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Gross Anatomical, Light and Scanning Electron Microscopic Studies on the Pharyngeal Roof of Turkey (*Meleagris gallopavo*): Comparative Study

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ABSTRACT

The present study was carried out to investigate the histomorphological features of the turkey's pharyngeal roof using light and scanning electron microscopy. Studied parts from the roof of the turkey's pharynx were processed and stained by convential stains to illustrate the mucosa, salivary glands, and connective tissue infiltration. Also, Scanning electron investigations were applied on pharyngeal papillae and pharyngeoseophageal junction. Grossly, the pharyngeal roof was shown to constitute about 12.27% of the oropharyngeal roof length, continue rostrally with the oral roof at the level located between the choanal and infundibular slits, and terminate caudally at the pharyngeoesophageal junction, demarcated by a transverse row of caudally directed conical papillae. Numerous different sized and shaped caudally directed pharyngeal papillae were distributed in the roof of the pharynx. Histologically, the pharyngeal mucosa demonstrated pharyngeal folds, intraepithelial mucous glands, abundant lymphoid infiltration and lymphatic nodules, in addition to intraepithelial sensory corpuscles. Numerous compound tubular mucous sphenopterygoid salivary glands were observed in the mucosa of the pharyngeal roof. This study in conclusion provided comprehensive information on the structure of the pharyngeal roof of the turkey, comparing these findings with those of other birds.

Introduction

Oropharynx morphology in birds gained special attention in earlier descriptions of Göppert (1903) which compared this structure of numerous avian species. It is previously described as a combined cavity extending from the beak to the esophageal opening, and the pharynx as a part of this cavity (Hamilton, 1952; McLelland, 1975; Nickel *et al*., 1977; Dyce *et al*., 2002). No obvious morphological distinction could be made between the oral cavity and the pharynx due to the lack of soft palate, so both cavities formed a common oropharyngeal cavity. This feature was previously informed in guinea fowl (Jayachitra *et al*., 2015), in ostrich (Tadjalli *et al*., 2008; Tivane *et al*., 2011), in emu (Crole and Soley, 2010), and in African pied crow (Igwebuike and Eze, 2010). Also, anatomy of the oropharyngeal cavity in the raven and magpie were excessively similar to the other avian species (Erdogan and Alan, 2011).

From embryological point of view, the homologous boundary between the oral and pharyngeal cavities was suggested to be between the choanal slit and the infundibular cleft (King and McLelland, 1984). Other studies in birds recorded the region analogous to the boundary between the mouth and pharyngeal cavities of mammals, was where the infundibular cleft began. Functionally, the boundary between the oral and pharyngeal cavities was informed to lie at the junction of the narrow and wide parts of the choanal slit (Nickel *et al*., 1977).

McLelland (1975) described the anatomy of the oropharynx in turkey as similar to that of the chicken, but recent studies on this species showed different characteristic features in the upper beak and palate in comparing with those of the other domestic birds (Sayed *et al*., 2014, 2016). In this regard, information available on the anatomy of the pharyngeal roof in turkey are still scanty; hence, the present study was undertaken to investigate its features.

Materials and methods

Birds

This study was conducted at Department of Anatomy and His-

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tology, Faculty of Veterinary Medicine, Assiut University, Egypt. Twenty adult healthy turkeys of bronze black species aged 12 months old and weighted 2.5-3 kg were obtained from a local farm in Assiut Governorate, Egypt. The birds were sacrificed by cervical dislocation, and the heads were cut off and washed under running tap water. The beak's angles were incised. Mouth cavity was exposed, and the pharyngeal roof was dissected. Different gross measurements in millimeters (mean ± S.E.) were studied on eight birds to investigate the different pharyngeal roof structures using Percision Digital Vernier Caliper and gross photographed were captured.

Histological examination

Cross and longitudinal specimens from the pharyngeal roof were studied from eight birds, fixed in 10% neutral buffer formalin. Decalcification of the bone was performed by formic acid and 10% formol saline (Geoffrey, 1969). Samples were then processed for histological examination. 5um-thickness paraffin sections were cut, mounted on glass slides, and stained with conventional stains including Haematoxylin and Eosin (Harris, 1900), Crossmon's trichrome (Crossmon, 1937), and PAS stains (Gurr, 1962). The stain techniques were adopted after Bancroft and Gamble (2002). The sections were examined with light microscope and digital images were acquired.

