# THE EFFECT OF AGE, SEX AND REGION ON HISTOLOGICAL STRUCTURES OF THE ESOPHAGUS IN BROILER CHICKENS

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**Abstract.** The purpose of this study was to determine the influence of age, sex and region factors on the histological structures of the esophagus in broiler chickens. 24 *Ross* broiler chickens (12 males and 12 females) were used. The chickens were allocated to one of three age groups; 3–7 days, 21–28 days, 49–56 days. The cervical and thoracic esophagi of all birds were used. The general histological characteristics of the esophagus were similar to those of the other birds. No significant histological differences in esophageal structures exist between the sexes. In all three age groups except the *tunica submucosa*, the other layers of esophagus were more developed in the thoracic region than that of the cervical region of the organ. The glandular units of simple branched *mucosal glands* which were more in thoracic part of esophagus than its cervical part were 7 or more around an *esophageal crypt*. Lymphatic tissues were not observed in the lamina propria of both regions of esophagus. An unusual finding of the esophagus in broilers was the presence of a capsule of reticular, elastic and collagenous fibres which separated the *mucosal glands*. By increasing age, all the histological layers of esophagus were increased.

**Keywords:** age, broiler chickens, esophagus, histology.

## Introduction

The avian esophagus is on the right side of the neck (mammals present it on the left side) (Sisson and Grossman, 1986). It has thin and dilatable walls that convey feed from the mouth to the stomach (Hodges, 1974). According to Sisson and Grossman (1986), the avian esophagus is divided into an S-shaped cervical and thoracic region. In many species (except *penguins, gulls, emu, ostriches*), the cervical region expands ventrally to form a diverticle, the crop, which is ventral and lateral to the esophagus and cranial to the clavicle and breast muscles. The avian esophagus consists of four tunica; *mucosa, submucosa, muscularis* and *serosa* (Dellmann and Eurell, 1998). Esophageal *mucosa* forms longitudinal folds (Banks, 1992). They are lined for stratified squamous epithelium in which several *mucous glands* open up (Sisson and Grossman, 1986). According to George et al. (1998), the *muscularis mucosae* of the fowl esophagus varies in species, in number of present layers (one or two) and in orientation of the muscular fibres bunches.

Numerous studies have been conducted on morphologic evaluations of the esophagus in the certain species of birds (Chikilian and DE Speroni, 1996; Bailey et al., 1997; Olsen et al., 2002; Rossi et al., 2006), but few studies have been done on histological structure of the esophagus (Geyikoglu et al., 2002; Shiina et al., 2005; Rajabi and Nabipour, 2009). However, to the author's knowledge, in birds, especially in chicken, studies of age, sex and region effects on the esophageal structure were limited. The purpose of this study was therefore to find out the histological differences of the esophagus among the different anatomical regions and investigate the influence of the sex and age factors on the histological structure of the esophagus of the *Ross* broiler chickens.

## Materials and Methods

A total of 24 clinically healthy *Ross* broiler chicks (12 males and 12 females) were obtained from the Research farm of household birds maintenance of Faculty of Veterinary, University of Shahrekord. The chickens were allocated to one of three age groups; 3–7 days (n=8), 21–28 days (n=8) and 49–56 days (n=8). The birds were killed by cervical sub-luxation method. After removing the whole esophagus, samples were taken from the middle parts of cervical and thoracic regions of esophagus. The samples were immediately fixed in 10% neutral buffered formalin solution for 24–48 hours and then submitted to the dehydration process with alcohol and embedded in Paraplast. The sections were cut at 6 µm. The following histological and histochemical methods of staining were employed: hematoxylin-eosin, Masson's trichrome stain, periodic acid-Schiff (PAS), Alcian blue (pH 1.0), and gomori's method for reticulum (Kiernan, 1999). The tissue sections were documented in Olympus microscope, model BX50, and described histologically. Finally, histological changes of different regions of esophagus were evaluated among the age groups in both sexes.

