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Ultrastructure of the Tongue of Turkeys (*Meleagris gallopavo*-hybrid breed) Reared in Türkiye Using Light and Scanning Electron Microscopy

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Abstract

The tongue is one of the organs involved in the digestive and sensory systems, which plays a vital role in feeding strategies in birds. In our study, macroscopic and microscopic characteristics of the tongue were determined in turkeys raised in Türkiye. Five turkey tongues, cut for consumption, were used. The tongue was divided into apex, corpus, and radix sections and fixed in formaldehyde. After fixation, routine histological tissue procedure was performed. The sections were stained with Crossman's triple staining method and histological features were determined. For scanning electron microscopy, tissue samples fixed by glutaraldehyde solution were coated with gold after the routine procedure and examined under a scanning electron microscope. As a result of the study, the tongue was triangular, and conical papillae were observed between the corpus and radix. Two cornified epithelial structures were identified through histological and electron microscopic findings. Microscopic papillae extending from the connective tissue to the epithelial layer on the dorsal surface of the tongue, extending in different directions were quite prominent. Anterior and posterior lingual glands were present in the corpus and radix of the tongue. Electron microscopic examination showed the opening holes of these glands and conical papillae. Although slight differences were observed, the tongue's structure was generally similar to that of Galliform birds.

Keywords: Morphology, scanning electron microscopy, tongue, turkey.

Introduction

In birds, the upper and lower jaws develop into a beak, while teeth, lips, and cheek formations are absent. The presence of the beak in birds, along with different feeding habits, and living conditions, has led to morphological differentiation of their tongues (1). Anatomically, bird tongues bear a basic resemblance to the tongue segments of mammals (apex, corpus, and radix) and form a triangular organ that fills the entire lower part of the beak. In mammals and aquatic and terrestrial poultry species (geese, ducks, swans, etc.), the tongue functions in solid food intake, grass cutting, drinking, and water filtration (2-6).

The avian tongue is characterized by mechanical papillae (7,8). The formation of a papillar crest consisting of mechanical conical papillae extending caudally and separating the tongue corpus and radix is remarkable (9,10). This crest structure can be observed as single or double. Conical papillae are known to assist in transporting food particles on the tongue, manupilating food, and filtration fluid (11,12).

Studies conducted in different bird species show determined that the histological structure of the tongue form, tongue skeletal apparatus, and tongue epithelium positively correlate with birds' feding habits (11, 12-15). The literature shows that the tongue has been examined macroscopically and microscopically in many bird species such as chicken, quail, parrot, penguin, goose, duck, and domestic

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turkey (6, 16-18).

Turkey (*Meleagris gallopavo*) is a large Galliform bird reported to have been domesticated before Christopher Columbus discovered the Americas, with various subspecies of its wild ancestor spreading over a wide area from southern Canada to southern Mexico (19,20). Turkey meat is widely preferred for its high nutritional value, low fat and cholesterol content, its ability to be processed into various products, and its flavor (21). In Türkiye, turkey meat consumption has increased to meet the increasing population's animal protein needs and with the tendency towards healthy nutrition.

This study aimed to investigate the general morphological structure of the tongue in turkeys raised in Türkiye by using light and scanning electron microscopy. It also aimed to determine the similarities and differences with birds from the same family.

Material and Methods

Supply of Animals

The tongue structures of five healthy adult turkeys slaughtered for consumption were analyzed. Tongues were dissected from each bird and removed from the oral cavities. Macroscopic features were noted.

The procedures used in this investigation were approved by the Siirt University Experimental Animals Application and Research Center under ethics committee report number 2024/05/27.

Light Microscope

The tongue was divided into the apex, corpus, and radix for histological procedures. Tissues were fixed in a 10% neutral buffered formalin solution for 24 hours at room temperature, and routine tissue processing procedures were performed. After dehydration in 70% ethanol, 80% ethanol, 96% ethanol, and absolute ethanol, the tissues were embedded in paraffin and 5 µm sections were taken. Tissue sections were mounted on polylysine-coated slides and incubated in an oven at 37°C for 1 hour. The slides were stained using Masson trichrome staining method modified by Crossman (22). All slides were examined under a light microscope (Nikon Eclipse 80i Microscope, Tokyo, Japan). Photographs were taken with a Nikon Ds Camera Control Unit DS-L1 (Tokyo, Japan).

