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Research Article

Comparative Anatomy, Histology, Histochemistry, and Immunohistochemistry of the Esophagus in Ostrich (*Struthio camelus*) and Turkey (*Meleagris gallopavo*)

Rabab Abd Alameer Naser[1]*, Sameer Ahmed Abid Al-Redah[2], Enas S. Ahmed[3], Fatimah S. Zghair[2], Ali Ibrahim Ali Al-Ezzy[4]

[1]Department of Anatomy and Histology, College of Veterinary Medicine, Diyala University, Iraq; [2]Department of Anatomy and Histology, College of Veterinary Medicine, Al-Qadisiyah University, Iraq; [3]Department of Biomedical Engineering, College of Engineering, Al-Nahrain University, Jadriya, Baghdad, Iraq; [4]Department of Pathology, College of Veterinary Medicine, Diyala University, Iraq.

Abstract | This study aimed to identify differences in the esophagus of ostriches and turkeys at the macroscopic, microscopic, histochemical, and immunohistochemical levels. Seven male turkeys and ostriches were obtained from Diyala province during the summer (July-September). Euthanasia was performed using anesthesia with Xylazine and Ketamine. Esophageal sections (cranial, middle, and caudal) were collected for microscopic, immunohistochemical, and biochemical analysis. Compared to turkeys, ostriches lacked a distinct crop (second esophageal segment). In bothostriches" and "turkeys, the first esophageal segment was longer than the thoracic segment. Histologically, the esophagus in both species had four layers. The mucosa was lined by a thick, keratinized stratified squamous epithelium. Immunohistochemical analysis revealed the presence of serotonin receptors in this layer. The density of glands in the lamina propria increased distally. Turkeys possessed tubular acinar glands, while ostriches had simpler tubular glands. The submucosa was less distinct in turkeys compared to ostriches, where it was well-developed. The muscularis layer also differed between the two species.

Keywords | Anatomy, Histology, Esophagus, Ostrich, Turkey

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***Correspondence** | Rabab Abd Alameer Naser, Department of Anatomy and Histology, College of Veterinary Medicine, Diyala University, Iraq; **Email:** rabab.a@uodiyala.edu.iq
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INTRODUCTION

The ostrich breeds are important animals in many ostriches and "turkeys industries and in the developing country in the world, the export of meat and skin is a valuable source of foreign currency (Cooper and Mahroze, 2004). The ostrich is the largest bird and belongs to the order Ratitae, and Family Struthionidae, these families include the cassowary, kiwi, and rhea. These families are characterized by healthy red meat and skin (Charles Gald Sibley, 1990). Ostrich Good growth and reproductive performance depend on good feeding and management (Cooper, 2000). The alimentary canal in ostriches and turkeys is composed of the esophagus, muscular stomach, glandular stomach, and small and large intestine (van Staaveren *et al*., 2020) domestic ostriches and turkeys are adapted to various environments according to different types of foods (Klasing, 1999). The ostriches and turkeys

have a coelomic cavity without a diaphragm The esophagus of ostriches and turkeys is a long tube connecting the oropharynx and the glandular stomach. It lies on the right side of the neck while in mammals located on the left side) dorsally to the trachea. It passes cranial to the thoracic entrance and crosses to the median line and expands ventrally to form the crop, in homing pigeons (*Columba livia domestica*) (Kadhim and Mohamed, 2015).The avian esophagus in this order was a long distensible tube that united the oropharynx with a glandular stomach, it situated dorsal to the trachea and cross the thoracic cavity, and passes through the medium in geese (Shehan, 2012), The wall of the elementary canals consists of four layers include (mucosa, submucosa, muscularis and Adventitia, and serosa) in rhea (Rhea American) (Rodrigues *et al*., 2012), in the barn owl (Oyelowo *et al*., 2017), in white-breasted Kingfisher (*Halcyon smyrnensis*) (Al-Kinany, 2017), in duck (Qureshi *et al*., 2017) and Kingfisher and Hoopoe (Upupa epops)(AbdElnaeem *et al*., 2019), in Muscovy duck (Cairina moschata) (Pourhaji and Hashemi, 2020).

