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# Histological Structure of Esophagus and Histochemical Profile of Mucins in Glands in Partridge (*Alectoris chukar*)

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**Abstract:** Mucins secreted by mucus-producing cells are glycoproteins with many important functions such as antimicrobial effect, lubricity and physical barrier. The structure and function of mucins may be different in various tissues. Therefore, we designed our study to reveal the current state of mucin chemistry in the glands located in the partridge's esophagus. The esophagus of total 10 adult partridges, five female and five male, whose carcasses were slaughtered in a private breeding farm were used. The extracted esophageal tissues were fixed in 10% formol-alcohol. Tissues were blocked following routine histological procedures, and histological and histochemical staining techniques were applied to 5 µm thick sections from the blocks. While distinguishing the cervical and thoracic parts of the esophagus, it was observed that the glands were localized in the lamina propria and had the characteristics of simple alveolar glands or simple branched alveolar glands containing two or three secretory units. It was also determined that esophageal glands contain neutral, acidic (COOH and sulfate groups) and N-acetylsialomucins. Thus, it was concluded that the histological structure of the esophagus and the histochemical properties of mucins were relatively similar to other poultry species.
**Keywords:** Esophagus, histochemistry, mucin, partridge

**Keklikte (*Alectoris chukar*) Özofagus'un Histolojik Yapısı ve Bezlerdeki Müsinlerin Histokimyasal Profili**

**Öz:** Mukus üreten hücreler tarafından salgılanan müsinler fiziksel bariyer, kayganlık ve antimikrobial etki gibi birçok önemli fonksiyona sahip glikoproteinlerdir. Müsinlerin yapısı ve fonksiyonu çeşitli dokularda farklı olabilmektedir. Bu nedenle, çalışmamızı keklik özofagusunda bulunan bezlerde müsin kimyasının mevcut durumunu ortaya koymak için tasarladık. Karkasları özel bir damızlık çiftliğinde kesilen beş dişi, beş erkek toplam 10 adet ergin kekliğin özofagusu kullanıldı. Elde edilen özofagus dokuları %10'luk formol-alkolde tespit edildi. Dokular rutin histolojik prosedürler izlenerek bloklandı ve bloklardan 5 µm kalınlığındaki kesitlere histolojik ve histokimyasal boyama teknikleri uygulandı. Özofagus'un servikal ve torakal bölümlerinin ayrımı yapılırken bezlerin lamina propriya'da lokalize olduğu ve basit alveolar bezler ya da iki veya üç salgı birimi içeren basit dallı alveolar bez özelliğinde olduğu görüldü. Ayrıca özofagus bezlerinin nötral, asidik (COOH ve sülfat grupları) ve N-asetilsialomusinleri içerdiği tespit edildi. Böylelikle, özofagusun histolojik yapısının ve müsinlerin histokimyasal özelliklerinin nisbeten diğer kanatlı türleri ile benzer olduğu sonucuna varıldı.
**Anahtar kelimeler:** Histokimya, keklik, müsin, özofagus

## Introduction

All poultry species have adapted to a wide variety of environments according to the distribution of food sources and nutritional characteristics. Therefore, different dietary habits reflecting the different lifestyles of the poultry caused physiological and histological differences in the structures of the digestive tracts (Koçak et al., 2019; Shojaei et al., 2016). According to the poultry species, the histomorphological structure of a system or organ and the secretion character of the gland localized in the organ vary according to their eating habits, habitat and nature of their lifestyle. This phenomenon is also called adaptation (Shojaei et al., 2016).

The esophagus, one of the important organs of the digestive system, acts as a bridge between the mouth and proventriculus in poultry, and in many species contains a pouch-like dilatation called a crop at the entrance of the thorax (Shojaei et al., 2016). The esophagus, located on the right side of the neck and dorsal to the trachea, is a thin-walled, muscular tube that consists of two parts, cervical and thoracic in birds (Koçak et al., 2019; Shojaei et al., 2016). As in all living things, the esophagus wall consists of three main layers: tunica mucosa, muscularis and adventitia/serosa (Kadhim et al., 2015; Koçak et al., 2019; Taşlıdere et al., 2013). The epithelial layer,

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which is part of the tunica mucosa layer, is special in non-keratinized stratified squamous epithelium (Aytürk, 2008; Demirbağ et al., 2012; Kadhim et al., 2015; Kum, 2002).

