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# Histological and Electron Microscopical Structure of Tongue and Lingual Papillae of Guinea Fowl (*Numida meleagris*)

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**Key words**
**Papillae, *Numida meleagris*, Lingual glands, SEM, Tongue**

## ABSTRACT

The aim of this study was to investigate the histology of the tongue of the guinea fowl, *Numida meleagris* by using light and scanning electron microscopy methods. Six chicks (6-7 weeks), six layer hens (9-13 weeks) and six studs guinea fowl were used to study and determine the histological features of the tongue of the guinea fowl. Histological structures were examined using a light microscope the tissues and papillae of the tongue were examined with a the scanning electron microscope, and photos of the general histologic structures were taken. The tongue was triangular shaped, and consisted of apex, corpus, and radix sections. The dorsal and ventral surfaces of the tongue were covered by a keratinised stratified squamous epithelium. Lamina propria and submucosal layers were distinguishable underneath the epitelium. SEM observations of surface of the radix linguae revealed conical papillae and tongue caudally directed lingual papillae. Thus, in this study, the anatomy and histology of the tongue of the guinea fowl tongue were examined in detail using light and scanning electron microscopy, and the similarities and differences between the tongue of the guinea fowl and the tongue of other poultry species were investigated.

## INTRODUCTİON

*Numida meleagris* is part of the order Galliformes (Dyke *et al*., 2003; Haaroma, 2003; Kristin, 2001; Monroe, 1993). In certain parts of the world, guinea fowl are used as experimental animals in biomedical research (Tabasi and Mohammadpour, 2019; Pourlis, 2014; Igwebuike and Anagor, 2013; Angus and Wilson, 1964).

The avian tongue is located inside beak, at the beginning of the digestive system. It is reported that the tongues's anatomical structure varies widely between species and is depending on the animal's eating habits (Dursun, 2014; Elsheikh and Al-Zahaby, 2014; Erdoğan and Iwasaki 2014; Getty, 1975, Nickel *et al*., 1977; Tabasi and Mohammadpour, 2019). The tongues of gallinaceous birds, which are located at the base of the oral cavity, have features that vary according to the shape and structure of the bird; for example, the tongue is large and wide in swimming species, shoveled in ducks, folded in birds such as the woodpecker (Karadağ and Nur, 2002; King and Mc Lelland, 1984).

In poultry, the tongue consists of the apex, corpus, and radix linguae sections. The tongue is attached to the base of the cavum oris by the radix linguae with the frenulum linguae, and the free-moving part of the tongue extends into the cavum oris (Tabasi and Mohammadpour, 2019; Dursun, 2014; Nickel *et al*., 1977; Getty, 1975).

Bird tongue epithelium is composed of two types of keratinized epithelium, which are layers called orthokeratinized and parakeratinized epithelium. The major criteria for the seperation of keratinized epithelia investigated in histological sections by light microscopy is the presence or absence of nuclei in the keratinized layer. In the orthokeratinized epithelium cell nuclei disappear in the keratinized layer, whereas in the parakeratinized epithelium flattened, highly condensed nuclei remain in the cell cytoplasm of the keratinized layer until exfoliation (Skieresz-Szewczyk, 2014; Tabasi and Mohammadpour,

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2019; Jackowiak *et al*., 2011; Jackowiak and Godynicki, 2005; Iwasaki *et al*.,1997).

Histologically, in poultry, the lamina propria is a thin layer of connective tissue, and part of the layers of the serous, mucous, sero-mucous glands that are located in the submucosa layer (Aytekin, 2016).

Kudo *et al*. (2008) stated that intra-epithelial taste buds are present in chickens, while Igwebuike and Eze (2010) reported that intra-epithelial taste buds are present in the pied crow. Erdoğan *et al*. (2012b) reported that intra-epithelial taste buds are also present in the partridge. On the other hand, it has been reported that some species such as quails, geese, ducks, and crows do not have taste buds (Erdoğan and Iwasaki, 2014; Pourlis, 2014; Karadağ and Nur, 2002).

The tongue muscularis (tunica muscularis) is situated under the lamina propria of the tongue (Aytekin, 2016; Erdoğan and Iwasaki, 2014). However, since the tongue muscles of poultry are less developed than mammalian tongue muscles, tongue movements are performed by the muscles of the tongue bone (Karadağ and Nur, 2002; Nickel *et al*., 1977).