Current study aims to compare the anatomical , histological, histochemical, and immunohistochemically structure of the esophagus in turkeys (*Meleagris gallopavo*) and ostrich (*Struthio camelus Linnaeus* 1758).

MATERIALS AND METHODS

Study design

Animals

Seven healthy turkeys (*Meleagris gallopavo*) and seven ostriches (*Struthio camelus Linnaeus 1758*) at the age of four months were used in this experiment obtained from the turkey and ostrich farm in Baqubah city. they performed the experiment during the summer season (July to September) and giving both ostriches and turkeys the same feed pellet then they weighed the turkey and ostrich 350±0.4 gm and 2660.2±0.418 gm, respectively.

Anatomy

All ostriches and turkeys were euthanized using Xylazine (10mg/Kg) and Ketamine (100 mg/Kg) (Murphy and Fialkwaski, 2001), and then they studied the topographical relationship of the esophagus with structures in the neck after removing the whole esophagus and washed with normal saline.

Histology

Esophageal tissue samples were collected from each region (cranial, middle, and caudal) and fixed in 10% formalin for at least 24 hours. Following fixation, the tissues underwent dehydration through a graded series of ethanol (70-100%) and were cleared using two xylene washes. Subsequently, they were infiltrated with paraffin wax and embedded in paraffin blocks. Four micrometer-thick transverse sections were obtained from each block using a disposable microtome blade.

For histological analysis, sections were stained with hematoxylin and eosin (H and E) for routine examination. Additionally, periodic acid-Schiff (PAS) stain was employed to visualize carbohydrates, and Masson's trichrome stain was used to identify collagen fibers (Bancroft and Gamble, 2008).

Histochemistry and immunohistochemistry

In immunohistochemical method, primary polyclonal serotonin antibodies raised in rats, diluted 1:100 and incubated overnight at 4°C. These antibodies were bought from Santa Cruse Biotechnology in the United States. 2.5% (v/v) donkey serum, 0.25% (w/v) Na azide, and 0.2% (v/v) triton X-100 were the ingredients in the buffer that contained antibodies (primary or secondary). Following that, each slide was washed in PBS for 5 x 5 minutes. We used FITC-conjugated IgG/IgY from by Stratech Scientific Limited in Suffolk, UK, diluted at 1:500. Finally, slides were mounted using Vectashield Hard Set Mounting Media and PBS for a 5 x 5-minute wash (Vector Laboratories Ltd, Peterborough, UK) (Bancroft and Gamble, 2008).

RESULTS AND DISCUSSION

Anatomy

In both ostriches and turkeys, the esophagus was a muscular tube, that began from the end part oropharynx to the stomach, it passes on the right side (Figures 1, 2), the cervical segment related with The jugular vein internal carotid artery and thymus but the thoracic segment related to the trachea, heart, and right and left lobes of the liver and gizzard the esophagus was composed of three in turkey (Figure 1A) and consisted of two segments in ostrich, (Figure 2A), in ostriches, the cervical part is longer than the thoracic part (Figure 2A), characterized by a small diameter, while in Turkey the cervical part extended to form the crop. Both esophageal segments contain highly longitudinal folds along the esophagus this result was acceptable the crop both esophageal segments contain highly longitudinal folds along the esophagus segments findings in common quail by (Zaher *et al*., 2012) in geese (*Anser anser*) (Shehan, 2012) and in Japanese quail (*Coturnix japonica*) by (Wilkinson *et al*., 2018). The numbers of the folds in turkey was (6-8) (Figure 1A), while in ostrich was (8-10) (Figure 2A) the internal surface of the both bird was thick covered by the stratified squamous non keratinized measurement of the esophagus of turkey and ostrich respectively (Table 1).

