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Histological and Histochemical Study of the Esophagus Before and After Crop in the Guinea Fowl (N*umida meleagris*)

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Abstract

ESOPHAGUS is an important organ of the digestive system and having different layers of tissue in every species of animal plays an important role in digestion. The length and diameter of the esophagus are also different in animals and without on the type of species and the diet it consumes. Because there is not enough information about the histology of Guinea fowl esophagus, this research was done. Eight adult Guinea fowls were the constituted materials of the study. Initially, each bird was humanely killed one by one and subsequently positioned on its back on an autopsy tray. Their abdominal cavity was opened and the esophagus was completely removed. In each animal, sampling was done from four parts of the esophagus, including the upper third, middle third, lower third, and after the crop, and were placed in fixative solution. Then, tissue sections were prepared and stained with routine and specific dyes; Hematoxylin and Eosin, Periodic Acid Shiff, Alcian Blue and Masson's Trichrome. In Guinea fowl, esophageal epithelium was covered by stratified squamous with numerous folds in the lumen. Also, its thickness was reduced from beginning (457.89µm) to the end (216.76µm). Lamina propria consisted of loose connective tissue with collagen fibers and mucous glands. Muscularis mucosa was made of smooth muscle and its thickness was reduced from beginning (86.76µm) to the end (62.79µm). Tunica muscularis was very thick and was composed of inner thick circular and outer thin longitudinal layers. In histometrical study of esophageal layers, there was significantly different ($P \leq 0.05$). In conclusion, the esophagus of the Guinea fowl exhibits distinct irregular longitudinal folds in the tunica mucosa that constrict the lumen, similar to observations made in chickens. The lining of its epithelium consists of nonkeratinized stratified squamous cells along with numerous ducts associated with esophageal glands. Interestingly, the thickness of the epithelium decreases from the beginning of the esophagus, measuring 457.89µm, to the end, which is only 216.76µm. Additionally, the thickness of the muscularis mucosa shows variation, being thinner at the start of the esophagus and just after the crop in comparison to the other two sections.

Keywords: Histology, Esophagus, Histomorphometry, Guinea fowl.

Introduction

Guinea fowl, sometimes called pintades or gleanies, are a family of birds originating from Africa, related to other game birds such as the pheasants, turkeys and partridges[1].

The guinea fowl has a long history of domestication, and the helmeted Guinea fowl is the most studied. The helmeted Guinea fowl (Numidia meleagris) is the best identified type that belongs to the Numididae family and is the only member of the Numida genus [1].

It is native to South Africa and is widely present in other regions such as West India, Brazil, Australia and South France. One of its distinctive characteristics is having a strong horn on the head and two fleshy appendages with skin under the beak. The color of the feathers around the neck and chest of the animal is simple gray, but on the back, there are white circular spots, which become larger as they move towards the wings and cover part of the feathers in this area. It is due to the presence of these spots that in some countries the horned chicken is also called the pearl chicken. Guinea fowl meat is

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very soft and has a low fat but high in protein and niacin. Their diet includes a variety of animal and plant food, grains, fruits, vegetables, snails, worms [1,2,3]. The female guinea fowl is generally smaller than the male, and its helmet is also smaller in size compared to that of the male. Additionally, the helmet of female guinea fowls tends to be short and narrow [4].

The digestive system of birds shows a great diversity according to their respective feeding and dietary habits. [5]. It is a continuous tube and composed of mouth, esophagus, crop, proventriculus, ventriculus intestines and cloaca [6].

Esophagus is a long and flexible tube that connects the mouth to the crop and then through a short part of the esophagus to the proventriculus. It is located on the right side of neck consists of two parts; cervical and thoracic, while in mammals there are three parts; cervical, thoracic and abdominal [7,8,9]. In many birds, except for penguins, seagulls, and ostriches, the cervical part of the esophagus is expanded in the ventral region and forms a diverticulum, called the crop, which is located on the ventrolateral side of the esophagus and in front of the clavicle and pectoral muscles [10].

In the past years, many studies have been conducted on the morphology of the digestive system of some birds, especially domestic chickens, whose results have been reported in several papers [11,12,13,14].

So far, there has been no study on the histomorphology of the Guinea fowl esophagus. The purpose of this research is to investigate the histological structure of different parts of Guinea fowl, and the results can be used by other researchers.

Material and methods

The use of animals and all stages of this research were carried out in accordance with the instructions of the ethics code of Ferdowsi University of Mashhad, Iran (2/48726-15/11/2018).