Papilla conica was aligned caudally of the behind tongue in the most of the native scanning electron microscope (SEM) images. The tongue plays a role in regurgitation, and also in the transmission of nutrients to the oesophagus (Tabasi and Mohammadpour, 2019; Erdoğan and Iwasaki, 2014; Jackowiak *et al*., 2010; Crole and Soley 2009a, b). Crole and Soley (2009a, b) identified (with SEM) the presence of multiple epithelial folds in the dorsal epithelial surface in ostriches, which enables the tongue's surface to become lubricious. Parchami *et al*. (2010) identified the same phenomenon in quail, and Pourlis (2014) also found this to be the case for Japanese quail.

There have been many studies on the morphological structures, histology, and SEM images of the tongues of different poultry species. Studies of this nature have been completed by Arthitvong *et al*. (1999) on domestic chicken, Erdoğan *et al*. (2012b) on the red-head partridge, and Liman *et al*. (2001) and Pourlis (2014) on the Japanese quail. There have also been studies on the oral cavity and tongue of the guinea fowl (Tabasi and Mohammadpour, 2019; Igwebuike and Anagor, 2013).

The aim of this study is to add new information to existing anatomical, histological and SEM-based studies on the tongue of the guinea fowl, and to provide resources for the scientific research to be done in relation to other morphological structures of the guinea fowl.

## MATERİALS AND METHODS

*Ethical aspects*

Protocols used in this research are approved by SUVEK, with the decision of the ethics commitee dated 30/12/2014, and numbered 2014/81.

*Chicks*

Six guinea fowl chicks (6-7 weeks), six layer hens (9-13 weeks) and six studs from an Aksaray guinea fowl breeder were weighed. Anaesthesia was injected intramuscularly, at 5 mg/kg xylazine for premedication and 30 mg/kg for anaesthesia, into guinea fowl held in special cages.

*Histological method*

For histological examinations, tissue samples were taken from different sections of the tongue. Tissues inspected by routine histological methods were embedded in paraffin wax. Tongue sections 6 mm thick were taken from the paraffin blocks and painted with Crossmann's triple stain to determine the general histological structure (Crossman, 1937), Periodic-acid schiff (PAS) and alcian blue (AB) (pH 2.5) stains were also used (Bancroft, 1994).

*Scanning electron Microscopy (SEM)*

For SEM images, the apex, corpus and basal tongue sections were separately kept in 10% formaldehyde solution for 24 h to modify the tissue samples, as per methods from Erdoğan and Alan (2012). After, 2.5% glutaraldehyde was allowed to stand for 6 h and washed for 10 min with 0.1 M phosphate buffer solution (PBS) twice, then washed five times with 0.1 M buffer in the solution. It was then placed for 10 min each in a 25%, 50%, 75%, 100%-ethyl alcohol series. Tissues were dried in desiccator (Nüve,EC160;Turkey) and gold-plating processes, images were taken with FEI-Quanta; FEG 250,USA scanning electron microscope. The Nomina Anatomica Avium (Baumel, 1993) was used for terminological expressions.

*Statistical analysis*

SPSS 20.0 was used for the statistical analysis of data (Büyüköztürk, 2011). It was determined whether there was a significant relationship between the three sample groups given the quantitative scale observations in the study. A non-parametric Kruskal-Wallis test was used. Data were shown with mean and standard error (Table I).

## RESULTS AND DISCUSSION

*Light microscopic structure*

The dorsal and ventral face of the tongue was covered with a multilayered flat keratinised epithelium. The epithelium covering the dorsal surface of the tongue was thicker than the epithelium covering the ventral surface (Fig. 1A, D). The presence of papillae (papillae linguales

**Table I. The average measurements of structures in the guinea fowl. (mm) (n:6).**

| | 6-7 weeks | | | 9-13 weeks | | | Studs | | | p |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean ± SE | Min | Max | Mean ± SE | Min | Max | Mean ± SE | |
| Va | 269 | 425 | 331.83±53.98 | 1025 | 1250 | 1127.66±77.37 | 1500 | 1750 | 1592±87.93 | p<0.01 |
| Du | 10.02 | 12.36 | 11.27-0.87 | 13.23 | 16.63 | 15.20±1.24 | 17.23 | 19.01 | 18.21±0.68 | p<0.01 |
| Dek | 3.12 | 4.17 | 3.5±0.39 | 5.22 | 6.91 | 6.03±0.69 | 8.05 | 9.58 | 8.64±0.56 | p<0.01 |

Ort±SH: Mean ± SE, (range; p<0,01); Va, Total weight of the body (g); Du, Lenght of the tongue (apex-radix distance) (mm); Dek, Cross-sectional length of the tongue (mm).