## Results

In all the age groups, the esophagus was composed of *tunica mucosa, tunica submucosa, tunica muscularis* and *tunica adventitia* (for the cervical region) or tunica serosa (for the thoracic region). There were no sex differences in histological features of the esophagus in *Ross* broiler among all the age groups. The *tunica mucosa* which was arranged in longitudinal folds was lined with keratinized stratified squamous epithelium. At 3–7 days of age, the lining epithelium was thin and with increasing age, the height of epithelium and mucosal folds were increasing (Fig. 1).


Fig. 1. **Photomicrograph of the cervical region of esophagus in the 3-7 days broiler chickens**
Lumen (L), epithelium (E), *lamina propria* (LP), *muscularis mucosa* (LM), *tunica submucosa* (SM), *inner circular* (IC), and *outer longitudinal* layer of *tunica muscularis* (OL), *tunica adventitia* (TA), blood vessels (Bv), adipose tissue (A), parasympathetic ganglia (G). Hematoxylin eosin × 109

The *lamina propria* contained dense connective tissue which consisted of ramified simple branched tubular *mucous glands* (Figs 2 and 3), reticular (Fig. 4), elastic and collagenous fibres and fibroblasts, but no lymphatic tissues were observed (Fig. 5). In the present study, there were 7 or more glandular units around an esophageal crypt.

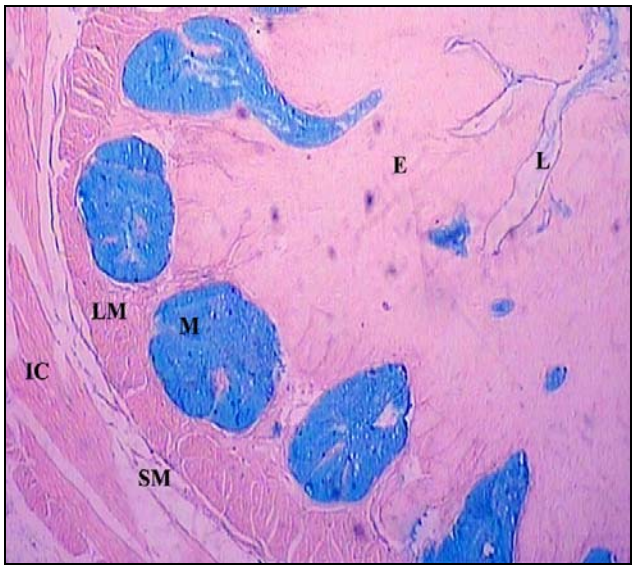


Fig. 2. **Photomicrograph of the thoracic region of esophagus in the 21-28 days broiler chickens**
Lumen (L), epithelium (E), purely *mucous glands* (M), *muscularis mucosa* (LM), *tunica submucosa* (SM), *inner circular* layer of *tunica muscularis* (IC). Alcian blue × 109

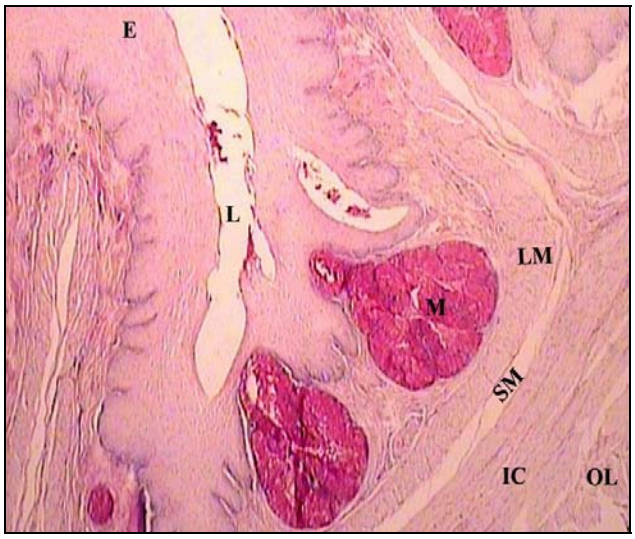


Fig. 3. **PAS-positive material in the *esophageal glands* (M) of the 49-56 days broiler chickens**
Lumen (L), epithelium (E), *muscularis mucosa* (LM), *tunica submucosa* (SM), *inner circular* (IC), and *outer longitudinal* layer of *tunica muscularis* (OL). PAS × 109