Scanning Electron Microscope

Tissue samples taken for scanning electron microscopy (SEM) images were kept in 2.5% (pH: 2.7) glutaraldehyde solution for 24 hours. After the first fixation step, they were washed three times at 10-minute intervals in 0.1 M Phosphate buffer (pH 7.4). The second fixation step was completed by rotating osmium tetraoxide at room temperature for 2 hours. The samples were then washed three times for 10 min each in phosphate buffer. Tissues were dehydrated in 25%, 50%, 75%, and 90% ethyl alcohol at +4°C for 15 minutes each. Following dehydration, they were kept in 96% and 100% ethyl alcohol for 30 minutes each. The drying phase was completed in an oven at 60°C for 2 days. Finally, the samples were coated with gold (23). After drying and coating in the incubator, images were taken with SEM (JEOL JSM 5600 LV) at Eskişehir Osmangazi University Central Research Laboratory Application and Research Centre. Nomina Anatomica Avium (24) was referenced for anatomical terminology.

Results

Macroscopic Findings

On macroscopic examination, the tongue was observed to resemble a triangle matching the shape of the beak. The tongue consisted of the apex, corpus, and radix sections. On the dorsal surface of the tongue, a groove structure extending from the apex to the radix and dividing it into two halves was observed. It was observed that the posterior part of the corpus was shaped like the letter "V", with two papillar crest on the right and left sides. Caudally oriented conical papillae were located at the end of the corpus (Figure 1). These papillae were short at the center of the letter "V" and grew larger toward the sides.

Figure 1. Dorsal view of the beak cavity in a turkey. p: palatinum; l: larynx; pc: papillar crest; c: corpus; a: apex; *: laryngeal cleft; arrow: papillae conicae.

Microscopic Findings

On histological examination, a multilayered epithelium covered the dorsal and ventral surfaces of the tongue (Figure 2a-d). Depending on the tongue region, three different types of epithelium were identified in the tongue mucosa: para-keratinized, ortho-keratinized and non-keratinized epithelium. The dorsal surface of the apex (Figure 2a) and corpus (Figure 2c) were covered with a multilayered par-

akeratinized epithelium, while the ventral surface of the both regions (Figure 2b) was surrounded by a multilayered orthokeratinized epithelium. Multilayered, non-keratinized epithelium covered the radix of the tongue (Figure 2d). The multilayered para-keratinized and ortho-keratinized epithelium consisted of basal, intermediate, and cornified layers (Figure 2a-c, 2f). In contrast, the multilayered non-keratinized epithelium consisted of basal, intermediate, and superficial layers (Figure 2d).