**Table II. Mean values from SEM images of papillae conicae in the guinea fowl. (mm) (n:6).**

| | 6-7 weeks | | | 9-13 weeks | | | Studs | | | p |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean ±SE | Min | Max | Mean ±SE | Min | Max | Mean ±SE | |
| Pce | 203 | 325 | 264±32.14 | 645 | 896 | 762±11.23 | 1685 | 1900 | 1740±32.15 | p<0.01 |
| Pck | 181 | 210 | 193±9.36 | 456 | 754 | 603±12.41 | 1150 | 1300 | 1196±37.85 | p<0.01 |

Ort±SH, Mean ± SE (range; p<0,01); Pce, The longest length of the papilla conicae (mm); Pck, The length of the shortest papilla conicae (mm).

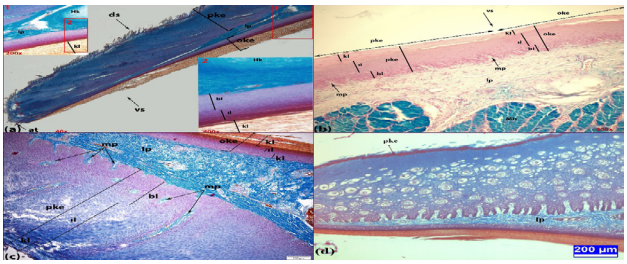


Fig. 1. (a) Apex-corpus view of the tongue ( 6-7 weeks). Triple stain 40x; 200x; 400x. (b) Corpus- radix view ventral surface of the tongue (studs). Alcian blue stain 200x. (c) Apex-corpus view of the tongue (studs). Triple stain 100x. (d) Apex view of the tongue (9-13 weeks). Triple stain, Scale bars: 200µm. vs)ventral surface of the tongue; ds) dorsal surface of the tongue; at) apex of tongue; oke) orthokeratinized stratified epithelium; pke) parakeratinized epithelium; lp) lamina propria; bl)basal layer; ıl)intermediate layer; kl)keratinized layer; Hk) Hyaline cartilage; mp) microscobic papillae; Mb)Mucous glands.

caudales) in the dorsal vertebrae was noted between the vertebral corpus and radix sections (Fig. 2A, B). Lamina propria were distinctive in the subepithelial regions. Starting from the lingual apex section, a hyaline cartilage extending toward caudal was seen. This cartilaginous tissue was ossified in the direction of the corpus region (Fig. 1A). The skeletal muscle of the tongue was found under the corpus linguae. In the apex of the tongue, the hyaline cartilage was surrounded by the conical tip, while in the corpus and radix, the glands were in the mucosa between the cartilaginous tissue and the dorsal facial epithelium (Fig. 3A, B). The majority of the epithelial cells forming the corpus glands in the apex of the epidermis were serous in the 6-7 week-old chicks (Fig. 3A, B), and the mucous cells were seen to increase in the 9-13 week-old layer hens. In the stud group, the glands in this region were mucous (Fig. 3B). As a result, cells stained with PAS and AB (pH 2.5) reacted positively, showing an age-related increase (Fig. 3C, D). The corpuscular epithelial cells in the corpus and radix regions reacted positively with PAS (Fig. 4A, B, C) and AB (pH 2.5) (Fig. 4D) to varying degrees, depending on the age and location.

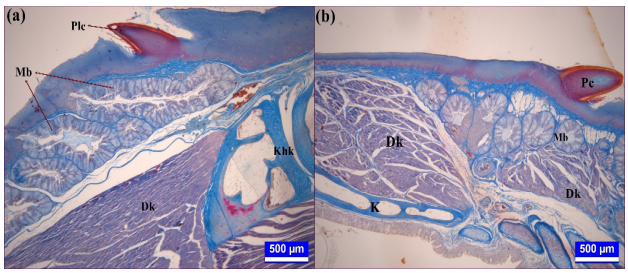


Fig. 2. (a) Corpus-radix view of the tongue (9-13 weeks). Triple stain. Scale bars: 500µm. (b) Radix view of the tongue (9-13 weeks). Triple stain. Scale bars: 500µm. Plc) papilla linguales caudales, Mb) mucous glands, Dk) tongue muscle, Khk) hyaline cartilage, Pc) papilla conicae, K) hyaline cartilage.