Scanning electron microscopy

Pharyngeal roof of four birds was washed for several times in normal saline and acetic acid 2% followed by fixation in 4% glutaraldehyde solution for 24 hour, and post fixed in 2% buffered osmium tetraoxide. The specimens were washed in 0.1 M cacodylate buffer (pH 7.3), dehydrated in ascending grades of ethanol, critical point dried in liquid carbon dioxide, and mounted on metal stubs then coated with gold palladium in sputtering device. Samples were examined and photographed by using JSM_4500 LV scanning electron microscope operated at 20 KV.

Results

Gross morphology and scanning electron microscopy

The roof of the pharyngeal cavity (*Cavitas pharyngealis*) of the turkey was found to begin from the line of separation between the choanal slit (Choana) and infundibular slit (*Rima infundibuli*). It extended rostrally with the oral roof and terminated caudally at the pharyngeoesophageal junction, which demarcated by a transverse row (14.30±0.5 mm long) of 11-14 caudally directed conical papillae. The pharyngeal roof measured 10.14±0.38 mm long constituting 12.27±0.30 % of the total oropharyngeal roof length, while its width was 18.94±0.31 mm at its rostral end and 13.58±0.23 mm at its caudal end, decreasing in width caudalwards. It is occupied on its middle by the infundibular slit. Fine papillae are shown to be scattered on the surface of the pharyngeal roof, and concentrated on both sides of the slit.

The infundibular slit was appeared as a median longitudinal fissure in the middle of the pharyngeal roof representing a common opening of the two auditory tubes, situated immediately caudal to the choanal slit and behind the level of the ventrally located laryngeal inlet. This slit was recorded to measure 6.93±0.13 mm long and constitute 68.34±1.75 % of the total length of the pharyngeal roof, with few fine papillae were demonstrated on its rim. The rostral and caudal commissures of the slit were narrow and had an acute angle. The caudal commissure continued caudally with a shallow groove till the level of the pharyngeoesophageal junction (Fig. 1).

Fig. 1. Photograph of the roof of the caudal part of the oropharyngeal cavity showing the pharyngeal cavity which extends from the line of separation between choanal slit (C) and infundibular slit (I) to the pharyngeoesophageal junction (arrows) which is characterized by transverse row of caudally directed papillae. Note the beginning of the esophagus (star).

According to the scanning electron microscopical findings, the infundibular slit was showed to extend rostrally with the choanal slit through a shallow groove which is occupied by longitudinal mucosal folds (Fig. 2). Moreover, the former slit continued caudally by a groove into the pharynx till its caudal end where it separated the pharyngeoesophageal junction into right and left parts. Directly before its termination few small elongated papillae were extended caudomedially from the inner edges of this groove.

Numerous different sized and shaped caudally directed pharyngeal papillae (*Papillae pharyngeales*) were distributed in the roof of the pharynx, concentrated near the edge of the infundibular slit. Some of them were wedge shape while the other elongated conical in shape or conical in shape with wide rounded base and pointed apices. A number of these papillae were arranged themselves into a short transverse row located on both sides of the infundibular slit. The bases of these pharyngeal papillae were partially surrounded by groove as shown by SEM. Between the previous pharyngeal papillae numerous openings of the sphenopterygoid salivary glands (*Glandulae sphenopterygoideae*) were scattered. The papillae of the pharyngeoesophageal junction were caudally directed and elongated conical in shape. The medially located papillae were generally larger than the lateral ones. Some papillae showed duplication pattern where two papillae were fused at their bases and separated at their apices (Figs. 3-4).

