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Morphometrical and Histological Study of Esophagus in Adult Partridge (*Alectoris chukar*)and Harrier(*Circus aeruginosus*)

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Abstract

This study was conducted to identify the morphometrical and histological characteristics of the oesophagus in adult Partridge (Alectoris chukar)and Harrier (Circus aeruginosus).To conduct such a project, twenty healthy adult partridges and Harriers, ten of each species, were obtained from local hunters, which were between 1 and 2 years old and weighed about 400 to 550 gm. for Partridge and 2 to 3 years, and the weight ranged from 390 to 750 gm. for Harrier. Birds were killed with an intramuscular injection of ketamine and diazepam, then dissected to collect esophageal specimens, which were cleaned with normal saline . The length and diameter of esophagus were measured, then specimens were stored in 10% formalin for 48 hours to undergo normal histological technique. Hematoxylin-eosin and Crossman's stain were used to stain sections. Gross findings of the esophagus revealed that the esophagus of Partridge and Harrier was a muscular, long and thin translucent tube divided into three parts: cervical, thoracic and abdominal parts. Histological structure of the esophagus wall in both consisted of three well-defined tunics. Tunica mucosa of partridge consists of stratified squamous epithelium, which was significantly higher than the epithelium of Harrier, and consists of high numbers of esophageal glands and lymphatic nodules compared with that of the Harrier's esophagus, while the thickness of the muscular tunica of Harrier's oesophagus was thicker than that of partridge. The variation in the esophagus of the partridge and harrier was related to the food types consumed and feeding behaviors.

Key words: Alimentary tract ,Esophagus, Harrier ,Partridge.

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Introduction

According to their differences in lifestyles, birds have different feeding habits, with corresponding differences in the structures of their digestive canal. The Chukar Partridges (*Alectoris chukar*) belong Partridges breed, Aves class, Galliformes order. The Chukar habitat is the mountainous areas in the north of Iraq (Sulaymaniyah, Sulaimani, Erbil, and Dhouk).(1)
The Harrier (Marsh Hawk) is a prey bird; the diet of Marsh Harriers mainly comprises mammals, birds, fish, and reptiles (2). The digestive passage or tract in birds is composed of the oral cavity, pharynx, esophagus, proventriculus (glandular part of the stomach), ventriculus (muscular part of the stomach), small and large intestine,ceca if present, colon and cloaca (3).

The esophagus is a long tubular, distensible organ running at the right side of the neck, connected between pharynx and the stomach it was pass through the cervical and thoracic regions (4). The esophagus plays double functions, transport-feeding material and acting as temporally store and maintaining food temperature.*The capacity of the gizzard is limited to storage. Its maximum capacity is ten grams of feed. Extra or large quantities of feed that taken at once, are stored in the esophagus or crop* (5,6).The oesophagus wall in birds is histologically made of four distinguished tunica mucosa, Tunica submucosa, Tunica muscularis and Tunica adventitia. The mucosa of esophagus was folding longitudinally (7,8). The avian alimentary duct has undergone some physiological and structural characteristic in varies with other animals to accommodate a wide variety of food kinds (9,10). According to the type of diet consumed, avian species are classified into granivorous that feed on seeds and grain (11). Omnivorous species feed on seeds, insects and fruits in their diets (12). The carnivorous birds, like prey birds, feed on meat (13). The present paper aimed to report the morphometrical and histometrical characteristics of the esophagus in two wild birds, Partridge (granivorous bird) and Harrier (carnivorous bird).

Materials and Methods

For gross examination, twenty adult apparently healthy of partridge and Harriers, ten of each species with no respect to sex. Birds were collected from local hunters, and the birds euthanized by intramuscular injection of a mixture of ketamine and diazepam at dose 25 and 5 mg /kg BW, which agree with (14).The length and diameter of the body of esophagus were measured (15).By measurement tape,ruler and digital electronic vernier were used, then esophagus of two birds were captured using a digital camera. Specimens were washed with normal saline and settled by 10% neutral buffer formalin for 48 hours at room temperature. Following fixation, the specimens were washed with tap water and underwent two hours of ethyl alcohol dehydration (70%, 80%, 90%, and 100%) followed by fifteen minutes of xylene clearing. In order to obtain paraffin blocks, specimens were first infiltrated with paraffin wax at a temperature of between 58 and 60 ºC, then a rotating microtome was used to cut paraffin slices that were six microns thick. (16). Paraffin block sectioning to (5-7) micrometre thick . Harries Hematoxylin and Eosin (H&E) stain for

demonstrating the general histological components, and Crossman's stain for connective tissue and muscle fibers used. The stained slides were examined by a light microscope. Thicknesses of each tunica for all parts of esophagus measured by ocular micrometer.