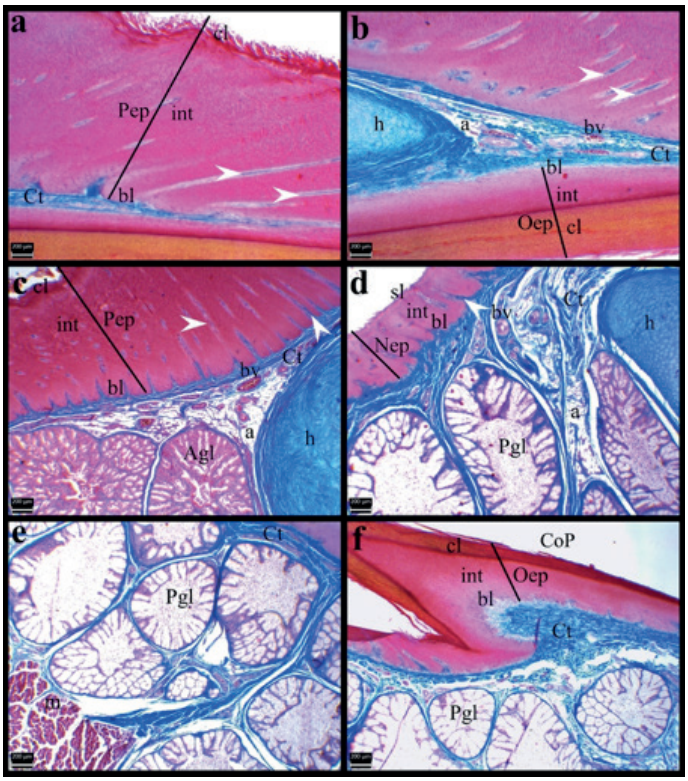

Figure 2. Histological appearance of the apex (a, b), the corpus (c), the radix (d, e) parts of the tongue, and the conical papillae (f). a: adipose tissue, Agl: anterior lingual glands; bl: basal layer, bv: blood vessel, cl: cornified layer, CoP: conical papillae, Ct: connective tissue, h: hyaline cartilage, int: intermediate layer, m: skeletal muscle fibers, Nep: nonkeratinized epithelium, Oep: ortho-keratinized epithelium, Pep: parakeratinized epithelium, Pgl: posterior lingual glands, sl: superficial layer, arrowheads: microscopic papillae. Crossman's triple staining. Bar: 200 µm.

Beneath the epithelial layer, connective tissue containing adipose tissue, nerve plexuses, blood, and lymphatic vessels was identified (Figure 2a, 2d). The connective tissue also contained hyaline cartilage starting at the apex of the tongue and extending towards the radix (Figure 2b-d). No lingual glands were found at the apex of the tongue. However, anterior lingual glands were determined in the connective tissue in the corpus of the tongue (Figure 2c), and posterior lingual glands were found in the connective tissue in the radix of the tongue (Figure 2d, 2e). Skeletal muscle fibers were observed in the connective tissue between the posterior lingual glands at the radix of the tongue (Figure 2e).

On the dorsal surface of the tongue, microscopic papillae extending from the connective tissue to the epithelial layer were prominent (Figure 2a, 2d). Microscopic papillae were denser on the corpus of the tongue (Figure 2c). The microscopic papillae extended at lateral angles at the apex of the tongue (Figure 1a, 1b), whereas they extended at steeper angles at the corpus (Figure 2c) and radix (Figure 2d) parts of the tongue.

Conical papillae were found between the corpus and radix parts of the tongue.
The conical papillae were V-shaped and covered with multilayered orthokeratinized epithelium. Posterior lingual glands were identified in the connective tissue under the conical papillae (Figure 2f).

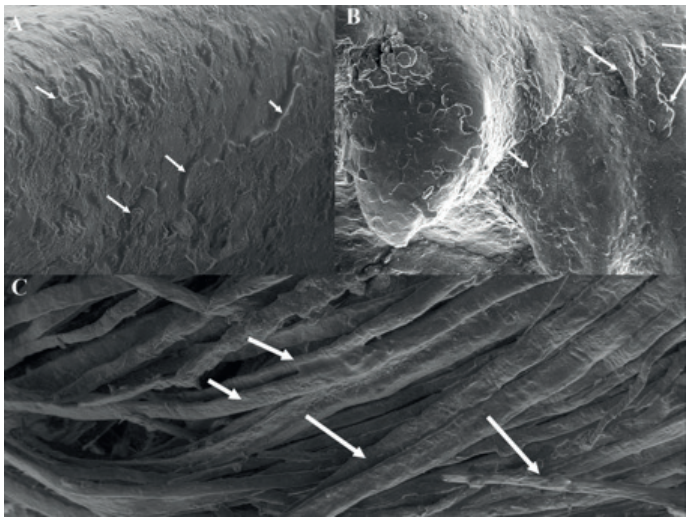

Figure 3. Scanning electron microscopy image of the apex and dorsal parts of the tongue of a turkey. A: Arrow: ortho-keratinized epithelium X100; B: Arrow: ortho-keratinized epithelium X100; C: Arrow: Para-keratinized epithelium X125.