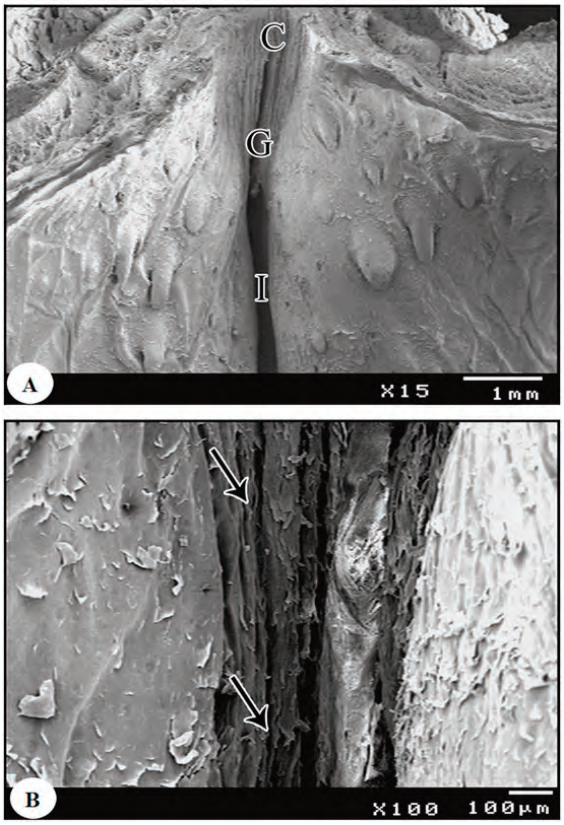

Fig. 2. A) Scanning electron micrograph showing the end of the wide part of choanal slit (C), beginning of infundibular slit (I) and communication between them through a shallow groove (G). B) Higher magnification of the junction between the choanal and infundibular slits showing presence of longitudinal mucosal folds (arrows).

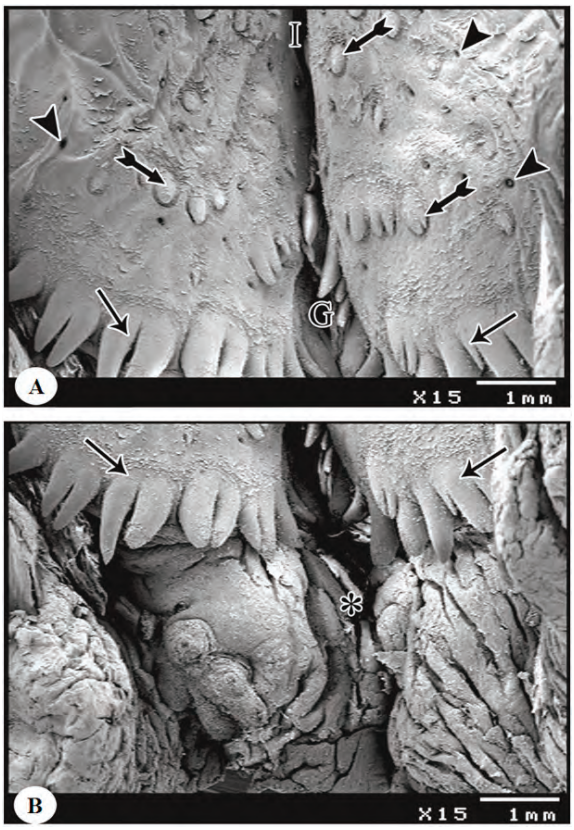

Fig. 3. A) Scanning electron micrograph of the pharyngeal roof showing the caudal part of the pharyngeal roof containing numerous different sized and shaped caudally directed pharyngeal papillae (barbed arrows), in between several openings of the sphenopterygoid salivary glands (arrow heads) are distributed. In addition, the infundibular slit (I) which continues caudally as a groove (G) which terminates at the pharyngeoesophageal junction that demarcated by transverse row of elongated conical papillae (arrows). B) Termination of the pharyngeal roof showing the row of pharyngeal papillae at pharyngeoesophageal junction (arrows) and beginning of the esophagus (star).