Statistical analysis

Statistical analysis of data performed using SAS (Statistical Analysis System - version 9.1). Independent T test used to assess significant differences between means. $P < 0.05$ is considered statistically significant (17).

Results

In the present study, the esophagus of Partridge and Harrier was a muscular, long and thin translucent wall tube located on the right side of the neck, dorsally to the trachea, and extending from the oropharynx to the proventriculus. The esophagus was wide and highly distensible. The ability of the esophagus to distend was facilitated by the occurrence of a huge amount of loss of connective tissue and flabby, wrinkly skin. The luminal surface of all esophagus parts showed large and extensive longitudinal parallel luminal folds, which help to accommodate large amounts of food or large pieces of food (Fig.1,2,3).In partridges, the esophagus was divided into three parts: the cervical, pre-crop, crop and another part called the thoracic or post-crop part. The first part extends from the oropharynx to the entrance of the thoracic cavity. Then the ventral wall was expanded to form the crop. After that, the thoracic (post-crop part) comes, which extends dorsally to the trachea and into the thoracic-abdominal cavity to end with the proventriculus.
The crop was an enlargement of the esophagus, it has a single pouch, and it was positioned at the entrance of the thoracic cavity, which had an important storage function in grain-eating birds (Fig.1). The length of the partridge esophagus was (8.03±0.04) cm, and its width was (1.01±0.03) cm (Table 1).

The light microscope examination of histological slides revealed that both bird species were similar in general structures, with a few differences. The general histological structure of the esophagus wall consisted of three well-defined tunics (mucosa, muscular, and adventitia or serosa) (Fig.5).
The tunica mucosa of the partridge esophagus consists of stratified squamous epithelium, and no keratin was noticed. The thickness of this layer was (161.90±0.83)µm, which was significantly higher than the epithelium of Harrier, which was (83.60±2.09)µm. (Table 2). This difference was related to the nature of the food each bird consumes. Partridges feed on dry, rough food; it was a grain for coarse birds.
The lamina properia submucosa consists of loose connective tissue with mucous glands and lymphatic nodules. The thickness of this layer was (159.70±0.36) µm thicker in partridges than in

Harriers, at (94.50±1.46) µm. (Table 2). This difference was caused by a high number of glands in the compeer with that of Harrier esophageal, glands were simple mucus oval in partridge, in harrier, it was circular and fewer in number than that of partridge. Each gland opened with a separate duct to the surface of the epithelium. The lining cells of esophageal glands were columnar; the cytoplasm was light-stained with a dark basally located nucleus (Fig.6). Lymphatic nodules were present in the lamina propria-submucosa, especially at the end of the thoracic part (Fig.7).
The muscular tunica was thick and very well developed; it consisted of two layers: longitudinal and circular, arranged in bundles of smooth muscle fibers (Fig.5).
The study revealed that the thickness of the muscular tunica of the Harrier esophagus was thicker than that of the partridge. They were (288.90±3.46)µm.in Harrier and (258.10±1.20)µm.in partridge (Table 2). The cervical part of the esophagus is covered by connective tissue adventitia, where the cervical part is covered with thin, loose connective tissue and mesothelium forming serosa membrane (Fig.5).

Table (1) shows the length and width of esophagus In two different adult birds (adult partridge and Harrier).