In some poultry species, keratohyaline granules appear in some epithelial cells due to their dietary properties (Aytürk, 2008). The second layer, the lamina propria, contains numerous mucous simple tubuloalveolar or compound tubular glands with aggregated lymph follicles called the esophageal tonsil in the adjacent part of the proventriculus (Aytürk, 2008; Çolakoğlu et al., 2018; Kadhim et al., 2015; Koçak et al., 2019; Kum, 2002; Sağsöz, 2005; Shibata et al., 1991). In addition, lamina muscularis consisting of smooth muscles with a longitudinal course of the mucosa layer and submucosa layers with a large number of blood and lymph vessels and nerves are also available (Koçak et al., 2019; Sağsöz, 2005; Taşlıdere et al., 2013). Tunica muscularis consists of inner circular and outer longitudinal smooth muscle layers (Aytürk, 2008; Kadhim et al., 2015; Koçak et al., 2019; Sağsöz, 2005). The tunica adventitia is a layer of loose connective tissue with blood and lymph vessels that surround the organ from the outside (Aytürk, 2008; Koçak et al., 2019; Sağsöz, 2005).

The esophagus mucosa is moistened by the mucous glands, which are always localized under the epithelium (Amano et al., 2012). The main functions of the esophagus glands are to produce mucus, which plays an important role in moisturizing and lubricating food. Mucus, called mucin, is in the structure of O-glycated glycoprotein since it also contains proteins (Alçay, 2006; Mater, 2016). In addition to lubricating, mucins also acts as a protection against harmful components such as microorganisms and toxinagents (Sağsöz, 2005; Ünübol et al., 2010). Histochemically, mucins are divided into neutral and acidic mucins (Sağsöz, 2005; Yakan, 1990). Neutral mucins do not contain reactive acid radicals but carry free hexose groups. Acid mucins are divided into sulfate (sulfomucin) and carboxylic (sialomucin) mucins (Sağsöz, 2005; Yakan, 1990). Carboxylic acidic mucins contain sialic acid. Groups with sialic acid and sulfate are important because they lubricate and protect the canal they are in (Demirbağ et al., 2012; Yakan, 1990). Acidic mucins with carboxylates; it consists of N-acetyl and N-acetyl-O-acetyl sialomucins. N-acetyl sialomucins have the function of forming a protective barrier by forming a thin film on the epithelium (Sağsöz, 2005; Yakan, 1990). It has been demonstrated in some studies that the histological structure of the esophagus and the secretion characters of its glands vary in different poultry species with different nutritional properties (Shojaei et al., 2016).

In this study we aimed to define the basic histological features of the esophagus in partridges and whether there are histomorphological differences between glands and histological structure of the esophagus in partridges and other poultry species; we also aimed to reveal the compositions of mucins secreted from esophageal gland epithelial cells, physiological functions and whether there are differences between partridges and other poultry species. However, since the histochemical method we used in our previous study (Sağsöz et al., 2009) best demonstrated the characterization of glycoconjugates, in our current study we aimed to determine the changes in carbohydrate side chains in the secretions of esophageal gland epithelial cells.

## Material and Method

In the study, the esophagus of 10 adult partridges, five female and five male, whose carcasses were slaughtered in a private breeding farm were used. Esophagus was totally removed. And the segments from pars cervical and pars thoracic ranging from course to proventriculus, the part of the esophagus from the beginning to the course were retrieved. Longitudinal directional organ parts were taken from three of the esophagus of female and male partridges, and transversal directional organ parts were taken from two of them and were fixed for 18 hours in formol-alcohol solution. The tissues were then blocked with paraffin following routine histological procedures. Serial sections with a thickness of 5 µm were taken from the prepared paraffin blocks. While Crossman's triple stain was used to determine the general histological structure, histochemistry [Periodic acid Schiff (PAS) for detection neutral mucins; Alcian blue (pH 2.5)-Periodic acid Schiff (AB-PAS) to distinguish between neutral and acidic mucins; combined Aldehyde fuchsin-Alcian blue (pH 2.5) (AF-AB) to distinguish carboxylated and sulfated mucins; Periodic acid-Phenylhydrazine-Schiff (PAPS) for detection N-acetyl sialomucins] was used to determine the properties of mucins in the esophageal glands (Bancroft et al., 1984). Harris hematoxylin was used for nucleus staining in histochemical procedures. The samples were evaluated and photographed under the light microscope of the Nikon-Eclipse E400 (Tokyo, Japan) with DS-RII video camera (Nikon, Japan) attachment.