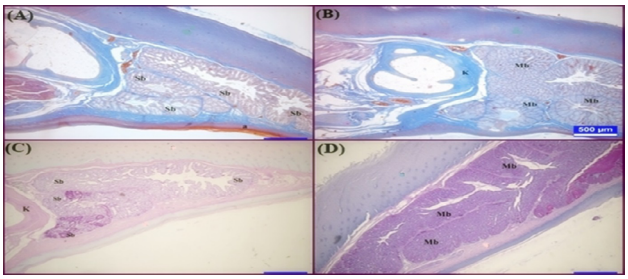


Fig. 3. (A) Apex-corpus view of the tongue ( 6-7 weeks). Triple stain. Scale bars: 500µm. , (B) Apex-corpus view of the tongue in the stud, Triple stain. Scale bars: 500µm. (C) Apex view of the tongue ( 6-7 weeks). PAS. Scale bars: 500µm. (D) Mucous glands of the stud. PAS. Scale bars: 500µm. Sb) Serous glands, a) parakeratinized epithelium, Mb) Mucous glands, K) Hyaline cartilage.

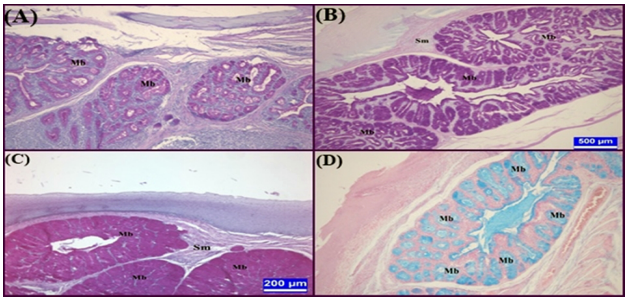


Fig. 4. (A) Corpus- radix view of the tongue (6-7 weeks) PAS. Scale bars: 200µm. The glandular epithelial cells in the corpus and radix regions show a PAS positive reaction at varying degrees of age, Mb) Mucous glands. (B) Corpus-radix view of the tongue (9-13 weeks). PAS. Scale bars: 500µm. The glandular epithelial cells in the corpus and radix regions show a PAS positive reaction at varying degrees of age. Mb). Mucous glands (C) Corpus-radix view of the tongue (stud). PAS. Scale bars: 500µm. The glandular epithelial cells in the corpus and radix regions show a PAS positive reaction at varying degrees of age. Mb) Mucous glands (D) Corpus- radix view of the tongue (6-7 weeks). Alcian blue stain. Scale bars: 200µm. Mb) Mucous glands.

*SEM structure*

In the SEM observations, the dorsal surface of the tongue had a smooth appearance (Fig. 6A). The shape of the tongue was triangular, which agreed with the feding habits. The tongue consisted of the apex, corpus, and radix sections. The structure of dorsal and ventral multilayered flat keratinised epithelial layers was distinctive (Fig. 5A). The dorsal multilayered flat keratinised epithelium was found to be thicker than the ventral. The apex section was divided into bands, in the cross-sections of flat epithelial layer (Fig. 5B). When we examined the cross-sectional surface from the apex to radix sections, multilayered flat keratinised tissue was detected, as were layers of the hypoglottis and hyaline cartilaginous tissues (Fig. 6B). A total of four caudally directed lingual were detected between the corpus and radix (Fig. 6C). While these papillae were in the form of a flat flattened protrusion in the 6-7 week-old guinea fowl, in the 9-13 week-old layer hens the papillae were observed to be thicker and more pointed (Fig. 5C).