Fig. 4. **Reticular fibres (arrows) are present around *esophageal glands* (M) of the 49-56 days broiler chickens**
Epithelium (E), *muscularis mucosa* (LM), *tunica submucosa* (SM), *inner circular* (IC), and *outer longitudinal* layer of *tunica muscularis* (OL), *tunica serosa* (TS), blood vessels (Bv). Gomori's method for reticulum × 109

The walls of connective tissue separated the glands and a great quantity of all the connective tissue fibres (reticular, elastic and collagen) and blood capillaries could be observed. By increase in age, in addition to the gradual increase in the number and branching of *mucous glands*, the amounts of the all connective tissue fibres were also increased in both sexes. The *muscularis mucosa* was made up of a developed continuous layer of smooth muscles (Figs 1 to 5). The thickness of *tunica mucosa, lamina muscularis* and the number and branching of

*mucous glands* were lesser in cervical region of esophagus than in its thoracic region. The thickness of *lamina muscularis* increased with age.

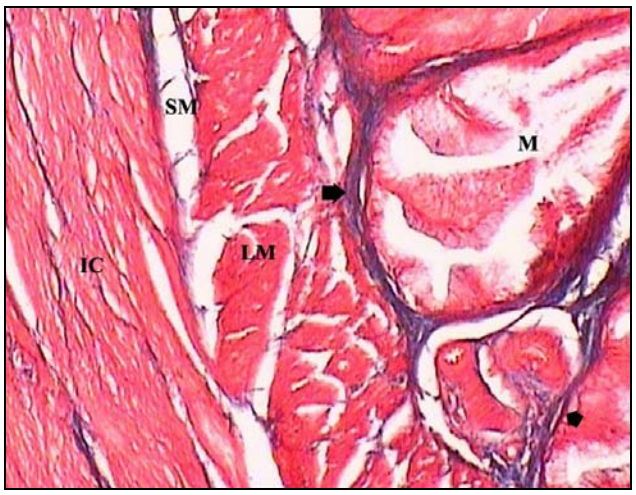


Fig. 5. **Higher magnification of the thoracic region of the esophagus in the 21–28 days broiler chickens**

*Mucous glands* (M), *muscularis mucosa* (LM), *tunica submucosa* (SM), *inner circular* layer of *tunica muscularis* (IC), Collagenous fibres (arrows). Masson's trichrome × 273

*Tunica submucosa* which consisted of very thin strands of loose connective tissue was associated with *submucosal plexus*, nerve fibers, blood vessels, fibroblasts, fine reticular, elastic, and collagen fibres (Figs 1 to 5). Unlike the tunica mucosa, the thickness of *tunica submucosa* was more in cervical region as compared to thoracic region of esophagus. This layer gradually increased with age.

The *tunica muscularis* which was formed only of smooth muscle fibres was consisted of a broad inner band of circular muscle and a thin outer layer of longitudinal muscle (Figs 1 and 4). The *myenteric plexus* can be seen between them. This layer was more developed in the thoracic region in comparison to cervical region of the organ. As the age increased, the thickness of this layer also increased.

The outermost tunica in the thoracic region of esophagus was found *serosa*, which loose connective tissue invested by mesothelium, whereas in the cervical region was *adventitia* and mesothelium was absent. This loose connective tissue was made up of adipose tissues, blood vessels, parasympathetic ganglia, and nerve bundles, reticular, collagenous and elastic fibres and binds of the esophagus to the surrounding tissues. Although this layer showed no histological difference between the various regions of esophagus, all the above structures, especially adipose tissues, became well developed with increasing age (Figs 1 and 4).

**Discussion**

In the present study, sex differences were not found in histological features of the esophagus in *Ross* broiler among all the age groups which is in agreement with the results reported by Olsen et al. (2002) in Adelie penguins.

