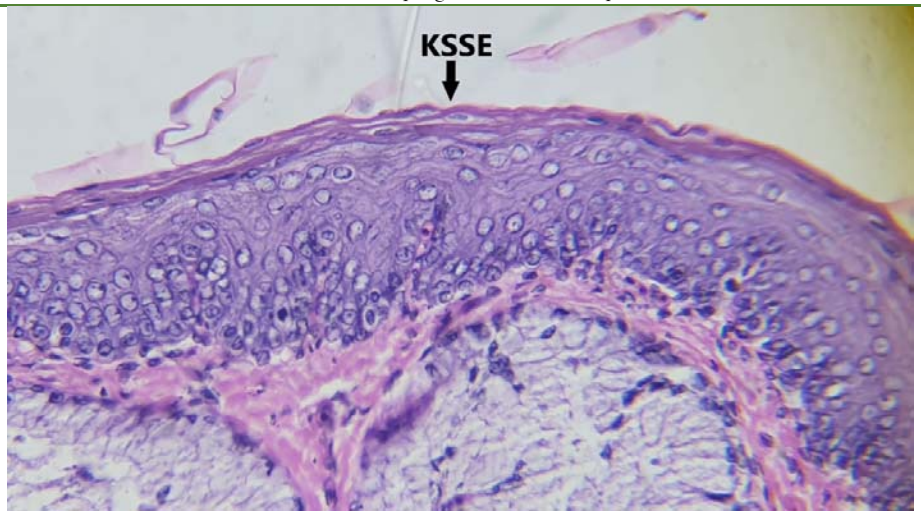


Fig.7 Cross section of Thoracic region in rock pigeon showed the mucosal epithelial cell the keratinized squamous epithelial tissue (KSSE). 400X

Fig.8 Cross section of thoracic region in Kestrel showed the collagen fibers in lamina propria (arrow), and submucosa (arrowhead), Masson trichome 40x

Fig.9 Cross section of thoracic region in hoopoe showed the collagen fibers in lamina propria (arrow) and submucosa (arrowhead). Masson trichome 40x

Fig10 Cross section of thoracic region in rock pigeon showed, the collagen fibers in lamina propria (arrow), and submucosa (arrowhead) and between the muscle fibers (arrows). Masson trichome 40x.

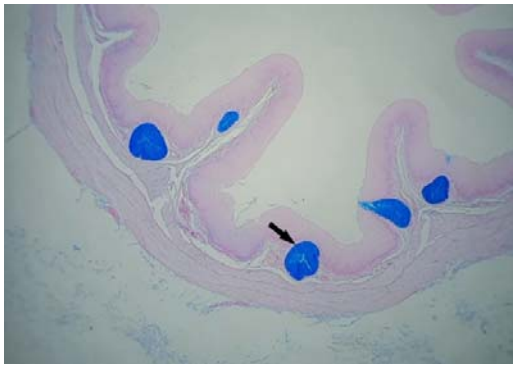


Fig11 A: Cross section of thoracic region in kestrel showed, the submucosa, the duct of the glands (arrow) blue color, AB 40x

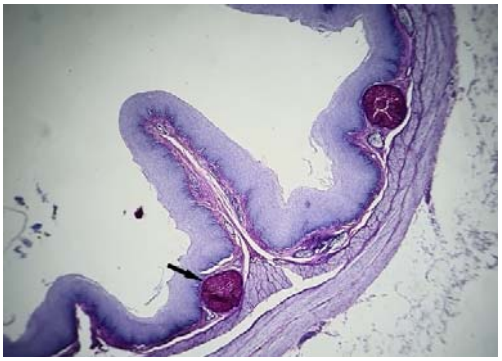


Fig11 B: Cross section of thoracic region in kestrel showed, the submucosa, the duct of the glands (arrow) blue color, PAS 40x