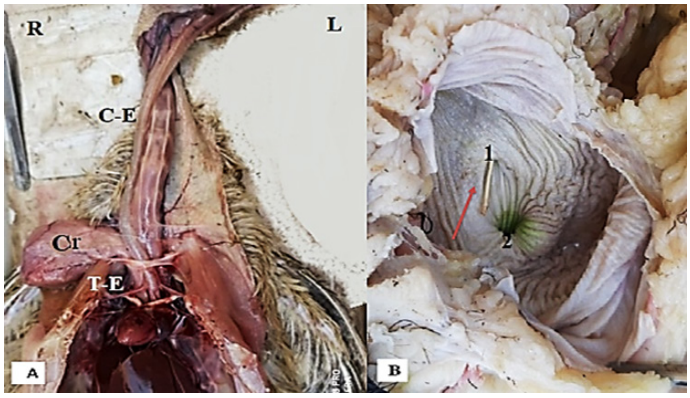

Figure 1: Photograph illustrate: A: The lesophagus in turkey consist from three parts cervical esophagus (C-E), thoracic esophagus (T-E).and crop (Cr), and relative with Trachea (T), B: The crop after fixed by 10% formalin, mucosal fold (brown arrow).

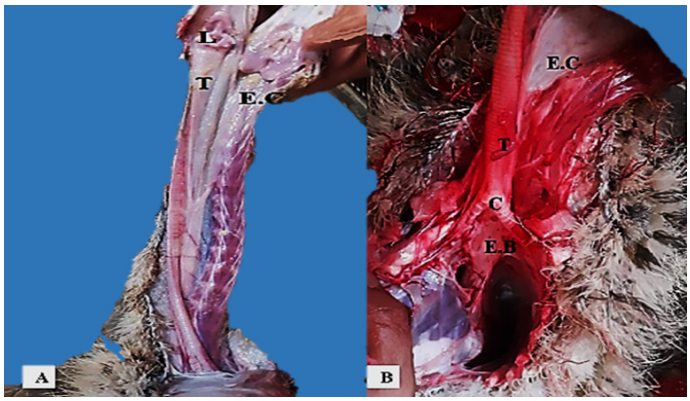

Figure 2: Photograph illustrate, A: The esophagus in ostrich (black arrow).(A):Cervical esophagus(E.C.) longitudinal folds (black arrow), (B):Thoracic Esophagus (E.T), (Trachea (T), Syrinx (C).

Table 1: Mean and standard error of morphological Measurement the esophagus male chicks turkey and chick male ostrich.

Measurement	Turkey	**Ostrich**
Weight (gm)	350±0.4	2660.2 ±0.418
Length (cm)	12.66 +0.8	21.7 +1.08
Diameter (cm)	6.05 ± 1.	8,90 ± 0.56

Crop

Crop was the second part of the turkey esophagus being an extended part located in the thoracic inlet (Figure 1A, B). The internal surface contains a longitudinal and circular fold and presents two orifices. The crop was absent in the ostrich esophagus. The middle part of the esophagus is run to the left and then returned to the right, before entering the thoracic inlet.

The mean total length of the esophagus, in the turkey, was 12.66+0.8cm, and in the ostrich was 21.0.7+1.08 while the diameter in the turkey was 6.05±1.024 cm .in the ostrich 8,90±0.56 cm. In both ostriches and turkeys, the cervical portion was longer than the thoracic portion this result agrees with the finding in a duck in Muscovy duck (*Cairina moschata*) by Pourhaji and Hashemi (2020) but different from in chicken (Nasrin *et al*., 2012).

The second portion of the turkey crop varied in shape and size depending on the eating habits of ostriches and turkeys species and the shape of the crop, Chickens and Pigeons were spherical, while waterfowl are spindle-shaped (Zaher *et al*., 2012), in Muscovy ducks (Pourhaji and Hashemi, 2020), but different from some ostriches and turkeys lack the crop such as in Grey-backed shrike (*Lanius tephronotus*.) by (Zhu, 2015) in barn owl by (Oyelowo *et al*., 2017). However, this was contradicted. the last part was short and wide. While the ostrich esophagus was a muscular longitudinal tube similar to the esophagus in Turkey but lack the crop this result was found to agree with Rhea (Rodrigues *et al*., 2012), in Grey-backed shrike (*Lanius tephronotus*) black–tailed crake (*Porzana bicolo*) by (Zhu, 2015), and in the barn owl (Oyelowo *et al*., 2017). The crop absences in ostrich are due to having a large glandular stomach that leads to storage of the food before digestion. Their digestive efficiency is comparable to that of herbivorous mammals because they can digest and degrade about 38% cellulose and 66% hemicellulose of their consumed meals (Cooper and Mahroze, 2004).