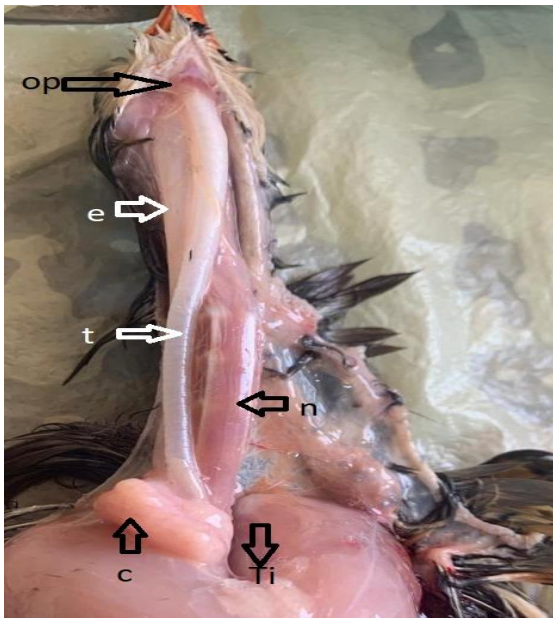
Bird	No.	Width (Cm.)	Length (Cm.)
Partridge	10	1.01±0.03	8.03±0.04
Harrier	10	1.80±0.01	11.52±0.04

Number: No., Mean± Stander error : M±SE, P-value <0.0001

Table (2) shows the Thickness of the esophagus wall layers in two different adult birds (adult partridge and Harrier).

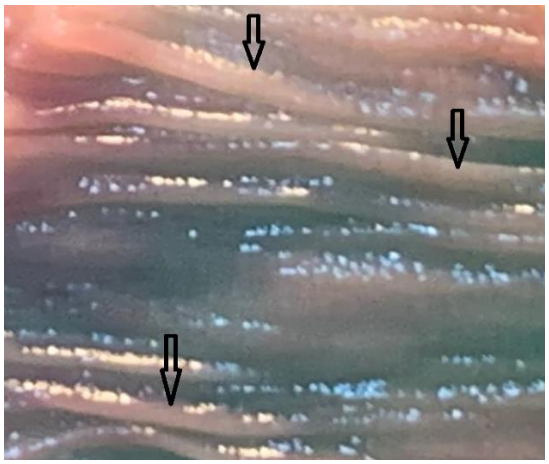
Bird	No.	Epithelium (µm.)	Propria-submucosa(µm.)	Muscular layer(µm.)
Partridge	10	161.90±0.83	159.70±0.36	258.10±1.20
Harr	10	83.60±2.09	94.50±1.46	288.90±3.46

Number: No., Mean± Stander error : M±SE, P-value <0.0001



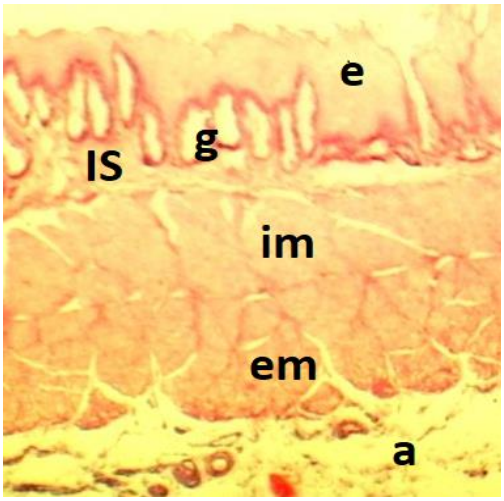
Figure(1) Macrograph of partridge shows:op.oropharynx,e.esophagus,c.crop,t.treachia,n.neck,Ti.Thoracic inlet

Figure(2) Macrograph of Harrier esophagus shows:op.oropharynx, fd.fusiform dilatation,esj. Esophageal stomach junction.

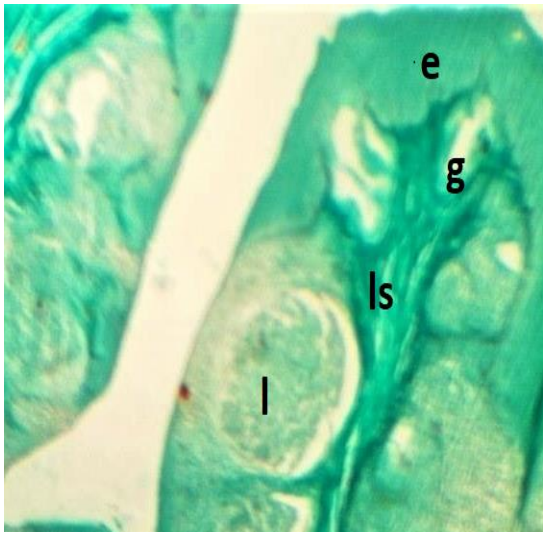


Figure(3) Macrograph of luminal surface of esophagus in Harrier shows: esophageal folds (arrows).