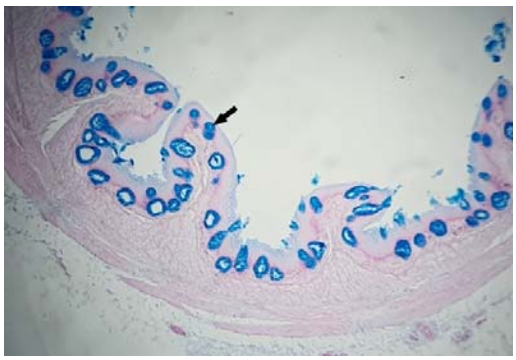


Fig12 A: Cross section of thoracic region in hoopoe showed, the submucosa, glands (arrow) blue color, AB 40x

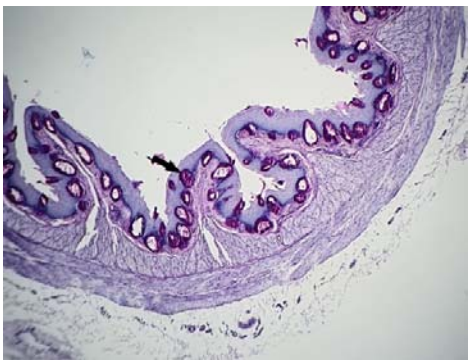


Fig12 B: Cross section of thoracic region in hoopoe showed, the submucosa, glands (arrow) blue color, PAS 40x

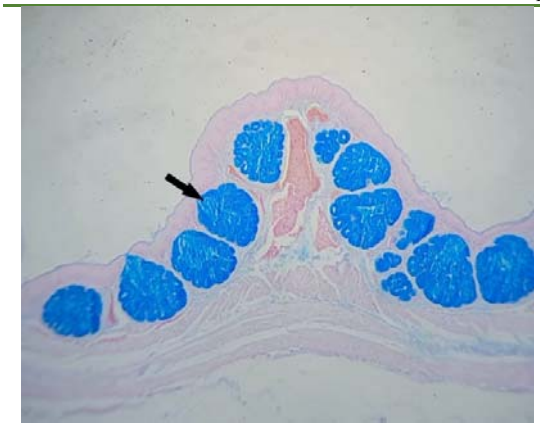


Fig13 A: Cross section of thoracic region in rock pigeon showed, the submucosa, lack glands (arrow) blue color, AB 40x

The large submucosal glands exhibited a strong alcian blue reaction

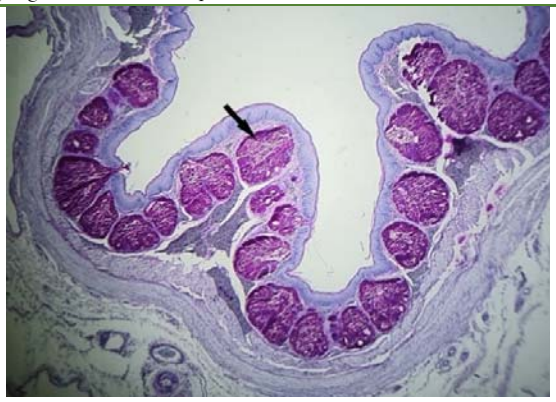


Fig13 B: Cross section of thoracic region in rock pigeon showed, the submucosa, lack glands (arrow) blue color, PAS 40x

the large submucosal glands exhibited a strong PAS-reaction

Fig14 Cross section of thoracic region in kestrel showed, the tunica muscularis was thin inner longitudinal (IL), and thick outer circular(OC) Masson trichome 100x

Fig15 Cross section of thoracic region in hoopoe showed, the tunica muscularis was inner longitudinal (IL), and thick outer circular(OC) Masson trichome 100x

Fig16 Cross section of thoracic region in rock pigeon showed, the tunica muscularis was thin inner longitudinal (IL), and thick outer circular (OC) Masson trichome 100x.