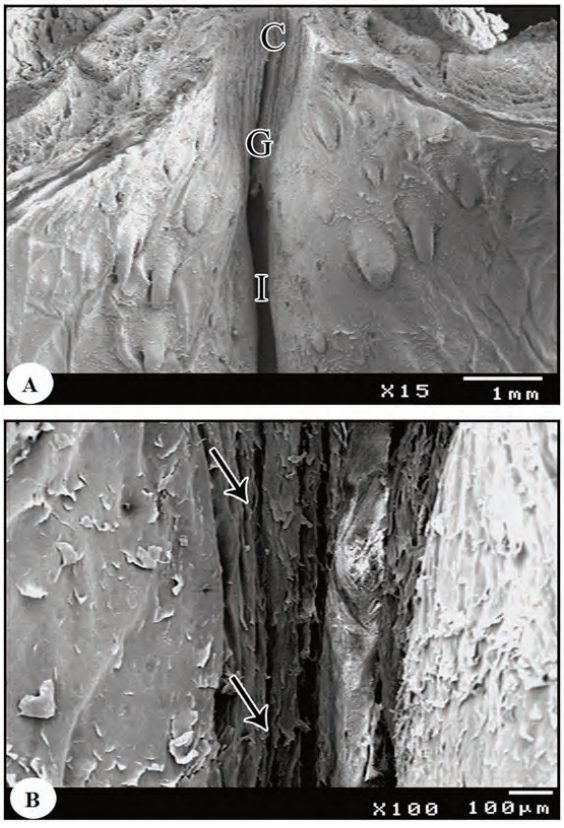

Fig. 4. Scanning electron micrograph of higher magnification of pharyngeal papillae (A) showing different shaped and sized pharyngeal papillae (arrows), and the groove which represents the caudal continuation of the infundibular slit (B). It contains elongated papillae (barbed arrows) extending caudomedially from its inner edges. Note the medial located papillae of the pharyngeoesophageal junction are larger than the lateral ones. Moreover, some of these papillae have duplication patterns (arrows).

Histological examination

Figs. 5-7 clarified the light microscopical structures of the pharyngeal roof. The lining mucosa of the pharynx in turkey was formed of lamina epithelialis and lamina propria. The lamina epithelialis consisted of stratified squamous epithelium cornified rostrally with small fine conical shaped pharyngeal papillae and none cornified caudally with thick epithelium showing intraepithelial sensory corpuscles. The lamina propria was constituted of connective tissue fibers containing compound tubular mucous secretory sphenopterygoid salivary glands. These glands were surrounded by a connective tissue capsule from which connective tissue septa arise dividing these glands into lobules. Each lobule showed secretory units lined by columnar epithelium with flat basally located nuclei and lightly stained basophilic foamy vacuolated cytoplasm.

The pharyngeal mucosa revealed pharyngeal folds and intraepithelial mucous glands. Abundant lymphoid tissues either diffused or in follicles were demonstrated in the lamina propria of the pharyngeal roof. These lymphatic tissues were observed in the connective tissue of the sphenopterygoid salivary glands especially around their ducts. Also lymphatic infiltrations were detected surrounding the intraepithelial mucous glands just close to the margin of the infundibular slit. Muscular layer are also observed in the pharyngeal roof, consisting of longitudinal and transverse layers.