## Results

### *Structure of esophagus*

The histological structure of both parts of the esophagus was similar and its walls consisted of three layers: tunica mucosa, tunica muscularis and tunica adventitia. It was detected that the mucosa occurs from pronounced longitudinal primary folds, which ranging from 4 to 6 in the cervical part and from 6 to 8 in the thoracic part. In addition, there were several small secondary folds between them. The height of

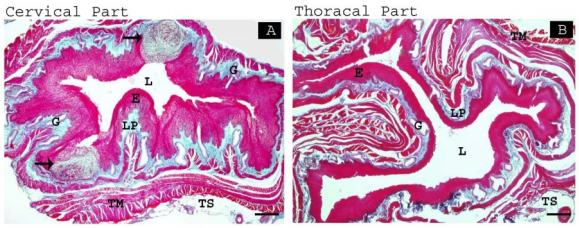


**Figure 1.** General view of cervical (A) and thoracic (B) parts of partridge esophagus. Dense tonsil units in the cervical part (arrow), L; lümen, E; epithelium, LP; lamina propria, TM; tunica muscularis, TS; tunica seroza, G; glandula, Crossman's Triple Staining. Bars; 100 µm (A, B).

the primary folds increased from the cervical part to the crop, and in the thoracic part it was higher and narrower than the cervical part (Figure1). In the partridges, the mucosa was cutaneous and covered with non-keratinized stratified squamous epithelium. Lamina propria consisted of fine collagen fiber (Figure1).

The esophageal glands were localized in the lamina propria under the basement membrane of the epithelium. They were either simple alveolar glands connected to the lamina epithelialis by a short excretory duct, or simply branched alveolar glands containing two or three secretory units. The secretory units of the glands were tube or drop-shaped in cross-sections (Figure 2C) and round-shaped in longitudinal sections (Figure 2B). The heterochromatic nuclei of the prismatic cells forming the secretory units were located towards the basal part. The neck regions of the glands extending to the luminal epithelium were covered with cuboidal epithelial cells, and the excretory ducts located in the upper part of the epithelium or in it were covered with squamous epithelium (Figure 2B). The lymphoid structures with aggregate character were localized in the transitional zone where the pars thoracic part of the esophagus joined with the proventriculus. The number of tonsil units was the same as the folds of the esophagus and consisted of a cryptlined with epithelium and surrounded by dense lymphoid tissue (Figure 2A). The lamina muscularis was a band of smooth muscle cells with longitudinal course. The submucosa was a narrow area of loose connective tissue. Tunica muscularis consisted of inner circular and outer longitudinal smooth muscle cells. It was surrounded externally by a loose adventitial connective tissue layer (Figure 1A/B).

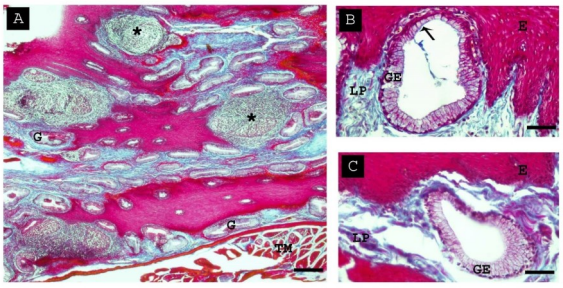


**Figure 2.** Microscopic images of cell shapes of dense tonsil units (A) and longitudinal (B) and transverse (C) sections in partridge esophagus. Tonsillar units (star), cubic shaped cells(arrow), E; epithelium, GE; glandular epithelium, LP; lamina propria, TM; tunica muscularis, G; glandula, Crossman's

### *Histochemistry of the esophageal glands*

Epithelial cells covering the glands and draining ducts in both the thoracic and cervical parts of the esophagus in partridges showed a strong PAS (+) reaction (Figure 3A/B). In AB (pH 2.5) / PAS combined staining, predominant PAS (+), moderately mixed and sporadic AB (+) reactions were observed in glandular