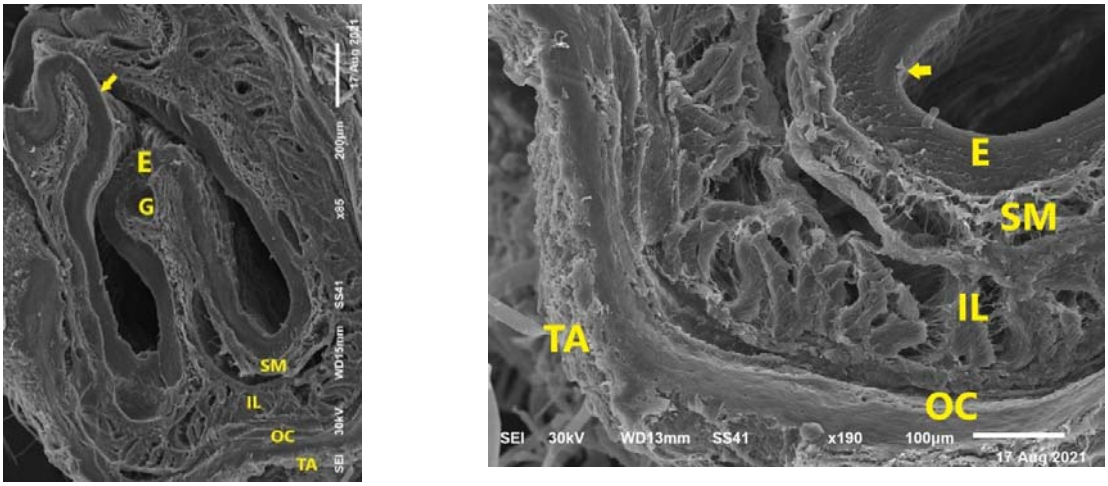
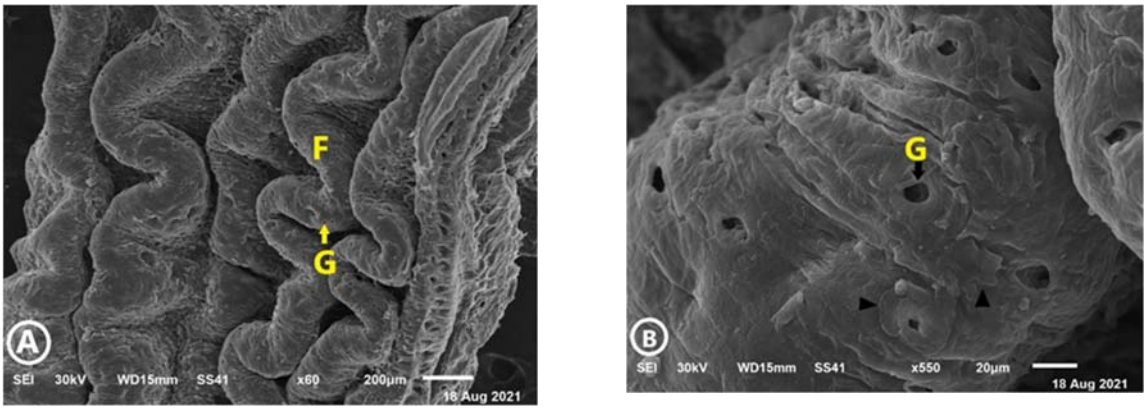


Figure17 . Scanning electron micrograph of kestrel thoracic region. (A)showing the thoracic region. (B) showing the high magnification large opening of gland (G), keratinized squamous epithelial tissue (arrow), the epithelium (E), submucosa (SM), inner longitudinal muscle (IL), outer circular muscle (OC) and Tunica adventitia (TA).



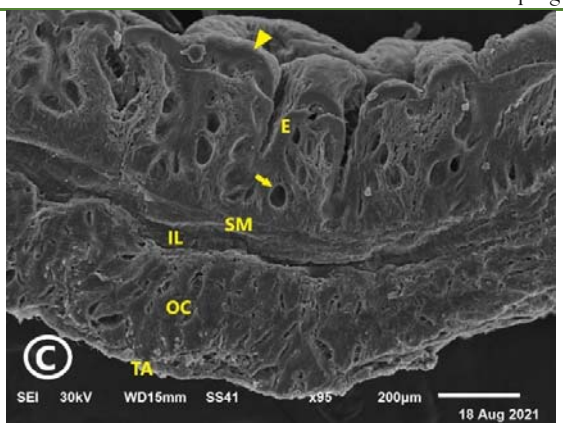


Figure18 . Scanning electron micrograph of hoopoe thoracic region. (A) showing the mucosal folds thoracic region of many mucosal folds (F) and glands (G) with numerous opening glands(arrow).(B) showing the high magnification arrow opening of gland circular shape, prominent boundary of squamous epithelial cells (arrowhead). (C) showing the high magnification large opening of gland (arrow), Non-keratinized squamous epithelial cells (arrowhead), the epithelium (E), submucosa (SM), inner longitudinal muscle (IL), outer circular muscle (OC) and Tunica adventitia (TA).