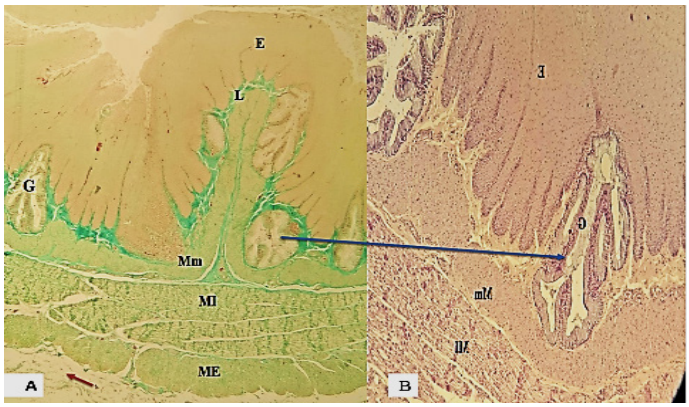

Figure 3: Photomicrograph section illustrated (A): the esophagus male turkey cervical part the epithelium (E),(lamina propria (L.P) ,musculais mucosa (Mm), two layers of tunica muscularis (MI), (ME) , tunica adventitia (brown arrow), Masson's Trichrome stain 40× (B)shows tubular acinar gland in turkey H and E) stain100 ×.

Histology

Under the microscope, the esophagus in turkey and ostrich is consist of four layers or tunica including the mucosa such as the alimentary canal composed of four layers. In Turkey the mucosal epithelium was a thick layer of stratified squamous epithelium cells non-keratinized (Figure 3A) as described in other avian species (Nagy *et al*., 2005; Zaher *et al*., 2012), in geese by (Shehan, 2012), in Blue and Yellow

Macaw by (Rodrigues *et al*., 2012), under the epithelium lies Lamina propria containing mucus glands (*Esophagus glands*), this finding reported in pheasant (*colchicus*) by (DehghaniTafti *et al*., 2017), in wild turkey (Rodrigues *et al*., 2012) and in Hoopeoe (*Upupa epops*) by (Al-Kinany, 2017) and disagreement by (Al-Kinany, 2017) and (AbdElnaeem *et al*., 2019) in Kingfisher (*Halcyon smyernensis*), The type of these glands are tubular alveolar glands in turkey (Figure 3B). While it is a simple tubular gland in the ostrich (Figure 4A) while these glands were different amounts depending on the age in wild turkey (Yovchev *et al*., 2017).The mucosal tubular acina gland less in number in crop that stated in pheasant (*Phasianus colchicus*) by (Parisa *et al*., 2019), that produce mucous material (Figure 4A, B).such as secreting acid mucopolysaccharide. The cells lining these glands are characterized as high columnar cells and basal nuclei that surrounding by smooth fibers (Figure 4B).These glands in both ostriches and turkeys are present along the esophagus and decrease in the distal portion of the esophagus but it is absent in the crop (Figure 5) such as in homing pigeon (*Columba livia domestica)* (Kadhim and Mohamed, 2015) and in Grey-Backed Shrike (*Lanius tephronotus*) (Zhu, 2015) and in pheasant (*Phasianus colchicus*) (Parisa *et al*., 2019) in ostrich disagreement with Kestrel and Linnet, (Rajabi and Nabipour, 2009). they have mucous glands. this gland is lined by columnar cells with a basal nucleus similar observed in grey-backed shrike (Zhu, 2015). These glands are embedded in dense connective tissue and rich with blood vessels (arteries, veins, and lymphatic) and nerve ends.