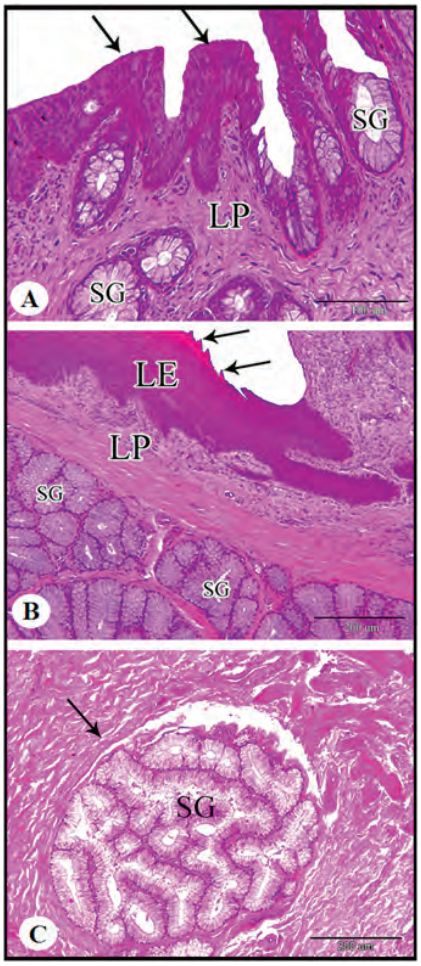

Fig. 5. Photomicrograph showing A) cross section of the pharyngeal roof showing pharyngeal folds (arrows) and sphenopterygoid glands (SG) in the lamina propria (LP). B) Longitudinal section of the pharyngeal roof on the side of the infundibular slit showing lamina epithelialis (LE) carry pharyngeal papillae (arrows) and lamina propria (LP) contains sphenopterygoid salivary glands (SG). C) Sphenopterygoid salivary gland (SG) surrounded by connective tissue capsule (arrow), H&E stain.

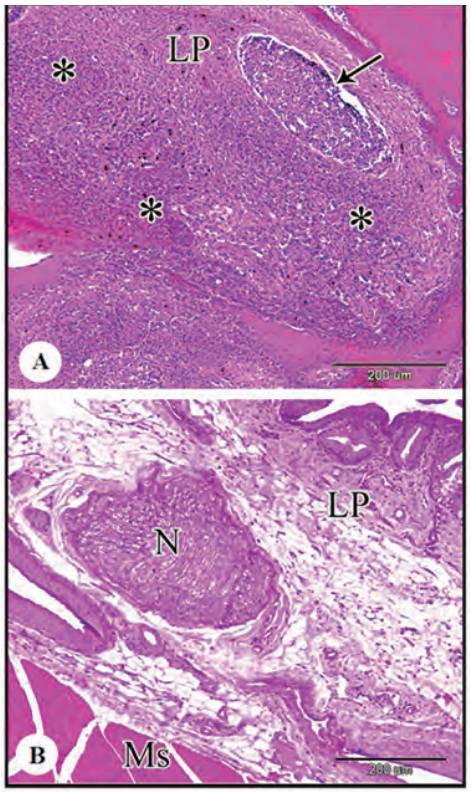

Fig. 6. Photomicrograph of longitudinal (A) and cross sections (B) of the pharyngeal roof showing lamina propria (LP) contains both diffused infiltration (stars) and lymphatic follicles (arrow), nerve bundles (N). Note presence of muscle fibers (Ms), H&E stain.

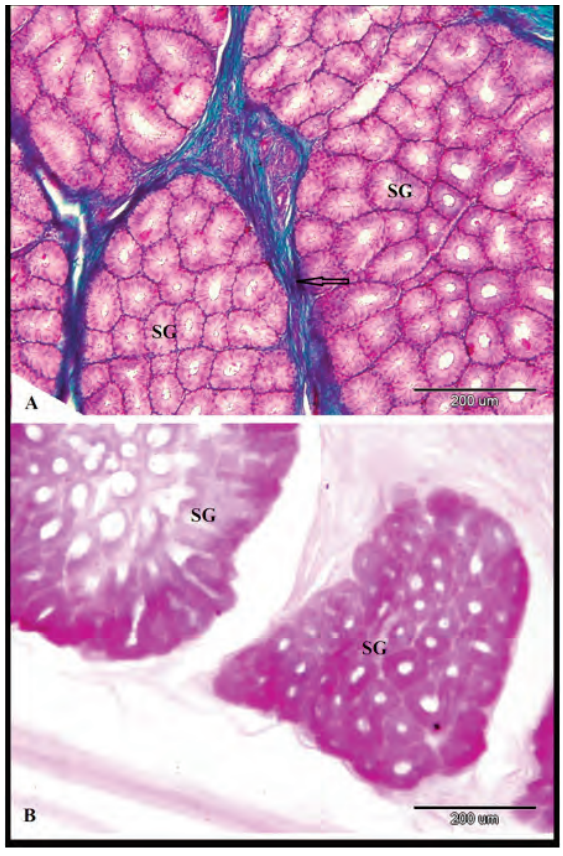

Fig. 7. Photomicrograph of the pharyngeal roof showing A) Sphenopterygoid salivary glands (SG) surrounded by connective tissue capsules (arrows), Crossmon's trichrome stain. B) PAS-positive sphenopterygoid salivary glands (SG), PAS stain.