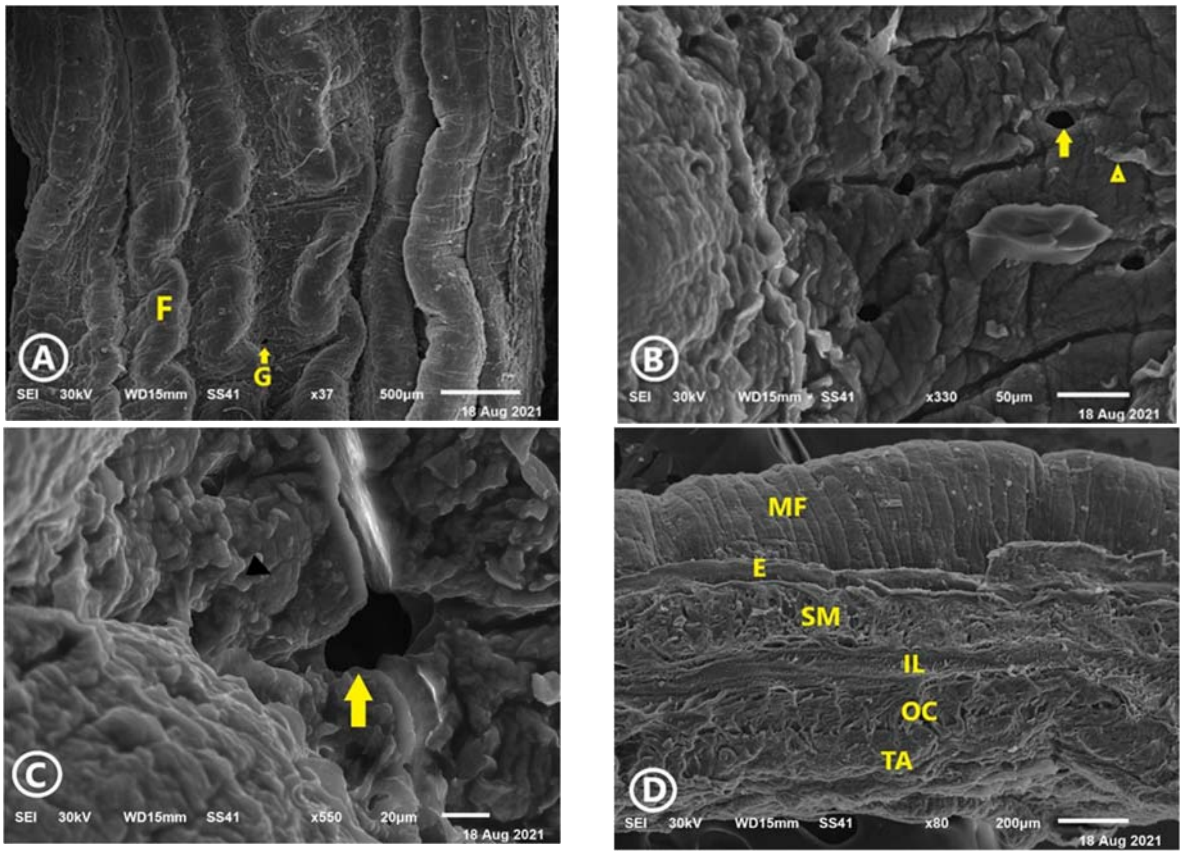


Figure. 19 Scanning electron micrograph of rock pigeon thoracic region. (A)showing the mucosal folds thoracic region of many mucosal folds (F), many glands (G). (B) showing the high magnification opening glands (arrow), prominent boundary of squamous epithelial cells (arrowhead). (D) showing the mucosa folds(MF), epithelium (E), submucosa (SM), inner longitudinal muscle (IL), outer circular muscle (OC) and Tunica adventitia (TA).

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