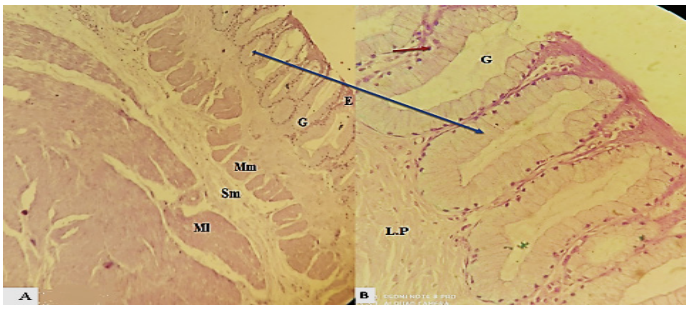

Figure 4: Photomicrograph section illustrated the esophagus in ostrich cervical part H and E stain 100×,(B): the gland in ostrich simple tubular gland (G) (mucus gland) PAS stain 200 ×:B: epithelium (E),(lamina propria (L.P), lamina muscularis (Mm); (Mm), tunica muscularis (MI) and (ME).

The lamina muscularis in both ostriches and turkeys was very development which extended to the tip of the mucosal fold (Figures 3, 4). this result is acceptable with finding instated in pigeons by (Batah, 2000), in Macaw by (Rodrigues *et al*., 2012) in Rhea (Rhea Americana) by (Rodrigues *et al*., 2012) in guinea fowl by (Gosomji *et al*., 2016). The measurement of the tunica mucosa of turkey and ostrich are presented in (Table 2).

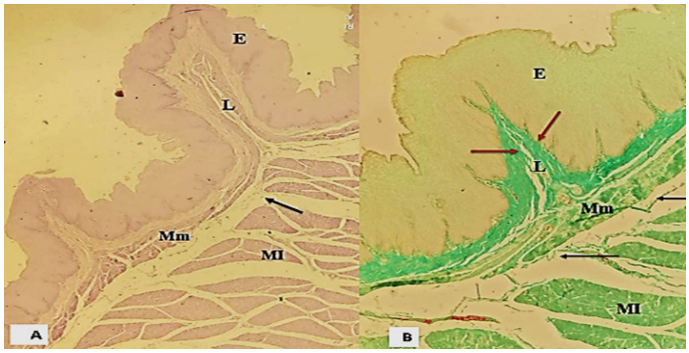

Figure 5: Photomicrograph section showing the mucosal fold in turkey (crop), epithelium (E), lamina propria contain high number of collagen fibers (brown arrow), lamina muscularis (Mm), submucosa (black arrow), inner tunica muscularis (MI), A: (H and E) stain 100 ×, B: Masson's Trichrome stain 100×.

Submucosa in turkey is very difficult to distinguish (Figure 6A). While in ostrich was very clear in the histological section (Figure 7). this state agreement with blue and yellow macaws Macaw by (Rodrigues *et al*., 2012) but disagreement with some ostriches and turkeys lack the submucosa such as geese (Anser anser domestic) by (Shehan, 2012) in Grey Backed Shrike by (Zhu, 2015) in Kingfisher (*Halcyon smyernensis*) by (Al-Kinany, 2017). They are located between the muscularis mucosa and tunica musculeris which is consist of connective tissue rich with blood vessel and nerve, that gives green color with Masson's stain (Figures 5B, 6A). The measurement of tunica submucosa of turkey and ostrich are presented in (Table 2).

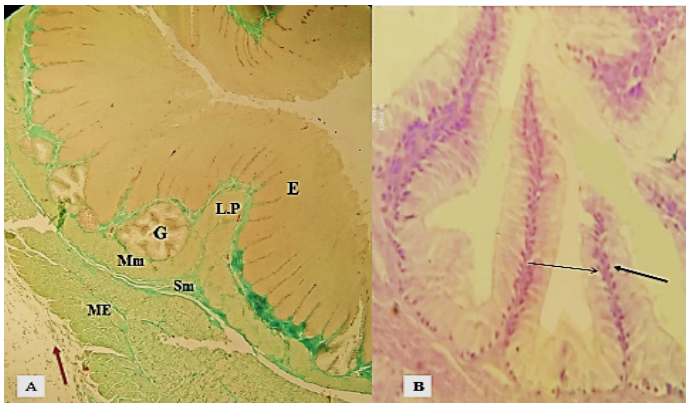

Figure 6: Photographic section of the thoracic part esophagus in chick turkey, (A): The mucosal folds become, elongated, and unbranched leaving only a narrow lumen, *T. serosa* (brown arrow) A: Masson's Trichrome stain A: 100 X, (B) mucus gland in turkey lined by columnar cell with basal nucleus: PAS stain 400 x.