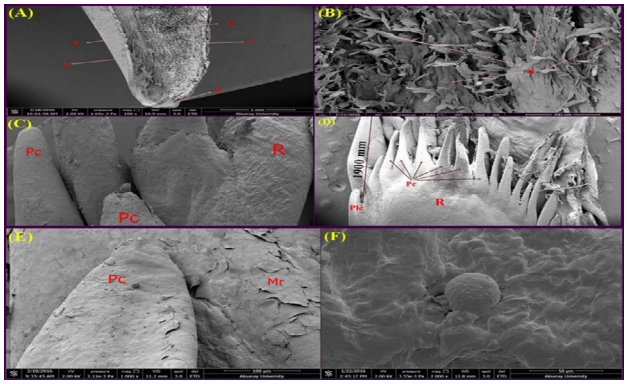


Fig. 5. (A) SEM view of the dorsal cross section of the apex part of the tongue in the stud. (a) Stratified Squamous Epithelium (Keratinized), (K) Keratin, (A) Apex, (C) Corpus. (B) A higher magnification of the tongue apex with the stratified squamous epithelium cells, (a) Stratified Squamous Epithelium (Keratinized), (C) SEM view of the papillae of the radix part of the tongue (9-13 weeks). (R) Radix, (Plc) Papilla linguales caudales, (Pc) Papilla conicae. (D) SEM view of the papillae of the radix part of the tongue (stud). (R) Radix, (Plc) Papilla linguales caudales, (Pc) Papilla conicae. (E) SEM view of the papillae of the corpus-radix part of the tongue (9-13 weeks). (Pc) Papilla conicae, (Mr) Epithelial folds, (F) SEM view of the surface of the tongue body and papilla linguales (stud).

The V-shaped conical papillae in the lingual radix showed a brush-like arrangement extending from the medial to the lateral direction, and the total amount of papillae ranged from 16-18 (Fig. 5D, Fig. 6D). As shown in Figure 5D, measurements of SEM images showed that the length of the conical papillae increased with age. This change was statistically significant at $p < 0.01$. The gap between the anterior and posterior tongue glands and the lingual surface epithelium increased in depth according to age.

In the literature (Igwebuike and Anagor, 2013; Tabasi and Mohammadpour, 2019), papilla conica was the given name for the papillae on the tip of the lingual radix, showing a cone-shaped, pointed, longitudinal sequence. The present study has therefore named the papilla conicae similarly in guinea fowl.

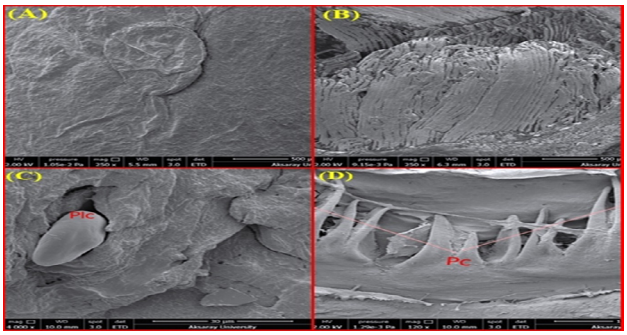


Fig. 6. (A) SEM view of the surface epithelium of the corpus portion of the tongue (9-13 weeks). (B) Cross-sectional SEM view of longitudinal tongue muscles (6-7 weeks), (C) SEM view of the surface of the tongue body and papilla linguales caudales (6-7 weeks), (D) SEM view of the papillae of the radix part of the tongue (6-7 weeks), (Pc) Papilla conicae.

It is stated that in the literature (Crole and Soley, 2009a; Erdoğan and Iwasaki, 2014; Jackowiak *et al*., 2010; Tabasi and Mohammadpour, 2019), the papilla conicae, which is arranged caudally in the majority of the radix in the poultry species, is involved in the transmission and regurgitation of food transported to the oesophagus. Erdoğan *et al*. (2012b) reports that the number of conical papillae in partridges changes between 12-14 weeks old. The papilla conicae were found in all of the poultry chickens examined, and the numbers ranged from 16-18 week.

As with reports by Erdoğan *et al*. (2012b) and Rossi *et al*. (2005) on partridges, Parchami *et al*. (2010) on quails, and Hassan *et al*. (2010) on geese, Igwebuike and Anagor (2013), reported that Nigerian guinea fowl's papilla conicae are arranged in a V-shaped sequence on the radix linguae. In the guinea fowl that examined in this study, a similar pattern was displayed in the same section.

Tabasi and Mohammadpour (2019) on guinea fowl and King and McLelland (1984) reported that the lingual glands in the tongue of poultry species were generally located in the anterior and posterior sections of the tongue. Jackowiak *et al*. (2006) reported that the gland was not found in the cormorant. In the present study, the lingual glands of the guinea fowl were located in the corpus, radix and apex section.