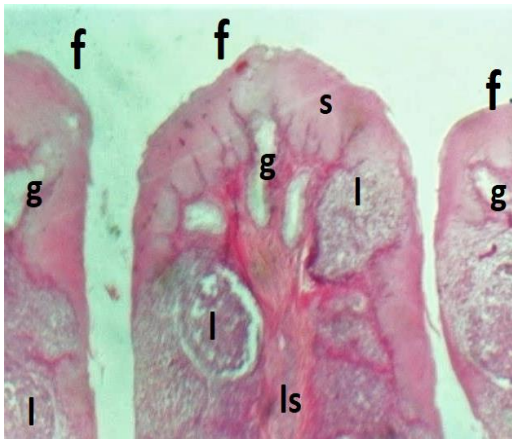
Figure(4)Micrograph of esophagus wall inpartridge shows: e. epithelium, g. glads ,ls. propria-submucosa (H&E stain×100



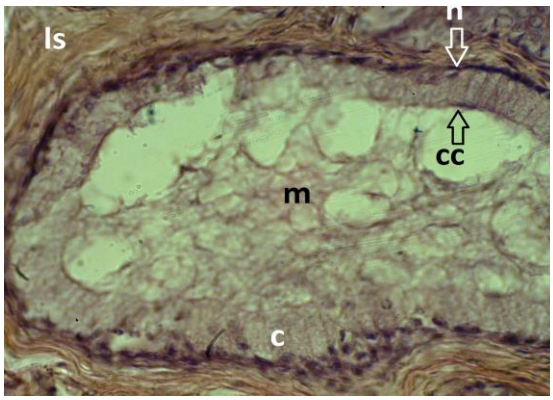
Figure(5): Micrograph of esophagus wall in Harrier shows: e .epithelium, g. glands, ls. properia- submucosa ,im.intenal muscls,external muscls, a. advantitia(H&E stain×40).



Figure(6)Micrograph of esophagus wall of partridge shows:e.epithelium,g.glnads,ls. propria-submucosa , l. lymphnodule(Crossmman´s stain ×100).



Figure(7)Micrograph of esophagus wall of partridge Shows :e .epithelium, g.glands, ls. propria-submucosa , l.lymph ,f.fold nodule(H&E stain ×100).



Figure(8)Micrograph of esophageal gland shows:e.,ls. propria-submucosa , n.nucleus ,cc. columnar cells,c.cytoplasm,m.mucous (H&E stain ×400).

Discussion

The esophagus of the Partridge and Harrier has the same morphological structure as a long, thin-walled, muscular tube, and it was wide, highly distensible, which was supported by loose connective tissue and flexible neck skin, allowing it to expand easily. The luminal surface showed

extensive longitudinal folds that helped accommodate large amounts of food. These results were constant in almost all bird species. (18) They reported that the esophagus of the common quail was a long tubular organ, while (19) writes the same description about the esophagus in the grey-backed shrike. The esophagus was long because the neck was characterized by its noticeable length in compression with most mammals (6) and (3).In this study, the highly flexible skin of lost connective tissue with the presence of luminal folds gives the esophagus the ability to expand and accommodate foods that play a role in food storage, according to (20, 21), who reported this observation.

All bird species that feed on grains need the presence of the crop because it is used for storing and softening the dry food before passing it to the stomach. (21) Proved the fact that reviewing the function of crops in birds. In wild birds and prey birds, like in black-winged kite birds, the esophagus had a spindle-shaped crop, hardly recognizable when it was empty of food (6,20). The esophagus in this carnivore's bird was relatively long with a poorly developed crop. The variation in the esophagus of the partridge and harrier was related to the food types consumed by the birds and different feeding behaviors, as proved by the study (10,18,21) on the esophagus of different bird species. This study suggests that the difference in length and width of the esophagus is a compensatory strategy for the absence of large crops, which play a role in food storage. The previous facts are in agreement with what was recorded by (22,23,24). They found in *Rhynchotus* rufescens birds, home pigeons, and kingfishers.