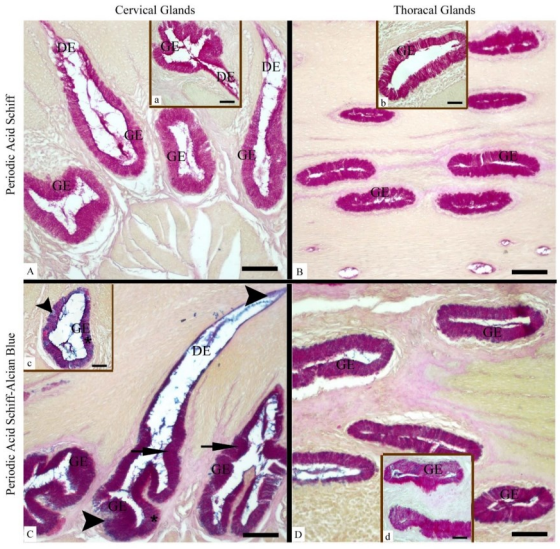


**Figure 3.** Type and localization of mucins expressed in both esophageal gland and drainage duct epithelium. Intense PAS(+) reaction with PAS staining in gland and drainage canal epithelium in both cervical (A, C) and thoracic (B, D) sections. As a result of PAS/AB (pH 2,5) reaction, dominant PAS (+) reaction (arrow) and mixed staining (*) (c, C) in gland epithelium, AB (pH 2,5) (+) reaction in some of the gland and duct epithelium (arrowhead). GE; gland epithelium, DE; drainage duct epithelium Bar: 25 µm (a, b, c, d), 50 µm (A, B, C, D).

epithelial cells in both parts of the esophagus. In general, mix and AB (+) reactions were noted in the draining duct epithelium of the glands (Figure 3C/D).

Although intense AB (+) reaction was observed in some glandular epithelial cells in the cervical part of the esophagus in AF/AB (pH 2.5) combined staining, AF (+) reaction was more dominant in the majority of glandular epithelial cells, especially in the neck part of the glands and in the draining ducts. There were also glands with combined reactions to both AB (+) and AF (+). In the thoracic segment, both AF (+) and

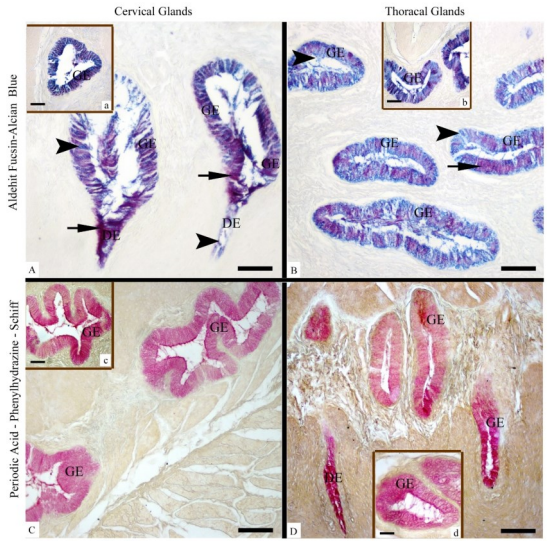


**Figure 4.** Type and localization of mucins expressed in both esophageal gland and drainage duct epithelium. In cervical (A, C) and thoracic (B, D) parts; AF (+) (arrow) and AB (pH 2.5) (+) (arrowhead) reaction in AF/AB (ph 2.5) staining, intensive staining of drainage canal epithelium in PAPS staining. GE; gland epithelium, DE; drainage duct epithelium Bar: 25 µm (a, b, c, d), 50 µm (A, B, C, D).

AB (+) reactions were at equal levels in glandular epithelial cells, whereas AF (+) reaction was dominant in the draining duct epithelial cells of the glands (Figure 4A/B). In PAPS staining, there were intense reactions of the glands which are localized in both cervical and thoracic segments, especially in the draining ducts and the neck parts close to the draining ducts. Glandular epithelial cells had a weaker reaction in both parts, and it was noted that glandular epithelial cells in the thoracic part also showed a more intense PAPS reaction than those in the cervical part (Figure 4C/D).