Ultrastructural examination revealed that the apex and corpus of the tongue were covered with ortho-keratinized and para-keratinized epithelium on the dorsal surface (Figure 3). There were diffuse conical papillae between the corpus and radix. Conical papillae and opening holes of the lingual glands were observed around the cohana half (Figure 4).

Figure 4. Scanning electron microscope image of the radix and laryngeal cleft of the turkey.
D: Arrow: Conical papillae X100; E: Arrow: Conical papillae X100.

Discussion

Different feeding habits have caused different tongue forms in birds. Tongue shape shows morphological differences in birds depending on habitat type, food intake, and beak anatomy (25, 26). A good understanding of these differences enables the selection of feed and the development of feeding strategies, especially for breeding species.

Our study found a triangular tongue structure was found in turkeys, similar to birds in the same family (18, 27, 28). The shape of the tongue was reported to be spade-like and oval-tipped in Falconiformes (8, 29), forked and oval-tipped in Strigiformes, and round-tipped in Anseriformes (30). Macroscopically, it was determined to consist of the apex, corpus, and radix as reported in many studies (1, 31). Although the groove dividing the apex and corpus of the tongue into two parts is reported to be absent in chickens (32, 33), we observed this groove structure in our study, similar to studies in domestic turkeys (17), common quail, and Japanese quail (26, 28, 34). The papillar crest consisted of caudally orientated conical papillae arranged in a 'V' shape, similar in structure to chicken (27), quail (26), partridge (35), and turkey (18). It has been reported that the conical papillae transmit food into the esophagus and prevent vomiting (28).

Salivary glands lubricate and protect the mucosa while transferring food to the esophagus (36,37). In a study on chickens (27), microscopic salivary gland opening holes were found on the lingual radix surface. These structures were also observed in our study's SEM examination.

Parakeratinized epithelium was found on the dorsal surface of the apex and corpus of the tongue, while orthokeratinised epithelium was found on the ventral surface of the apex and corpus (18). It has been reported that para-keratinized epithelium is found in the parts where food is collected and food is transported. The ortho-keratinized epithelium is found in the parts of the tongue associated with food intake, grass cutting, and food filtering from water (6, 18). Unlike our study, which was similar to the study conducted on geese, the non-keratinized epithelial structure was determined in the tongue radix, which is considered an exception in the tongue radix. This finding may be attributed to low food contact, consistent with the literature (6).

In our study, as reported in different galliform birds (10, 11, 18, 38, 39), anterior and posterior lingual gland structures were found in the connective tissue in the corpus and radix parts of the tongue. In addition, unlike the literature, more dense microscopic papillae were observed on the corpus in our study. These structures extended laterally at the apex and right angles at the stem and radix.

Although two conical papillae on the posterolateral part of the corpus were reported in domestic turkeys (18), we did not find these structures in our study. Filiform papilla-like structures (or distinct projections of deciduous epithelial cells) have been reported to be found on the anterior parts of the dorsal surface of the tongue in chickens (32) and the posterior parts of the dorsal surface of the tongue in domestic geese (6). In Galliform birds such as *Gallus gallus* (33), *Coturnix coturnix* (26), *Alectorix chukar* (35), and *Meleagris gallopavo* (18), *filiform papillae* structures were not found on the tongue surface. In our study, no *filiform papillae* structure was observed that was similar to that of galliform birds.

Harrison (1964) categorized the tongue in birds into three groups. Domestic turkey tongue was included in the third group because it fills the beak cavity except for the space in the front part of the beak and the mobility in the beak cavity is low (40).

As a result of the study, macroscopic and microscopic descriptions of the tongue were made in turkeys raised in Türkiye. As mentioned in the previous study in turkeys, two cornified epithelial structures were observed. Microscopic papillae extending from the connective tissue to the epithelial layer on the dorsal surface of the tongue with different directions were quite prominent. Our findings indicate that turkey tongues raised in Türkiye are generally similar to those of Galliform birds, with minor differences. The results can be used as a basic data source for the selec-

tion of feed material and veterinary anatomy and surgery in this bird species.

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