Animal preparation

Eight adult Guinea fowls of male weighing between 1200g and 1900g (1410.38±67.23g) were the constituted materials of the study. They were prepared from one of the breeding farms around the city of Mashhad and transferred to the Animal Department of Ferdowsi University of Mashhad, Iran. They fed with grains, vegetables; and water is consumed. Each bird was examined for its health.

Histological study

Birds were transferred to the anatomy hall for anatomical studies. At first, all of birds were slaughtered individually in humanly method and placed on their back on an autopsy tray. Their abdominal cavity was opened and the esophagus was completely removed. In each animal, sampling was done from four parts of the esophagus, including the upper third, middle third, lower third, and after the crop, and were placed in 10% buffered formalin solution for 24-48 hours.

Then, the fixed specimens were placed in the tissue processor or Autotechnicon by passing them through alcohol solutions of different degrees (70,80,90 and 100) for dehydration, Xylene solutions for clarity and melted paraffin wax for embedding respectively. Paraffin blocks were prepared by paraffin dispenser and were sectioned at 6 micrometer thickness.

For histological and histochemical studies of esophageal tissue, the following stains were used: Hematoxylin-Eosin to examine the general structure, Masson's Trichrome to distinguish collagen fibers from other tissue components, Periodic Acid Schiff (PAS) and Alcian Blue(AB) to identify the type of neutral or acidic mucins of esophageal gland secretions[11].

For histomorphometric measurement, the thickness of different layers of the esophagus (Tunica mucosa, Tunica submucosa, Tunica muscularis and Tunica adventitia) were measured using optical histomorphometric measurement (Image J Sofware, Version 1.44p) and Olympus microscope (model BX51) and images were prepared from tissue sections.

For statistical analysis, the mean and standard deviation of the measured parameters were determined using SPSS software. Then They were compared between the four parts of the esophagus by using the ANOVA test, and the difference with a P value of less than five percent was considered significant.

Results

Histological findings

In the histological examination of the esophagus of Guinea fowl, it was determined that the wall of the esophagus consists of four layers including tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia.

The esophageal lumen had numeral irregular longitudinal folds in tunica mucosa and its mucous membrane was lined with nonkeratinized stratified squamous epithelial tissue, where numerous ducts of esophageal glands were located in the mucosal folds (Fig.1), also its thickness was reduced from beginning (457.89µm) to the end (216.76µm) (Table 1).The lamina propria was composed of scattered collagen fibers and loose connective tissue, also there were fine collagen fibers, fibroblasts and simple branched mucous glands in this layer. Esophageal glands were large mucous glands and existed from the lamina propria to the sub-epithelium of the

esophagus (Fig. 1). In the present study, there were 6 – 7 glandular units around an esophageal crypt of the esophagus of Guinea fowl. The cells that make up the mucous glands of the esophagus were high and simple columnar, and their nuclei were arranged in a row at the base. These cells and their lumen secretions strongly reacted positively with Alcian Blue and Periodic Acid Shiff staining. (Fig. 5), also its mucosal glands were reduced from beginning or upper one third to end or lower one third (FigS. 1,2,3). The muscularis mucosa which is a very well-developed continuous layer was formed of longitudinal smooth muscle fibers. Its thickness was increased from upper part to lower part but it was reduced after crop. It was 86.76µm in the beginning and 62.79µm in the end part of esophagus, after the crop. The submucosa layer was not wide compared to other layers of the esophagus and contained fine fibers of connective tissue including collagen and elastic fibers, blood and lymphatic vessels, and Meissner plexus. The tunica muscularis was made up of two separate layers of smooth muscle, the inner layer was circular and thick, and the outer layer was longitudinal and thin (Figs.1,2,3). In addition, Auerbach's neural plexus was observed between the two muscle layers. The outer adventitia layer of loose connective tissue with elastic fibers, blood vessels and nerves were formed (Figs. 1 and 4). In histochemistry of the esophageal glands, the majority of cells showed purple staining, thus indicating the occurrence of a mixture of neutral and acidic mucins (Fig.5).

Histometrical findings

The various components of the three layers of the esophagus were defined and the thickness of them was measured and recorded using the micrometric method. The mean and standard deviation of the measured parameters were calculated using the SPSS statistical software and then compared using the ANOVA statistical test among the four parts of the esophagus, and their significant difference was calculated with a P value of less than 5 percent (Table 1).

Discussion

In Guinea fowl ,like other birds, the esophagus has two parts; cervical and thoracic part. The cervical part of esophagus was a distensible segment that extended from the caudal end of the pharynx to the cranial end of the crop and it was longer than thoracic part.