The results obtained from the present study clearly showed that general histological characteristics of the esophagus in broiler chickens were similar to those of the other birds (Hodges, 1974; Chikilian and DE Speroni, 1996; Whittow, 2000; Olsen et al., 2002; Rossi et al., 2006; Islam et al., 2008). In all the three age groups, the *mucosa* of the broiler esophagus similar to other species (Banks, 1992; Chikilian and DE Speroni, 1996; Dellmann and Eurell, 1998; Olsen et al., 2002; Rossi et al., 2006; Islam et al., 2008; Rajabi and Nabipour, 2009) was constituted of keratinized stratified squamous epithelium. The *mucosal folds* of the esophagus in *Ross* broilers were similar to those of the other birds (Banks, 1992; Olsen et al., 2002; Rossi et al., 2006; Islam et al., 2008).

The results obtained from the present study also showed that in both regions the *lamina propria* was formed of dense connective tissue. In contrast to this finding, the *lamina propria* tends to be loose in *Adelie* penguins (Olsen et al., 2002) and some species of wild birds (Rajabi and Nabipour, 2009).

In all the three age groups, the *mucous glands* were simple branched tubular glands. Banks (1992) and Olsen et al., (2002) reported that the type of esophageal glands was branched tubuloalveolar.

Like in the other birds (Suganuma et al., 1981; Chikilian and DE Speroni, 1996; George et al., 1998; Bacha and Bacha, 2000; Olsen et al., 2002; Rossi et al., 2006; Islam et al., 2008; Rajabi and Nabipour 2009) and unlike in the mammals (Banks, 1992; Dellmann and Eurell, 1998; George et al.,. 1998), these glands were located in *lamina propria* and were present in both cervical and thoracic regions. Also Suganuma et al. (1981) and Bacha and Bacha (2000) explained that the fowl's esophageal glands are always of mucous type and they are only on the *lamina propria*.

These glands were numerous and present throughout the length of the esophagus in the dog (Samuelson, 2007). The *mucous glands* were more developed in thoracic region as compared to cervical region of esophagus which was in agreement with those reported in some wild birds (Rajabi and Nabipour, 2009). In contrast to this finding, they were more numerous in cervical region than the thoracic esophagus in chickens (Hodges, 1974).

Our results revealed that the glandular units of *mucous glands* were 7 or more, while in Kasilla broiler were found 6–7 glandular units around an esophageal crypt (Islam et al., 2008). In the present study, the *esophageal glands* in both cervical and thoracic regions were surrounded by a capsule which consisted of reticular, elastic and collagenous fibres in all three age groups. Reports in this regard were not found in the available literature. Unlike in the other birds (George et al., 1998; Olsen et al., 2002; Olah et al., 2003; Rossi et al., 2006), *lymphatic tissues* or nodules were absent in the *lamina propria* of both cervical and thoracic regions of esophagus.

In the present study, the *muscularis mucosa* was made up of a developed continuous layer of smooth muscles, which correlate with the findings of Chikilian and DE

Speroni (1996) in Tinamou, Olsen et al. (2002) in the *Adelie* penguins and the other birds (Dellmann and Eurell, 1998; Rajabi and Nabipour, 2009).

The results obtained from the present study also showed that in both regions, the *tunica muscularis* formed only of smooth muscles which was in agreement with those reported previously (Banks 1992; Chikilian and DE Speroni, 1996; Dellmann and Eurell, 1998; George et al., 1998). It consisted of a broad inner circular and a thin outer layer of longitudinal muscles, which was in agreement with the results of Chikilian and DE Speroni (1996). In contrast to this finding, the *tunica muscularis* has two inner and outer longitudinal and a medium *circular layers* in partridge *Rhynchotus rufescens* (Rossi et al., 2006) and in the *Adelie* penguins (Olsen et al., 2002). It consisted of the skeletal muscles in ruminants and dogs (Banks, 1992; Dellmann and Eurell, 1998).

The cervical and thoracic regions of esophagus show differences in histological and histometrical characteristics in birds (Chikilian and DE Speroni, 1996; Olsen et al., 2002; Shiina et al., 2005; Rossi et al., 2006). The present study showed that in all three age groups, except the *tunica submucosa*, the other layers of esophagus were more developed in the thoracic region than that of the cervical region of the organ. Our results revealed that the *esophageal glands* and *tunica muscularis* were greater in thoracic region compared to cervical region. These differences might be due to the genetic variations in the different avian species.

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