Discussion

In turkey, the boundary between the oral and pharyngeal cavities was found to be located between the choanal and infundibular slits. This finding simulates that of Madkour (2011) in duck and the embryological result of Lucas and Stettenheim (1972). In this connection, Ali (2004) mentioned that this boundary is established at the caudal end of the choanal cleft. McLelland (1975) recorded that, in most domestic fowls, the caudal limit of the oral cavity is conveniently described to be at the level of the most caudal transverse row of papillae on the hard palate and the papillae on the base of the tongue. However, Hodge (1974) reported that the point of junction of the buccal and pharyngeal cavities in the fowl is more caudally situated where it reaches the opening of the glottis. In this concern, Hassouna (2002) stated that the pharyngeal cavity is extended from the rostral end of the choanal cleft to the pharyngeo-esophageal junction. Functionally, Nickel *et al*. (1977) pointed up that the boundary between the oral and pharyngeal cavities lies at the junction of the narrow and wide parts of the choanal slit, as the rostral narrow part did not participate in process of respiration, while the wide part is the only part that remained opened during respiration. The boundary between the oral and pharyngeal cavities in turkey was observed to lie behind the level of the angle of the mouth by 14.10 mm. This distance was measured 25.62 mm by Madkour (2011) in the duck at 60 days old. Embryologically, this boundary is stretching laterally to the angles of the jaws (Lucas and Stettenheim, 1972).

The present morphometrical study explained that the length of the oral roof in the turkey was 72.12 mm constituting 87.73% of the total oropharyngeal roof length, while that of the pharyngeal roof was 10.14 mm representing 12.27%. In 60 days old duck, the length of the oral roof was 82.96 mm constituting 84.47% of the oropharyngeal roof length, and that of the pharyngeal roof was 15.15 mm performing 15.19% (Madkour, 2011).

It was obvious from the present findings that the caudal limit of the pharyngeal roof which was represented by the pharyngeoesophageal junction had a transverse row of 11-14 caudally directed conical papillae. Similar to that mentioned in other birds, the roof of the pharynx is separated from the dorsal wall of the esophagus by transverse row of papillae (Nickel *et al*., 1977; King and McLelland, 1984; Hassouna, 2002; Erdogan and Perez, 2014; Jayachitra *et al*., 2015). Unlike to the description of Ali (2004) in ostrich, that the oropharyngeal roof is separated from the esophagus by a transverse mucosal ridge. In the latter bird, the dorsal surface of the pharynx was informed to lack a transverse row of papillae at the junction with the esophagus (Tadjalli *et al*., 2008). As shown by SEM, the medially located papillae of the transverse row were generally larger than the lateral ones, with some papillae of this row had a duplication pattern.

The present work clarified that the pharyngeal roof contains scattered fine papillae, which were concentrated on both sides of the infundibular slit. However, a numerous different sized and shaped caudally directed pharyngeal papillae were distributed in the roof of the pharynx as revealed by SEM. In the duck, numerous small fines caudally directed papillae with pointed ends were observed around the infundibular cleft. These papillae were observed to increase in number and length towards the esophageal opening (Hassouna, 2002). In budgerigar, the pharyngeal roof has several filiform papillae distributed primarily along the midline (Evans, 1996). Functionally, the caudally directed papillae on the laryngeal eminence and the roof of the pharynx facilitate the caudal movement of the bolus towards the esophagus (McLelland, 1975; King and McLelland, 1984).

This study confirmed the basic observations by previous authors (Koch, 1973; McLelland, 1975; King and McLelland, 1975; Tadjalli *et al*., 2008; Madkour, 2011; Tivane *et al*., 2011) that the pharyngeal roof was occupied by the infundibular slit which was a median longitudinal fissure representing common opening of the two auditory tubes. This slit in the turkey was situated immediately caudal to the choanal slit and behind the level of the ventrally located laryngeal inlet. Madkour (2011) in the duck, Tadjalli *et al*. (2008), and Tivane *et al*. (2011) in the ostrich stated that, this slit was located caudal to the choana. Erdogan and Alan (2011) informed that, the position of this slit in European magpie and Common raven at the rear of the palate and separated from the choanal cleft by transverse fold.