This study showed that the esophagus of Guinea fowl had numeral irregular longitudinal folds in tunica mucosa which close the lumen as demonstrated in chicken [16,17] and Japanese quail [18]. Esophageal mucosa was folded and composed of stratified squamous epithelial tissue, and numerous ducts from mucous glands were opened on the sides of the mucosal folds, and these observations are similar to some birds that reported by many researchers [16,17,19]. Like in other avian species [19,20]. In guinea fowl, the mucous glands of the esophagus were located in the lamina propria and the shape of its secretory units was branched tubular-alveolar type. There are reports that the shape of the secretory units of these glands can be different in animals of the same species [19].

Shibata et al. (1991) stated that the mucous glands in domestic chickens are usually of simple tubular type, but Kum (2002) reported it of simple branched tubular-alveolar type. In addition, it has been reported that the shape of the secretory unit of the esophageal glands of duck [11], geese [21] and the Germain's swiftlet [19] are simple tubular or simple branched tubular glands. In Guinea fowl, the cells and secretions of the lumen of the esophageal glands showed an intensive positive reaction to Alcian Blue and PAS staining, which results show that the esophageal glands in guinea fowl have acidic and neutral mucins, which are similar to adult chickens [22,23], and the Germain's swiftlet [19].

The muscularis mucosa was well developed and consisted of longitudinal bundles of smooth muscle in Guinea fowl. This layer is unstable in birds and absent in some birds such as partridge [24].

In Guinea fowl, the tunica muscularis consists of two distinct layers of smooth muscle: the inner layer is thick and circular, while the outer layer is thin and longitudinal. These findings are consistent with the results reported by Nagay et al.,[16], who studied the histology of chicken esophagus. They also reported that tunica muscularis of the chicken esophagus was composed of two layers, an inner thick circular and thin outer longitudinal layer. It was similar to our results in Guinea fowl and also similar with results that found by Al-Saffar and Al- Smawy 2016[25], who study the pigeon esophagus, while disagreement with Rossi et al., [24] which has reported, tunica muscularis of the esophagus in *Partigad Raynchotus* consists of three layers of smooth muscle, two inner and outer longitudinal layers and a circular middle layer.

Unlike mammals, there is no striated muscle in the esophagus of birds, including guinea fowl.

The muscles of the tunica muscularis of the esophagus, with their contractions, cause the food to be transferred to the crop and then from the crop to the proventericulus. The tunica adventitia consists of connective tissue with vessels and nerves, and there is no difference between this layer and the tunica serosa in birds [26].

Conclusions

The current research indicates that the thickness of the esophageal epithelium in Guinea fowl decreased from an initial measurement of 457.89 µm to a final measurement of 216.76 µm. The thickness

of the muscularis mucosa exhibited variability, being less pronounced at the beginning of the esophagus and following the crop compared to the other two sections, also this study showed that the esophagus of Guinea fowl had numeral irregular longitudinal folds in tunica mucosa with large tubuloalveolar mucosal glands which close the lumen as demonstrated in chicken.

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Conflict of Interest

The authors report no conflicts of interest or financial support.

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Ethical Approval

The use of animals and all stages of this research were carried out in accordance with the instructions of the ethics code of Ferdowsi University of Mashhad, Iran (2/48726-15/11/2018).

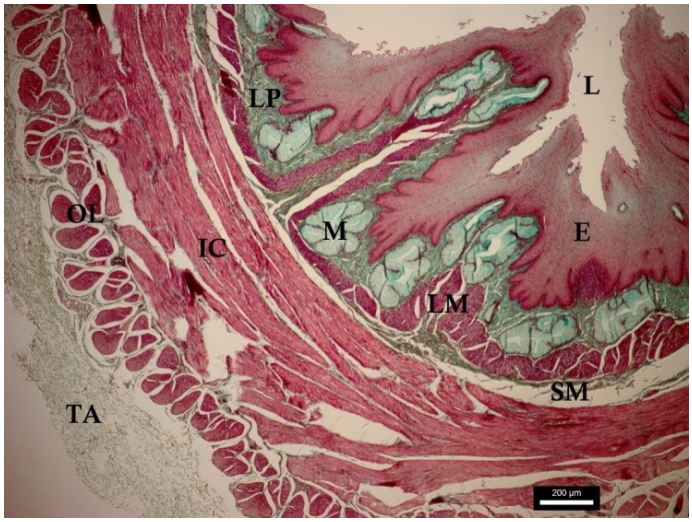

Fig. 1. Photomicrograph of upper one third of the esophagus in the Guinea fowl. Lumen (L), Epitelium(E), Lamina propria (LP), Mucous gland (M), Muscularis mucosa (LM), Tunica submucosa (SM), Inner circular (IC), and Outer longitudinal layer of tunica muscularis (OL), Tunica adventitia (TA). Masson Trichrome stain; bar=200 µm