Tunica muscularis in turkey is made up of two thick layers' outer (longitudinal) and inner (circular) that form from the skeletal fibers muscle especially in the cervical part of the esophagus in addition to the smooth muscle fibers

Table 2: The mean and standard error of histological Measurement of the esophagus. turkey and ostrich.

Measurement (µm)	Turkey			Ostrich			Crop of turkey
	Neck region	Thoracic	Abdominal	Neck region	Thoracic	Abdominal	
Height of epithelium	130±0.2	110±0.1	70±1.2	90±3.03	25±0.4	50±2.4	100±2.1
Tunica mucosa	830±4.03	140±0.6	110±2.8	947±2.07	105±1.3	130±0.6	90±0.1
T. sub mucosa	20±0.3	20±0.4	20±1.4	45±3.3	30±0.3	50±1.3	30±0.4
Tunica muscluris	595.20±4.26	290±0.2	125±0,1	1600±3.67	230±1.4	180±1.2	600±1.2
T. adventitia/ serosa	40±2.5	50±2.1	25±1.7	10±2.4	50±1.1	70±1.6	100±3.2

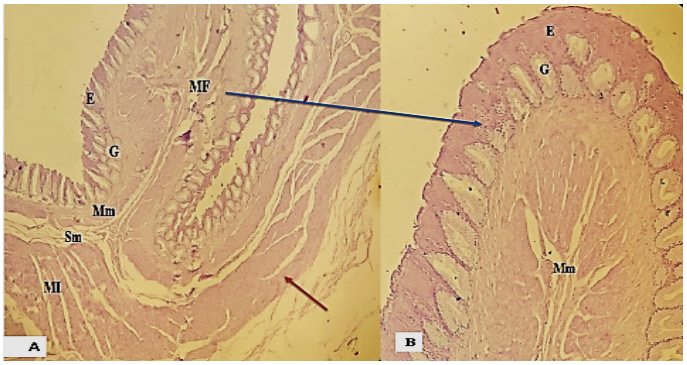

Figure 7: photographic section ostrich showing the mucosal fold (MF) was elongated (H and E) stain 100×, (B): thoracic part of esophagus ostrich B: (H and E) stain 200 x. epithelium gland (G), submucosa (SM), lamina muscularis (Mm).

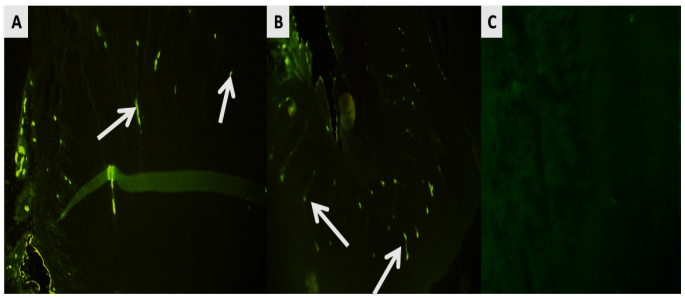

Figure 8: Immunohistochemical image of the esophagus in (A: turkey; B: ostrich, C: negative control) show immunoreactive cells (white arrows) (lable by FITC with the secondary antibody) in the mucosal layer that expression of serotonin cells. And negative control (C) when the omitted of primary antibody and used secondary antibody. (A and B: X200; C: X400).

(Figure 3A). While in ostrich tunica muscularis consist of two layers of smooth muscle bundles that are divided according to orientation circular inner layer and longitudinal outer layers (Figures 4, 7). this result reported agreement with those observed in blue and yellow macaw by (Rodrigues *et al*., 2012), in pheasant (*Phasianus colchicus*) by (Parisa *et al*., 2019) and in Kingfisher (*Halcyon smyrnensis*) (AbdElnaeem *et al*., 2019). The tunica adventitia of the cervical portion and crop in both ostriches and turkeys was thickness it is composed of loose connective tissue with blood vessels, while the thoracic part in both ostriches and turkeys was covered by a tunica serosa, (Figure 4A). these results same as reported in chicken by (Rossi *et al*., 2005), in the homing pigeon (*Columba livia domestica*) by (Kadhim and Mohamed, 2015).