In birds the parakeratinized epithelium covers the dorsal surface of the apex and body of the tongue, while in Anserinae it also covers the lingual prominence and root (Iwasaki *et al*., 1997; Jackowiak and Godynicki, 2005; Jackowiak *et al*., 2011; Skieresz-Szewczyk *et al*., 2014). Skieresz-Szewczyk *et al.* (2014, 2018) reported that the ventral surfaces of the tongue is covered with a orthokeratinized stratified epithelium. The findings obtained in this study are consistent with the results of the above mentioned studies.

In the literature (Aytekin, 2016; Erdoğan and Iwasaki, 2014), a muscular layer (tunica muscularis) was reported under the lamina propria in poultry species. In the guinea fowl, it was determined that the muscles of the tongue, which are skeleton-specific, were found just below the lamina propria in the corpus and radix sections.

According in literature (Karadağ and Nur, 2002; Nickel *et al*., 1977) on poultry, Tütüncü and Onuk (2012) reported on stork, it was found that along the median line of the tongue, there was a tongue bone, which began with hyaline cartilaginous tissue from the apex and extended along median into bone tissue. In accordance with this information, in the materials examined, conical hyaline cartilage was found extending from caudal to caudal was seen initiating at the lingual apex. In other words, the cartilage began to ossify in the direction of the corpus region.

Crole and Soley (2009a, b), Erdoğan *et al*. (2012a, b), and Liman *et al*. (2001) reported that the lingual glands differ, consisting of tubular, tubulo-alveolar, alveolar, serous, and sero-mucous glands, in accordance with the different types of secretions needed by poultry species.

In the examined guinea fowls, the majority of the epithelial cells forming the corpus glands in the apex of the tongue were serous cells in the 6-7 week-old chicks, while the majority of these cells were mucous cells in 9-13 week-old layer hens. In studs, it was determined that the glands in this region were mucous. The corpus glandular epithelial cells were increased in the corpus and radix regions, and composed of mucous cells to varying degrees depending on the age and location.

Arthitvong *et al*. (1999) found that the neutral mucin in the domestic chicken was secreted from all lingual glands, and acidic mucin secreted from the anterior and posterior lingual glands. Neutral mucin and acidic mucin secretion for red-headed partridges are secreted from the anterior and posterior lingual glands, as per Erdoğan *et al*. (2012b). In quail, Liman *et al*. (2001) stated that the neutral mucin was secreted from all lingual glands, and acidic mucin was secreted from the anterior and posterior lingual glands. In the guinea fowls examined in this study, acidic mucin and neutral mucin secretions were similar to those of chickens, red-headed partridges, and quails, but both mucins were secreted from both the anterior and posterior lingual glands.

Erdogan and Iwasaki (2014), Nickel *et al*. (1977), and Onuk *et al*. (2015), Tabasi and Mohammadpour (2019) reported that the dorsal epithelial surface of the tongue and the tongue itself are shaped according to nutritional

patterns, food type, and living environment, through SEM images of poultry tongues. In the SEM images of the present study, the tongue epithelium surface of guinea fowl was flat, long, and triangular.

According to reports by Parchami *et al*. (2010) on quail, Crole and Soley (2009a, b) on ostriches, and Pourlis (2014) on Japanese quails, it was found that the presence of a large number of epithelial folds allowed the tongue surface to become slippery in SEM images of the dorsal lingual epithelial surface. In present study, numerous epithelial folds were observed on the surface of the dorsal lingual epithelium in guinea fowl.

Tabasi and Mohammadpour (2019) reported that conical papillae and salivary glands are present on SEM images on the back surface of the guinea fowl tongue. The same findings were obtained in present study.

Pourlis (2014) noted that the tongue of the Japanese quail resembles a triangular shape in SEM images, which is divided into apex, corpus, and radix sections, and that the dorsal lingual epithelial surfaces are covered with keratinized stratified squamous epithelium. In the present study, dorsal keratinized stratified squamous epithelium was thicker than that of the ventral section.

## CONCLUSION

The histology, and SEM findings of the tongue and lingual papillae of the guinea fowl were examined, and their similarities and differences with other poultry species were determined. When all the findings obtained in the study are evaluated together, it can be said that tongue and lingual papillae of the guinea fowl show a great similarity with other poultry species.

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*Statement of conflict of interest*

The authors declare there is no conflict of interest.

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