Histologically, the wall of the esophagus consisted of three layers (mucosa, muscularis, and adventitia or serosa). These results were in agreement with the results of (10) and (25), who recorded that the mucosa showed no evidence of a separate tunica, and the muscularis mucosa was absent. The wall of the esophagus in wild birds, pigeons, and Kingfishers consisted of three tunics, like that of studded birds. These results disagree with the results of (18), who found that the wall of the esophagus in common quail was formed of four tunics (mucosa, submucosa, muscular, and adventitia). They found a layer of smooth muscles forming the muscularis mucosa, which separated the tunica mucosa from the tunica submucosa.

There, the epithelium was thicker to give proper protection. The thickness of the epithelium was significantly higher than that of Harrier. This difference was related to the nature of the food each bird consumes. Partridges feed on dry, rough food; it was a grain for coarse birds. This fact was proved by (25,26), who proved that the stratified epithelium withstands mechanical stress by increasing the cell-division rate and thickness of the dividing region in the epithelium.

The present histological findings recorded that the thickness of the lamina properia submucosa was thicker in partridge than harrier, which consists of loose connective tissue with mucous glands and lymphatic nodules. This difference was caused by a high number of esophageal glands in comparison with those of the harrier.

These results were similar to the findings of (22) in partridge, (10) in Hoopoe and Kingfisher; they stated in their studies that the esophagus was lined throughout its length with non-keratinized stratified squamous epithelium. The lamina properia -submucosae consist of connective tissue with lymphatic tissue. Esophageal glands were abundant within the lamina properia mucosae. There were small tubule alveolar glands with ducts penetrating the stratified squamous epithelium. The present study disagrees with the results of (23), who reported that the stratified squamous epithelium of the esophagus in the home pigeon had keratin.

The presence of esophageal glands in connective tissue of properia-sub mucosa was common in all birds, as mentioned by (18) in common quail, (20) in kingfisher, (23) in home pigeon. The esophageal glands secreted mucin to the luminal surface, which acts as a lubricant and moisture medium to facilitate the movement of food through the esophageal tube. This function is accomplished due to the chemical composition of mucus, as stated by (27), he found that the mucus contains oligosaccharide glycoprotein, proteins and water. In addition to lubricating, it is reported that the mucin protects from the harmful mechanical and toxic effects of food intake. The number of glands in the partridge was more than in the harrier.

These variations in the number of glands are related to the differences in the type of foods consumed by the birds. The meat, which is the main food of Harrier, was moist in nature. The water percentage was about (65-75) %in meat, which was higher than the water percentage in partridge, which depends on its feeding on grains. The moisture of the meat facilitated swallowing. This suggestion was in parallel with the analysis data reported by (28; 29), who found high moisture in the fish and poultry meat. Grains or seeds' moisture was (25-33.3) percent, as mentioned by (32), who used a different method to measure the water content in seeds. The presence of lymphatic nodules in the properia submucosa was in parallel with the findings and the result of (30,31), they mention that the esophageal lymphatic tissue forms nodules or tonsil located in the mucous submucosa of the junction between the caudal part of the esophagus and the glandular stomach. The presence of this lymphatic nodular tissue underneath the esophagus epithelium, which contains (T and B) lymphocytes, is important in digestive tract immunity. This finding was proved in papers published by (32,33,31). Current microscopic findings indicated that the muscular tunica was thick and consisted of longitudinal and circular smooth muscle fibers. This result was consistent with the studies of many researchers (25) in gees (34) in wild birds' esophagus, they found that the two layers of smooth muscle fibers, the fibers lie in circular and longitudinal orientation. Such muscular layers facilitated the food swallowing and move to the stomach, as proved by (6, 9), they review and study the physiology of birds' digestive system. Some authors had different findings. (10) Claimed that the muscular layer, underlying the submucosa, consists of one layer of circular type.