From the morphometrical data, the infundibular slit in the turkey measured 6.93 mm long and constituted 68.34% of the total length of the pharyngeal roof. This slit measured 8.04 mm in the 60 days old duck (Madkour, 2011). In the ostrich, the length of the infundibular slit was 1.97 cm (Tadjalli *et al*., 2008) and 1.8-2.2 cm long (Ali, 2004). Erdogan and Alan (2011) mentioned that this length was 1.41 mm and 3.69 mm in the European magpie and Common raven respectively. It was cleared that the length of the infundibular slit in the turkey was nearly equal to that of the duck, but shorter than that of the ostrich.

In the same line with Madkour (2011) in all studied ages of post hatching ducks, the present scanning electron microscopical results showed numerous openings of sphenopterygoid salivary glands scattered between the pharyngeal papillae. Moreover, the above mentioned author added that in 60 days old ducks few openings of these glands were observed in the shallow groove which represents the caudal continuation of the infundibular slit. These findings were confirmed by Hodges (1974) and McLelland (1975) in chicken that the sphenopterygoid glands were associated with the pharyngeal roof. In this connection, Nickel *et al*. (1977) mentioned that the Gland. pterygoidea and Gland. tubariae were situated in the mucosa of the pharyngeal roof. In addition to the sphenopterygoid salivary glands the caudal palatine glands in the duck were present in the roof and lateral walls of the pharyngeal cavity. The openings of these glands were filled with secretory droplets and desquamated epithelial cells as shown by electron microscope (Hassouna, 2002).

Corresponding to the current study, the pharyngeal mucosa demonstrated pharyngeal folds. In this respect, Tivane *et al*. (2011) stated that the massive pharyngeal folds in the ostrich were seen to overlap on the midline as also noted by McLelland (1979). The latter author stated that these folds made a sharp boundary with the esophagus. Hassouna (2002) observed temporary mucosal folds on the roof and lateral walls of the pharyngeal cavity of the duck. The scanning electron microscopic observations of both the rostral and caudal parts of the infundibular folds in the ostrich detected that the mucous membrane possesses short numerous microvilli at the free apical surface of the cells at the ventral surface of the infundibular fold (Ali, 2004).

The present study supported results of Madkour (2011) in duck that the lymphatic infiltration could be observed surrounding the intraepithelial mucous glands near the edge of the infundibular slit. Nickel *et al*. (1977) stated that the mucosa of the mouth cavity and pharyngeal cavity contains glands and also lymphoreticular tissue, the latter distributed both diffusely and in follicles. This lymphoreticular tissue was particularly prominent in the palatine and infundibular clefts. Hassouna (2002) mentioned that in duck much lymphoid aggregations were observed in the infundibular mucosa. This tissue was described in birds as pharyngeal tonsil (King and McLelland, 1984) and tubal tonsils (Nickel *et al*., 1977). The latter authors added that the lymphoid centers were abundant

in geese than in pigeon. With this regard, Koch (1973) reported that in the mucous membrane folds of the lateral boundary of the infundibular slit were throat tonsil (Tonsilla pharyngica). In this connection, several authors (Hodges, 1974; Ohshima and Hiramastsu, 2000; Hassouna, 2002) mentioned that, lymphoid accumulation were located in connective tissue septa of the salivary glands and around their ducts. The pharyngeal tonsils aid in the protection of the body against invading bacteria, viruses and other foreign bodies, therefore, these tonsils are known to be frequent portals of infections (Tadjalli *et al*., 2008).

Conclusion

The present study provided comprehensive information on the structure of the pharyngeal roof of the *Meleagris gallopavo*. Distribution of numerous caudally directed pharyngeal papillae in the roof of the pharynx which helps in swallowing of the food and facilitates it's caudally movement towards the esophagus. Compound tubular mucous sphenopterygoid salivary glands scattered in the mucosa of the pharyngeal roof participating by their secretions as a barrier against microbial development, and involved in lubrication and humidification of the food.

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