Fig. 2. Photomicrograph of middle one third of the esophagus in the Guinea fowl. Lumen (L), Epithelium(E), Lamina propria (LP), Mucous gland (M), Muscularis mucosa (LM), Tunica submucosa (SM), Inner circular (IC), and Outer longitudinal layer of tunica muscularis (OL), Tunica adventitia (TA). Masson Trichrome stain; bar=200 µm

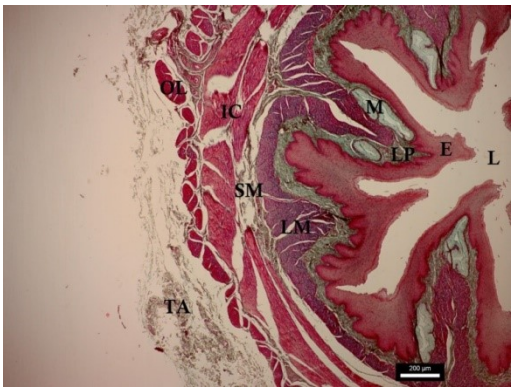

Fig. 3. Photomicrograph of lower one third of the esophagus in the Guinea fowl. Lumen (L), Epithelium(E), Lamina propria (LP), Mucous gland (M), Muscularis mucosa (LM), Tunica submucosa (SM), Inner circular (IC), and Outer longitudinal layer of tunica muscularis (OL), Tunica adventitia (TA). Masson Trichrome stain; bar=200 µm

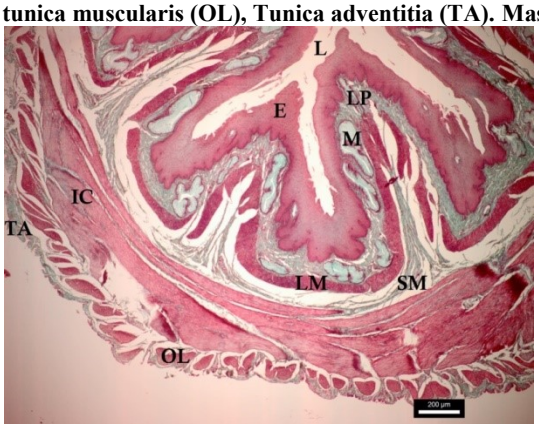

Fig. 4. Photomicrograph of the esophagus after the crop in the Guinea fowl. Lumen (L), Epithelium(E), Lamina propria (LP), Mucous gland (M), Muscularis mucosa (LM), Tunica submucosa (SM), Inner circular (IC), and Outer longitudinal layer of tunica muscularis (OL), Tunica adventitia (TA). Masson Trichrome stain; bar=200 µm

Fig. 5. Photomicrograph of the esophageal glands in the Guinea fowl. Aarrows showing that mix stained mucous glands. Periodic Acid Schiff (PAS) - Alcian Blue (AB) stain; bar=200 µm

TABLE 1. Summarized histometric results (Mean ±SD) in different areas of the Guinea fowl esophagus.

Esophageal parts Parameters(µm)	**Upper one third**	**Middle one third**	**Lower one third**	**After the crop**	Duncan test
Epithelium	457.89±83.5^{a}	372.80±86.2^{bcd}	292.31±78.4^{bcd}	216.56±76.7^{bcd}	.000*
Lamina propria	187.32±76.2	109.41±40.3	82.81±36.1	116.56±36.7	.005
Muscularis mucosa	86.76±35.6^{ac}	102.44±51.7^{b}	164.37±76.5^{ac}	62.79±16.9^{d}	.000*
Tunica submucosa	68.24±18.7^{ad}	56.42±32.3^{bc}	62.24±17.8^{bc}	68.27±20.6^{ad}	.000*
Tunica muscularis (Inner circular)	337.72±55.2^{abcd}	204.75±39.6^{abcd}	222.38±45.1^{abcd}	236.57±52.9^{abcd}	.000*
Tunica muscularis (Outer longitudinal)	116.52±28.6	99.27±22.3	98.03±34.6	88.06±36.8	.281
Tunica adventitia	142.72±62.3^{a}	89.29±52.7^{bc}	126.22±70.6^{bc}	63.68±21.4^{d}	.000*

* Mean values of esophagus in the Guinea fowl differ significantly P<0.05

a, b, c, d mean values in the esophagus of features in rows with distinct letters, differ significantly

Average each column having at least one common letter are significantly different.

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