According to the present study, the Harrier esophageal muscle tunica was thicker than the partridge's tunica muscularis. This variation could explain, depending on the findings of (13,35). They found that the prey birds that feed on different prey get rid of undigested material, such as

bones, feathers, and hair, by compacting it into pellets, then move toward the oral cavity by the esophageal anti-peristalsis contraction of the muscular layers.

The microscopic present study showed thin loss of connective tissue and mesothelium creates the serosa membrane on the cervical portion of the esophagus, which is covered by connective tissue adventitia. This result was proven in all bird species. The cervical part of the esophagus, with the crop, is covered with wide, extended connective tissue adventitia. The thoracic one is covered with a serosa membrane of thin connective tissue enveloped by mesothelium. The current study suggests that this variation in coating may belong to the early formation of the foregut in the embryo. Otherwise, the adventitia helps in esophagus expansion during food swallowing, as mentioned by (20), they stated that the esophagus of the falcon is a highly distensible tube.

The serosa covers the part of the esophagus in the body cavity. The reason for that is related to the structure and function of the serosa. (36) stated that the mesothelium extends as a monolayer over the entire surface of the serosa of the body cavity. The mesothelium rests on a thin basement membrane supported by thin connective tissue. This serosa membrane protective barrier guarantees free movement of the organ in the body cavity.

Conclusions

The conclusions of the present study revealed variations in structural characteristics of the esophagus of partridge and harrier, which were related to their feeding habits.

Conflict of Interests

There are no conflicts of interest revealed by the author.

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Ethical Clearance

This work is approved by The Research Ethical Committee.

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دراسة شكلية قياسية ونسيجية للمرئ في الحجل والمرزة البالغ

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الخلاصة

أجريت هذه الدراسة للتعرف على الجوانب المورفومترية والنسيجية للمريء في الحجل والباز البالغ .لإتمام هذا المشروع تم الاستعانة بعشرين من طيور الحجل والمرزة البالغة السليمة، عشرة من كل نوع بدون الاهتمام الى نوع جنس الطيور من الصيادين المحليين وتراوحت الاعمار بين 1الى2 سنة وبوزن 400 الى550غرام لطائر القبج وبعمر 2الى 3 سنة وبوزن 390 الى 750غرام بالنسبة لطائر المرزة. تم القتل الرحيم للطيور عن طريق الحقن العضلي لخليط من الكتامين والديازيبام، وتشريحها ثم تم جمع عينات المريء ثم غسلها بمحلول ملحي عادي. تم أخذ قياس طول ووزن جسم المرئ وتم ثبيت العينات باستخدام10٪من الفورمالين.تم إخضاع العينات لعمليات روتينية مثل التجفيف والتطهيروالطمر، وتحضير البلوكات. تم تحضير مقاطع من 5-7 ميكرومتر وصبغها باستخدام الهيماتوكسيلين وألايوسين صبغة كروسمان . كشفت النتائج الإجمالية أن المريء في الحجل والمرزة كان عبارة عن أنبوب عضلي طويل ورفيع شفاف.تبين انه في الحجل ينقسم المريء إلى ثلاثة أجزاء حيث كان طول وعرض المريء في الحجل 8.03 ± 0.04 سم و1.01 ± 0.03 سم على التوالي، في حين كان الطول والعرض في المريء 11.52 ± 0.04 سم و 1.80 ± 0.01 سم على التوالي.كشفت النتائج المجهرية أن التركيب النسيجي لجدار المريء في كليهما يتكون من ثلاث غلالات او طبقات محددة جيدًا. تتكون الغلالة المخاطية للحجل من ظهارة حرشفية طبقية والتي كانت أعلى بكثير من ظهارة المرزة . تتكون الغلالة المخاطية من أعداد كبيرة من الغدد المريئية والعقيدات اللمفاوية التي تتنافس مع تلك الموجودة في مريء المرزة. وكشفت الدراسة أن سمك الغلالة العضلية لمريء المرزة وأكثر سمكا من سمك الحجل.كان التباين في المريء لدى الحجل والمرزة مرتبطًا بأنواع الطعام التي تستهلكها الطيور وسلوكيات التغذية المختلفة.

الكلمات المفتاحية: المسلك الهضمي, المرئ, الحجل, المرزة.