**Discussion and Conclusion**

Our results showed that partridge esophagus consists of a longer cervical and shorter thoracic part, as in other bird species, however, the diameter of the esophagus begins to narrow as one moves towards the crop, and the diameter of the cervical part is larger than the thoracic part (Kadhim et al., 2015; Kum, 2002; Sağsöz, 2005; Sağsöz et al., 2009; Shibata et al., 1991). This study revealed that partridge esophagus contains four to eight longitudinal mucosal folds that extending into the lumen, as shown in chicken (Nagy et al., 2005) and duck (Shyla et al., 1991; Olah et al., 2003;). Although Shyla et al. (1991) reported that the mucosal folds in the thoracic part of the esophagus in ducks (Shyla et al., 1991) are wider and shorter than those in the cervical part, in partridges as in quails (Sağsöz, 2005; Sağsöz et al., 2009), the height of the primary folds in the cervical esophagus began to increase towards the crop, and the mucosal folds in the thoracic esophagus part compared to those in the cervical part of the esophagus is narrower and higher. In partridges, the wall of the esophagus also had all the layers of a typical tubular organ (Kadhim et al., 2015; Kum, 2002;Sağsöz, 2005; Sağsöz et al., 2009; Shibata et al., 1991; Shyla et al., 1991). Our findings showed that the mucosa of the esophagus in partridges, as in other bird species, is composed of stratified squamous non-keratinized epithelium, lamina propria, lamina muscularis, and submucosa (Hodges, 1974; Klem et al., 1982; Klem et al., 1984; Kum, 2002; Nagy et al., 2005; Olah et al., 2003; Sağsöz et al., 2009; Shyla et al., 1991; Srisai et al., 2002). Similar to the studies performed in chicken (Kum, 2002), American red robin (Klem et al., 1982) and sparrow (Klem et al.,1984), it was found that the lamina muscularis in partridges is in the form of a longitudinal band of smooth muscle fibers, and as in many poultry species (Hodges, 1974; Klem et al., 1982; Klem et al., 1984; Kum, 2002; Nagy et al., 2005; Olah et al., 2003; Sağsöz et al., 2009; Shyla et al., 1991; Srisai et al., 2002), the tunica muscularis has a layer of smooth muscle cells with an inner circular and outer longitudinal direction and externally surrounded by tunica adventitia which has loose connective tissue.

As with various poultry species (Hodges, 1974; Klem et al., 1982; Klem et al., 1984; Kum, 2002; Srisai et al., 2002), in partridges, the esophagus glands were made of mucous cells and localized in lamina propria. Although the structural features of these glands differ according to the species, it has been observed that there are different definitions even in studies on the same species. In chicken, Shibata et al. (1991) found that esophageal glands are typically compound tubular glands (Shibata et al., 1991); in contrast, Kum (2002) reported that these are simply branched tubuloalveolar glands.

It has been reported that esophageal glands are simple tubular or simply branched tubular in duck (Shyla et al., 1991) and Germain's swiftlet (Srisai et al., 2002), and bottle-like in sparrow (Klem et al., 1982)

and thrush (Klem et al., 1984). In this study, when the transverse and longitudinal sections of partridge esophagus were examined, it was seen that the glands had an inflated balloon-like shape. The partridge had one or two secretory units of these glands and a short excretory duct. Therefore, partridge esophageal glands were histologically defined as simple alveolar and/or simple branched alveolar glands. It has been reported that in many poultry species there is lymphoid tissue in the esophagus and these are especially observed at the junction of the esophagus with the proventriculus (Klem et al., 1982; Klem et al., 1984; Kum, 2002; Kum et al., 2006; Nagy et. al., 2005; Olah et. al., 2003; Rahman et al., 2004; Shyla et. al., 1991). This lymphoid tissue, called esophageal tonsil (Nagy et al., 2005; Olah et al., 2003), is defined as an important member of the gut-associated lymphoid tissue (GALT) in poultry. In the present study, there are lymph follicles in aggregate character in the transition region from the esophagus to the proventriculus in partridges, and it was named as esophageal tonsil in partridges, as reported in other poultry species (Sağsöz, 2005).