Crop

The second part of the turkey esophagus was lined with epithelium stratified squamous non-keratinized epithelium beneath this a lamina propria is characterized by a lack of glands. The muscular mucosa consists of smooth muscle (Figure 5A). Submucosa tunica is similar to the submucosa in the cervical part, and muscularis tunica is delimited by two layers, the inner and outer skeletal muscle, tunica adventitia was a typical layer' consist loose connective tissue (Figure 5A). The crop in turkey similar in blue and yellow macaw by (Rodrigues *et al*., 2012), lined by keratinized stratified squamous epithelium and containing four tunica was identical to those observed in the esophagus (cervical part), this result finding in Kestrel (*Falcon iununculus*), house sparrow (passer domesticus) (Rajabi and Nabipour, 2009). The measurement of Adventitia of turkey and ostrich are presented in (Table 2).

Histochemical study

The PAS stain was used to determine the type of mucin secreted by the esophagus glands in both the ostriches and turkeys and used the Masson's Trichrome stain to determine the collagen fibers in lamina propria and to separate the muscle fibers in tunica muscularis (Figure 3A, 5B).

Histochemical and immunohistochemistry study

Expression serotonin immunoreactivity in the esophagus of male turkey and male ostrich, endocrine-IR cells were detected in the mucosal Epithelium Layer (Figure 8). Morphologically, these cells were totally colored by FITC label by secondary antibody , lead to it possible to visualize a nuclear halo. The nucleus of these cells is small, occupying a tiny area inside them.

Esophagus glands was giving positive reaction to PAS that indicated the gland was secreted mucous polysaccharide this finding agreement (Li *et al*., 2015) in guinea fowl. esophageal glands which secrete acid mucopolysacchride,

the mucins that act as a resistant barrier and protect the mucosa from chemicals, enzymes, mechanical injuries and microorganisms (Zhu, 2015)

As the main neurotransmitter, serotonin (5-HT) acts on 5-HT receptors to regulate a number of processes, including locomotor activity, cognition, emotion, appetite, and endocrine function. Immunohistochemistry techniques have been used to identify 5-HT receptors as of late (Li *et al*., 2015; Khaleel *et al*., 2021).

CONCLUSIONS AND RECOMMENDATIONS

Gross observations

Examination of the esophagus in both male ostriches and turkeys revealed a muscular, membranous tube. In turkeys, the esophagus comprised three distinct segments, while ostriches possessed only two. The cervical portion, the longest segment in both birds, was located on the right side of the neck, dorsal to the trachea.

Microscopic observations

Microscopic analysis demonstrated that the esophagus in both male turkeys and ostriches was composed of four distinct layers. The mucosal layer displayed folding throughout its length in both species. The cervical region in both birds harbored numerous mucous glands situated within the lamina propria. The distribution and density of these glands varied along the length of the esophagus. Notably, the submucosal layer exhibited a significant difference in thickness between ostriches and turkeys. The muscularis layer in turkeys consisted of skeletal muscle fibers, whereas in ostriches, it comprised only smooth muscle fibers. Finally, the outermost layer, the adventitia or serosa, was composed of loose connective tissue.

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NOVELTY STATEMENT

Comparison of the anatomical , histological, histochemical, and immunohistochemically structure of
the esophagus in turkeys (Meleagris gallopavo) and ostrich (*Struthio camelus Linnaeus* 1758).

AUTHOR'S CONTRIBUTION

All authors are equally contributed in planning, writing a draft and final manuscript, experimental design and laboratory work, statistical analysis.

Ethical approval

Current study approved by Ethics Committee at the Department of Anatomy and Histology, College of Veterinary Medicine University of Diyala.

Conflict of interest

The authors have declared no conflict of interest.

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