In classical carbohydrate histochemistry, PAS (+) reactions indicate the presence of neutral carbohydrates, AF (+) and AB (pH 2.5) (+) reactions indicate the presence of acidic sulfate and carboxylic mucins, and PAPS (+) reactions indicate the presence of N-acetyl sialomucins (Bancroft et al., 1984; Erdoğan et al., 2015; Schumacher et al., 2004; Spicer et al., 1992). Sagsöz and Liman (2009) reported that the glands and draining ducts located along the esophagus in adult quails show strong PAS (+) reactions and contain neutral mucins (Sağsöz et al., 2009). Again, Kum (2002) and Nakhoul et al. (2007) stated that the esophageal glands gave strong PAS (+) reactions and in this case, they showed the presence of neutral mucins (Abdulnour-Nakhoul et al., 2007; Kum, 2002). In the presented study, the intense PAS (+) reaction of glands and draining duct epitheliums in partridges in both parts of the esophagus revealed the widespread presence of neutral mucins. It has been stated that the epithelial cells that react PAS (+) or AB (+) are in equal proportions in the esophageal glands of chickens and that these glands contain neutral and carboxylic acid mucins (Kum, 2002). In a study conducted in geese, it was reported that many cells in the esophageal glands contain only AB (+) glycoconjugate, while fewer cells contain PAS (+) glycoconjugates (Demirbağ et al., 2012). In AB (pH 2.5)/PAS combined staining of partridges, the presence of mixed-reacting glandular epithelial cells and only AB (+) reacting glandular epithelial cells were observed, along with PAS (+) reactions in glandular epithelial cells. In addition, it was noted that the dominant reaction in the canal epithelial cells was the AB (+) reaction. In this case, it revealed that the esophagus glands and duct epithelial cells of partridges contain sulfated and carboxylic acid mucins along with neutral mucins. Demirbağ et al. (2012) reported that glands and draining ducts along the esophagus in geese contain sulfated and carboxylic acid mucins (Demirbağ et al., 2012). Kum (2002) revealed that in general AB (+) reaction is dominant and AF (+) reaction is rarely found in esophageal gland and duct epithelium in chickens (Kum, 2002). Pastor et al. (1988) also revealed that esophageal glands in chickens contain a mixture of sulfo- and sialomucins (Pastor et al., 1988). It was noted that in AF/AB (pH 2.5) combined staining, esophageal gland epithelial cells showed AF (+) and AB (+) reactions in partridges, but these reactions were in the form of weak or strong AF (+) reactions in gland epithelial cells, especially towards the thoracic region. In some gland epithelial cells, only AB (+) reactions was observed. In partridges, it was determined that epithelial cells covering the glands, especially the neck parts and draining ducts, gave AF (+) rather than AB (+) reactions. These results revealed the presence of weak and strong sulfated and carboxylated (COOH group) acid musins in the glandular and duct epithelial cells along the esophagus in partridges. Sağsöz and Liman (2009) reported that weak PAPS reactions were found in the esophageal glands of adult quails and they were especially localized in the neck parts and draining ducts of the glands. In the present study, we determined that PAPS reaction in partridges is found in the neck regions and ducts of the glands in both the cervical and thoracic part of the esophagus. This reveals the presence of N-acetyl sialomucins, one of the carboxylic acid mucins, in partridge esophagus.

In all species, the surface of the mucous membranes of the digestive, respiratory and urinary tracts is protected by a layer of sticky and persistent viscoelastic mucus, which provides low permeability for many molecules and creates a physical barrier (Gendler et al., 1995). The mucins protects the epithelium against chemical, enzymatic and mechanical destruction, as well as pathogenic microorganism invasion (Corfield et al., 2000; Sağsöz et al., 2009). Especially sulfated mucins play an important role in the protection of mucous membranes against bacterial adesions. Because the sulfation event gives resistance to the mucosa caused by bacterial glycosidase in the mucus barrier (Brockhausen, 2003; Ferri et al., 2001; Roberton et al., 1997; Sağsöz et al., 2009). In this context, we can say that sulfated mucins in the partridge esophageal glands provide primary protection against microorganisms that try to colonize the epithelial surface. In addition, it has been reported that carboxylated mucins also settle on cell surface membranes (COOH-terminal domains) and contribute to epithelial protection by forming the basic infrastructure of gel-forming mucins (MUC2, MUC5AC, MUC5B and MUC6) (Kesimer et al., 2010). The presence of carboxylated mucins in partridge esophageal glands

suggested that in addition to the above-mentioned epithelial protection, it may help to lubricate the ingested foods and aid swallowing.

As a result; we found that the general histological structure of the esophagus in partridges is relatively similar to other poultry species. We determined that the glands localized in both the cervical and thoracic parts of the esophagus contain neutral, acidic (COOH and sulfate groups) and N-acetylsialomucins. We can say that these mucins secreted by the esophageal glands of partridges form a barrier in the esophageal mucosa, protecting the mucosa against mechanical destruction, drying, external and microbial toxic substances and bacterial adhesions.

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